Agela Technologies

Bonna-Agela

Sample Preparation Products

Official Website

Best Value
Guaranteed Product Quality
Innovation to Benefit Customers


Sample Preparation
Better Solutions for Chromatography
Chemical Analysis
Solid Phase
Agilent Technologies

Bonna-Agela Technologies — A Global Supplier for Chromatography Solutions

As Bonna-Agela is poised to enter its new development stage with confidence and pride in its innovative separation, purification, and sample preparation products, we would like to thank our many loyal customers for your continuous support and trust. With your support and our effort in delivering the highest quality products to you, our company has grown remarkably. This has allowed us to expand our research and development effort, and thus introduce more innovative products to better service your application needs.

We had tremendous accomplishments: We cataloged over one thousand different products. Our manufacturing and R&D operation were certified in compliance with ISO 9001 and passed many quality audits by customers and distributors, including VWR International. As a global wide company, this will allow us to reach higher goals and to provide our customers with even better quality products and faster service in the new year.

Our mission statement and commitment:

- Provide products with our innovative technologies at the best performance to cost ratio.
- Deliver products with guaranteed quality.
- Provide global support with quick responses.

How to Place Orders

Our office is open from 9:00 am to 6:00 pm Eastern Standard Time, Monday through Friday.

To place an order or receive a quote, you may choose from the following contacts:

Bonna-Agela Technologies US
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Wilmington, DE 19808, USA
Tel: (302) 438 8798
Fax: (302) 636 9339
Website:http://www.bonnaagela.com
E-mail: info@bonnaagela.com

Bonna-Agela India
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Phone : 91-120-4225466-71
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Bonna-Agela China
179 South Street, Teda West Zone, Tianjin 300462, China
Tel:+86(22)25321032/7023
Fax:+86(22)25321033

Please include the following information with your order or request: Account number (if you have one), purchase order number, contact name, organization name, shipping and billing address, telephone number, fax number or email address, product number, brief description and quantity, method of payment and preferred method of delivery. A written confirmation will be sent to you by email or fax. We accept business checks, wire transfers and major credit cards as methods of payment.

Checks:
Please make checks payable to:
Bonna-Agela Technologies Inc. and send to:
Bonna-Agela Technologies Inc.
2038A Telegraph Road, Wilmington, DE 19808, USA

Wire Transfer:
Please contact us by phone, fax or email for account information.

Credit Cards: (USA Only)
Please include card type and number, expiration date, and card holder name. Due to security concerns, please do not email the information. Please call or send a fax to provide your credit card information.

Terms and Conditions

PLEASE READ THESE TERMS BEFORE ORDERING. IF YOU HAVE ANY QUESTIONS, PLEASE DO NOT HESITATE TO CONTACT US AND OUR STAFF WILL BE GLAD TO ASSIST YOU.

Acceptance and Availability
All orders placed are subject to the agreement of Bonna-Agela Technologies Inc. The catalogue does not constitute an engagement of the company to sell all listed products. You are guaranteed to be notified at the time of ordering if the ordered items are in back-order or discontinued.

Price and Payment
The prices are in effect at the time of printing. Bonna-Agela Technologies reserves the right to change the prices without notice, though we do our best to provide our customers with advance notice. The prices quoted at the time of ordering will be guaranteed. The general payment term is net 30 days, F.O.B., Newark, Delaware,USA. However we reserve the right to ask for prepayment if customers' account information is not satisfactory. A 1.5% per month service charge will be added to delinquent accounts. If a purchase order is less than $1000.00, a $50.00 extra charge will be added to the invoice.

Changes
Bonna-Agela Technologies reserves the right to change product specifications, quantities, designs or prices without prior notice and without liability for such changes.

Shipping Policy
The standard shipping method is 2-day FedEx within the United States and Canada. We will try to accommodate requests for other shipping methods if they are available. All shipping and handling charges will be billed separately. Should you receive damaged goods, it is imperative that you notify us immediately and save all packing materials for inspection by the carrier.

Application
All products in this catalog should be used for laboratory or manufacturing use only. They are not intended for direct medicinal or food use. Bonna-Agela Technologies assumes no liability for any misuse of the products.

Returns
Bonna-Agela Technologies tries to accommodate all requests for returns of unused goods. However, return of some items may be restricted by the original manufacturers. Please contact us for return authorization before returning any items. A restocking charge may apply to certain products.

Warranty
All Bonna-Agela Technologies products are warranted to be free of defects in materials and workmanship. They are not warranted for any other particular purpose. Bonna-Agela Technologies shall not under any circumstance be liable for any incidental, consequential or compensatory damage in conjunction with its products. The maximum liability shall not exceed the invoice price of the product.

Best Value
Guaranteed Product Quality
Innovation to Benefit Customers

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Sample Preparation Products

Introduction

Solid phase extraction (SPE) is one of the most used techniques in sample preparation. It is replacing the conventional time-consuming liquid-liquid extraction (LLE) method. The process of SPE is a miniature version of liquid column chromatography. SPE, utilizing the same type of stationary phases as used in liquid chromatography columns, are practiced in different mechanisms including normal phase, reversed phase, ion-exchange, affinity chromatography and mixed-mode in the fields of agricultural and food, bio-analytical, environmental and forensic applications. Bonna-Agela as a leader of chromatographic media products offers a variety of polymer and silica based SPE products.

Cleanert® SPE Cartridges, Well Plates and Media

Filtration Plate

Our Filtration Plates can effectively intercept most of the precipitated proteins. The plates can replace time-consuming manual protein precipitation (PPT) procedure to achieve a more efficient bio-sample cleanup result.

Agela select high purity raw materials ensuring to produce high quality products.Residue determination has been tested for each lot, and all materials be used are inert to avoid extra adsorption of analysis.

Various membranes with different pore size and specification are available for 96 well plate format.

Common Filters

Materials of the filters: PP, PTFE, PVDF, Cellulose mixed esters, Regenerated cellulose and glass fibre;

Pore size: 0.22 μm, 0.45 μm, 1 μm, 5 μm…

MAS (Multi-function Impurity Adsorption SPE)

MAS is a simple sample treatment method that applies multi-function impurity adsorption to minimize matrix effect caused by phospholipids in plasma sample. It achieves a faster and easier approach in comparing to SPE, PPT method.

MAS method is more proper for early stage development for series compounds with a similar property.

Outstanding clean up performance offer enhanced effective sensitivity. Method development is much simpler by following the method protocol, making lab work more effectively.

MAS-QuEChERS is an application of modified QuEChERS method which is suitable for most of pesticide residue analysis, drug or antibiotic residue analysis in vegetable, fruit, grain, and animal issues.

SLE (Solid Supported Liquid/liquid Extraction) Cartridges and Well-plates

Specially treated diatomite materials are packed in columns and well plates. The liquid/liquid extraction happens on the surface of the materials, and SLE method can effectively avoid emulsification and removing the phospholipids in the biological samples, also its operation is easily automated in parallel processing for time saving.

Traditional line:

Different pH values, neutral and basic;
Different particle size, small and big particle lines provide different applications;
Surface modification and deactivated for sensitive compounds;
Bonna-Agela is one of the very few original manufacturers of diatomite for chromatography. Bonna-Agela can provide diatomite at different pH values, as well as a variety of surface modification, to meet different application needs. The particle size distribution was narrowed and the surface activity was controlled to avoid unwanted adsorption of analytes.

Bonded Silica SPE Cartridges

Silica based SPE series are made of high quality and low metal contents silica particles. Using the special surface modification methodology, the activity of silica surface is reduced, which will reduce the tailing of basic compounds and ensure high recovery and reproducibility.
Average particle diameter: 50 μm (spherical particles)
Average pore size: 60 Å
Specific surface area: 600 m^2/g(spherical particles); 480 m^2/g(irregular particles)

Traditional line:

C18, C18-N, C8, NH_2, COOH, Silica, PSA, PRS, SCX, SAX;

Unique: AQ-C18, HILIC (Amide), PEG/C18

OMM SPE Products (Optimized Molecular Modification)— PEP series, PAX, PCX, PWAX, PWCX and PS are all based on polystyrene/divinylbenzene while each phase has different functionality and unique selectivity. They are highly recommended for the extraction of a wide range of compounds in pharmaceutical, agricultural, food, and environmental industries.
Average particle diameter: 40-60 μm and 30 μm;
Average pore size: 70 Å;
Specific surface area: 600 m^2/g.

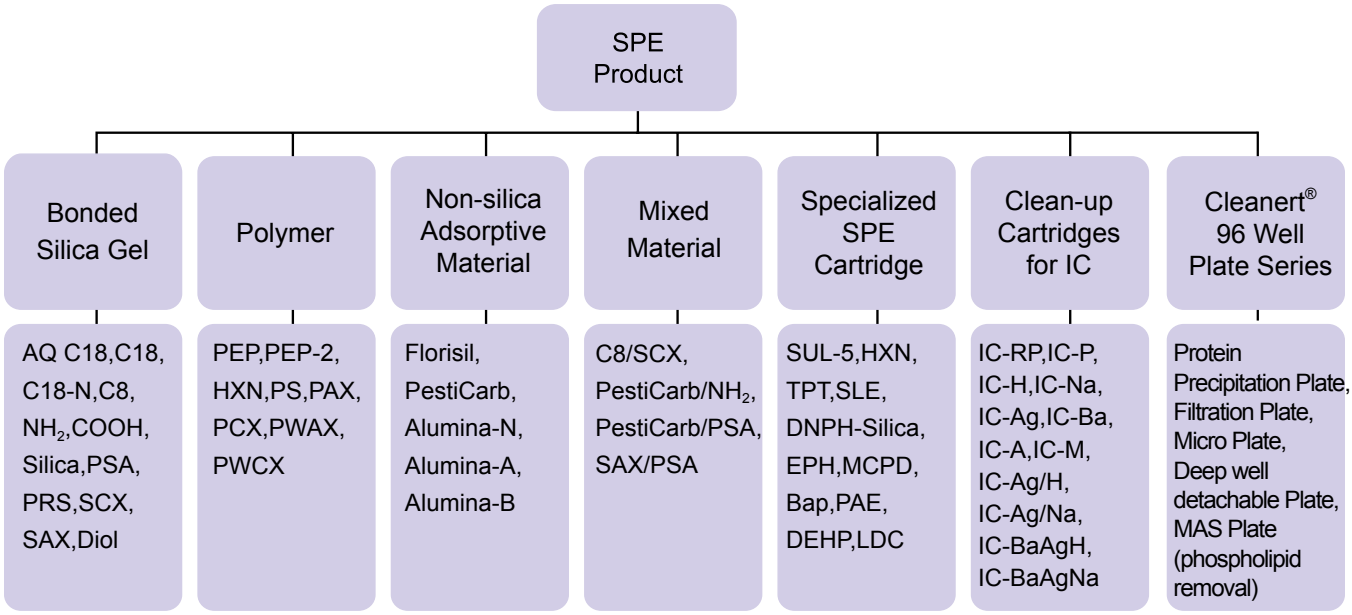
Non-silica Adsorption Phase Cartridges - Florisil, PestiCarb, Alumina (Neutral, Acid, Basic). They are commonly used to remove polar interference from non-polar samples. Cleanert® adsorption sorbents have high purity, high recovery and good reproducibility. They are widely used in sample preparation for environmental and food analyses.

Mixed and Layered Phases - C8/SCX, PestiCarb/NH_2, PestiCarb/PSA, SAX/PSA

Specialized Phases - SUL-5 (Determination of five kinds of sulfonamides in pork sample)
TPT---Triple-phase for tea leaves (Multi Pesticide...Pesticide residues analysis)
TPH---Triple-phase for herb (Multi Pesticide...Pesticide residues analysis)
DNPH-Silica (Aldehyde ketone analysis in air)
HXN (Determination of 10 kinds of sulfonylureas herbicide residues in soil samples)
MCPD (Chloropropanol detection in food)
ACA (Polar compounds analysis in water solution, such as acrylamide)
PAE (Plasticizer detection in food matrix containing fats and oils)
DEHP (Plasticizer detection in aqueous sample)
BAP (Benzopyrene detection in food matrix containing fats and oils)
EPH (Extractable petroleum hydrocarbon analysis in environmental samples)
LDC (Large disk column for water contaminant analysis)

Clean-up Cartridge for Ion Chromatography Application - IC-RP, IC-P, IC-H, IC-Na, IC-Ag, IC-Ba, IC-A, IC-M, IC-Ag/H and IC-Ag/Na; IC-BaAgH, IC-BaAgNa; Remove matrix interferences such as phenolics, metals, cations, anions, or hydrophobic substances encountered in many ion chromatography applications

Specialized cartridge and well plate format - LRC cartridge; Deep array 96-well plate series; Glass tube and PTFE frit without plasticizer.



Classification According to The Type of The Products

1. SPE Cartridges

SPE cartridge

- The common SPE cartridges consist of three parts: high purity polypropylene tube, porous PE frit and packing materials. (40-60 μm).
- Common specification: 100mg / 1mL, 200mg / 3mL, 500mg / 3mL and1g / 6mL etc. Take 100mg / 1mL of cartridge as an example, 100 mg is the quality of packings and 1 mL is the volume of the tube.
- Disposable usage: SPE is disposable to avoid cross-contamination.

2. 96-well plates

96-well plate 2 mL

96-well plate is the product for high throughput applications. Each well contains a small amount of sorbents (10-100 mg) with max. Total volume per well is 1mL & 2 mL . With the main applications in the cleanup of multi-sample in the area of bio-analysis and clinical analysis, it is compatible with the automated sample handing work station for high throughput operation.

96-well plate 1 mL

3. Micro plate series

Micro plate allows a small amount of sample loading and elution.
Array series provide base plate and individual removal wells, and they allow different SPE materials in one plate, which are suitable for method development.

Cleanert® micro plate

4. Deep well detachable plate

Deep well detachable plate has a bigger storage room for sample and elution solvent, a totally 3mL volume is available, also all the cartridge could be detached from the base plate.

Deep well detachable plate

5. Clean-up Cartridges for Ion-chromatography application

Cleanert® IC series are used for removing matrix interferences such as phenolics, metals, cations, anions, or hydrophobic substances encountered in many ion chromatography applications.

Cleanert® IC cartridges

6. MAS-QuEChERS

MAS (Multi-mechanism Adsorption SPE) -QuEChERS, which is in centrifuge tube format, has been widely applied in pesticide and veterinary drug residue analysis in vegetable, fruit, grain, and animal tissues.

Cleanert® MAS -QuEChERS

7. Bulk Media

Bulk packing materials are available for customized usage in sample preparation.

8. Cartridge Format

General cartridge

Flangeless cartridge

LDC cartridge

LRC cartridge

Glass cartridge

Bulk media

Featured Products

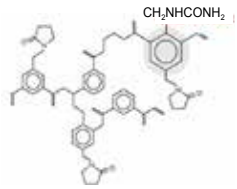
OMM Technology (Optimized Molecular Modification) for SPE

Bonna-Agela SPE products have been developed based on a thorough understanding of interactive natures of chemical molecules. Our SPE products thus can better meet customer's needs. Our R&D results demonstrated that the adsorption/ desorption property of the polymeric SPE materials are regulated by the types of the functional groups and the degree of substitution of the surface modification.

In general, modification with electron donor groups will help to retain the electron-deficient molecules, while modification with electron-withdrawing groups will prefer to retain the electron-rich molecules. Different SPE materials have been developed by incorporating proper types of functional groups and the degree of substitutions on the surface, and thus providing optimized and balanced performance for all types of molecules.

Cleanert® PEP-2

Cleanert® PEP-2 is made of polydivinylbenzene on which the surface is functionalized with vinyl pyrrolidone and urea. In addition to a balanced hydrophilic and hydrophobic property, the PEP-2 is also an electron donor in a polar-polar interaction, as well as a strong hydrogen donor and acceptor in hydrogen bonding. As a result, the PEP-2 can retain most of acidic, basic and neutral polar compounds without adjusting the pH of the samples. PEP-2 has stronger retention of polar compounds than PEP.



Particle Characteristics

Functionalized polymer sorbents;
Average particle size: 40-60 μm;
Average pore size: 70 Å;
Specific surface area: 600 m^2/g.

Suggestion Processing Method

Neutral and Basic Compounds (metoprolol)

Condition: MeOH or ACN; Water

↓

Sample Loading: Diluted biological sample

↓

Wash: Water (or 5% organic solvent water solution) Dry the cartridge by vacuum

↓

Elution: MeOH or ACN

Acidic Compounds (salicylic acid)

Condition: MeOH or ACN; Water

↓

Sample Loading: Diluted biological sample

↓

Wash: Water (or 5% organic solvent water solution) Dry the cartridge by vacuum

↓

MeOH or ACN (For PEP-2, add some acid in the solvent will improve the recoveries for acid compounds.)

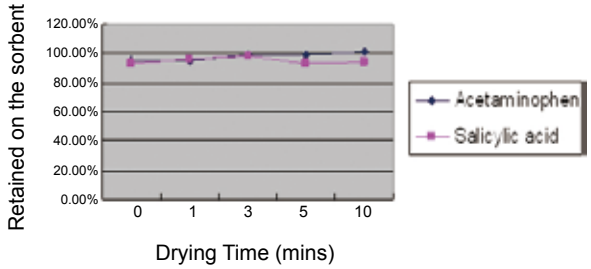
*This processing method could be used for all the PEP series.

Good Water-Wettability

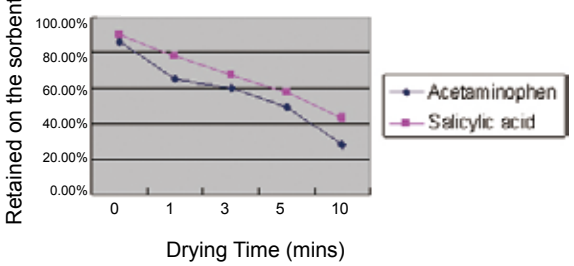
The Cleanert® PEP-2 (polar polymer) sorbent is a unique hydrophilic-lipophilic balanced material. It provides excellent wettability since the hydrophobic surface of the sorbent is highly modified with polar functional groups. The SPE mechanism follows the reversed phase separation principle.

Effect of Drying on Recovery - PEP-2 Versus C18 Sorbents

Cleanert® PEP-2 (60mg / 3mL)



Cleanert® C18 (200mg / 3mL)



High Retention for Polar Compounds and Acid Compounds

The electron donating and with-drawing functionalities of the sorbent allow it to have enhanced retention for polar compounds. For high polar compounds such as acid analytes, there is no need to adjust the pH condition to restrain the ionization of the target analytes, which make method more simple and robust.

The recoveries of three compounds on different SPE materials

	PEP	PEP-2	Other RP phase similar to PEP	
Caffeine	98.58%	100.37%	100.12%	103.53%
Metoprolol	80.11%	88.25%	91.23%	90.05%
Salicylic acid	21.36%	109.73%	8.03%	18.79%

* No pH adjustment for sample loading solution

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® PEP-2	30 mg	1 mL	100	PE0301-2
	60 mg	3 mL	50	PE0603-2
	100 mg	3 mL	50	PE1003-2
	200 mg	6 mL	30	PE2006-2
	500 mg	6 mL	30	PE5006-2
	30 mg/well	2 mL	96-well plate, 1/PK	PE0302-2W
	50 mg/well	2 mL	96-well plate, 1/PK	PE0502-2W
	10 g	-	bottle	PE0010-2
	100 g	-	bottle	PE0100-2

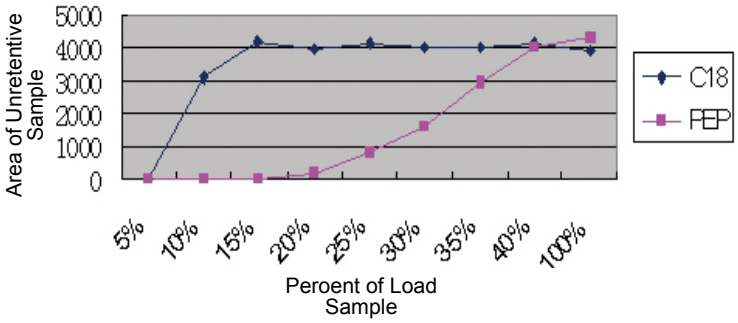
Cleanert® PEP

Cleanert® PEP is made of polydivinylbenzene on which the surface is functionalized with vinyl pyrrolidone. The material has a balanced hydrophilic and hydrophobic property and can be used in the entire pH range of 1-14.
PEP can be used to extract a variety of polar and non-polar compounds. Some highly hydrophilic compounds which have little retention on C18 columns, such as chlorinated phenols, phosphate esters and drug metabolites, can be effectively retained on PEP.

High Capacity

The Cleanert® PEP series sorbents have much bigger surface area and show a dramatic increase of sample capacity compared to silica-based C18. The volume of elution solvents can be reduced, and subsequently the total operation time (including solvent evaporation) will be shortened.

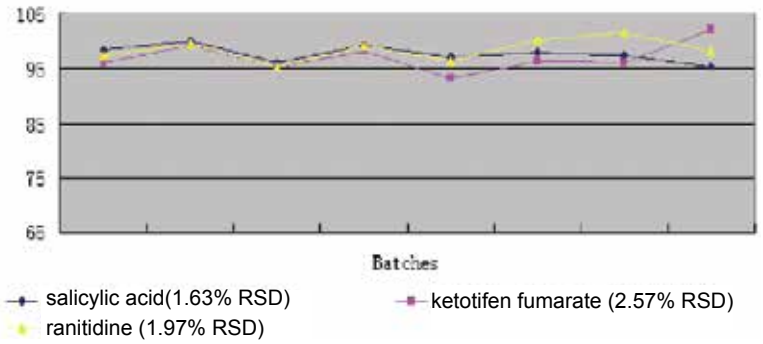
The Capacity of Acetophenone on C18 (200mg / 3mL) and PEP (60mg / 3mL)



Excellent Batch-to-Batch Reproducibility

The OMM technology offers consistent surface modification, resulting in excellent batch-to-batch reproducibility.
Multiple batches of Cleanert® PEP series have been successfully used for various compounds with consistent results.

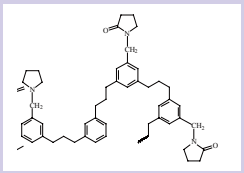
Batch to batch reproducibility of PEP



Particle Characteristics

Functionalized polymer sorbents; Average particle size: 40-60 µm.
Average pore size: 70 Å; Specific surface area: 600 m^2/g.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® PEP 	30 mg	1 mL	100	PE0301
	60 mg	1 mL	100	PE0601
	60 mg	3 mL	50	PE0603
	100 mg	3 mL	50	PE1003
	200 mg	6 mL	30	PE2006
	500 mg	6 mL	30	PE5006
	30 mg	2 mL	96-well plate, 1/PK	PE0302-W
	50 mg	2 mL	96-well plate, 1/PK	PE0502-W
	10 g	-	bottle	PE0010
	100 g	-	bottle	PE0100

Cleanert® PAX (RP/Strong Anion Exchange)

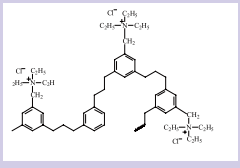
Description

It is designed to overcome the limitations of traditional silica based mixed-mode SPE sorbents such as C18/SAX. It is a RP/strong anion exchange mixed-mode polystyrene/ divinylbenzene sorbent, stable from pH 0-14.

Particle Characteristics

Based on functionalized polystyrene/divinylbenzene;
Average Particle Diameter: 40-60 μm;
Average Pore Size: 70 Å;
Specific Surface Area: 600 m^2/g

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® PAX 	30 mg	1 mL	100	AX0301
	60 mg	1 mL	100	AX0601
	60 mg	3 mL	50	AX0603
	100 mg	3 mL	50	AX1003
	200 mg	6 mL	30	AX2006
	500 mg	6 mL	30	AX5006
	30 mg/well	2 mL	96-well plate, 1/PK	AX0302-W
	50 mg/well	2 mL	96-well plate, 1/PK	AX0502-W
	10 g	-	bottle	AX0010
	100 g	-	bottle	AX0100

Cleanert® PWAX (RP/Weak Anion Exchange)

Description

Cleanert® PWAX provides the dual modes of retention, weak anion exchange and reversed-phase on a stable polymer sorbent, which improves the retention for acidic analytes.

Particle Characteristics

Based on partially functionalized aminopolystyrene/ divinylbenzene;
Average Particle Diameter: 40-60 μm;
Average Pore Size: 70 Å;
Specific Surface Area: 600 m^2/g.

Suggested processing Method for PAX and PWAX

Condition with Methanol and then Water

↓

Sample loading (adjust pH with acid solution)

↓

Wash with 2% $NH_3 \cdot H_2O$ in water

↓

Wash with methanol

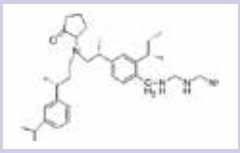
↓

Elution with 2% formic acid in methanol

↓

Evaporation and reconstitution

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® PWAX 	30 mg	1 mL	100	WA0301
	60 mg	3 mL	50	WA0603
	100 mg	3 mL	50	WA1003
	200 mg	6 mL	30	WA2006
	500 mg	6 mL	30	WA5006
	30 mg/well	2 mL	96-well plate, 1/PK	WA0302-W
	50 mg/well	2 mL	96-well plate, 1/PK	WA0502-W
	10 g	-	bottle	WA0010
	100 g	-	bottle	WA0100

Cleanert® PCX (RP/Strong Cation Exchange)

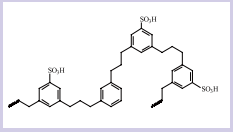
Description

Cleanert® PCX is a mixed-mode, strong cation exchange sorbent which provides dual retention modes of reversed-phase and cation-exchange. It has high surface area and a wide usable pH range of 0-14.

Particle Characteristics

Based on Functionalized polystyrene/divinylbenzene;
Average Particle Diameter: 40-60 μm;
Average Pore Size: 70 Å;
Specific Surface Area: 600 m^2/g.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® PCX 	30 mg	1 mL	100	CX0301
	60 mg	1 mL	100	CX0601
	60 mg	3 mL	50	CX0603
	100 mg	3 mL,	50	CX1003
	200 mg	6 mL	30	CX2006
	500 mg	6 mL	30	CX5006
	30 mg	2 mL	96-well plate, 1/PK	CX0302-W
	50 mg	2 mL	96-well plate, 1/PK	CX0502-W
	10 g	bottle	bottle	CX0010
	100 g	bottle	bottle	CX0100

Cleanert® PWCX (RP/Weak Cation Exchange)

Description

Cleanert® PWCX provides dual modes of retention, weak cation exchange and reversed-phase on a stable polymer sorbent, which improves the retention for basic analytes.

Particle Characteristics

Based on partially functionalized polystyrene/divinylbenzene;
Average Particle Diameter: 40-60 μm;
Average Pore Size: 70 Å;
Specific Surface Area: 600 m^2/g.

Suggested processing Method for PCX and PWCX

Condition with Methanol and then Water

↓

Sample loading (adjust pH with basic solution)

↓

Wash with 2% formic acid in water

↓

Wash with methanol

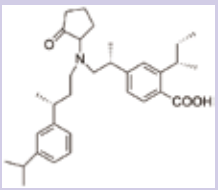
↓

Elution with 2% $NH_3 \cdot H_2O$ in methanol

↓

Evaporation and reconstitution

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® PWCX 	30 mg	1 mL	100	WC0301
	60 mg	3 mL	50	WC0603
	100 mg	3 mL	50	WC1003
	200 mg	6 mL	30	WC2006
	500 mg	6 mL	30	WC5006
	30 mg/well	2 mL	96-well plate, 1/PK	WC0302-W
	50 mg/well	2 mL	96-well plate, 1/PK	WC0502-W
	10 g	-	bottle	WC0010
	100 g	-	bottle	WC0100

Cleanert® PS

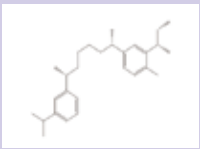
Description

Cleanert® PS is made of non-substituted polydivinylbenzene. It has larger surface area (>600 m^2/g.) and thus greater capacity than reversed phase bonded silica. Cleanert® PS can be used for the extraction of non-polar and polar compounds simultaneously.

Particle Characteristics

Based on polystyrene/divinylbenzene;
Average Particle Diameter: 40-60 μm;
Average Pore Size: 70 Å;
Specific Surface Area: 600 m^2/g.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® PS 	30 mg	1 mL	100	PS0301
	60 mg	3 mL	50	PS0603
	100 mg	3 mL	50	PS1003
	200 mg	6 mL	30	PS2006
	500 mg	6 mL	30	PS5006
	30 mg/well	2 mL	96-well plate, 1/PK	PS0302-W
	50 mg/well	2 mL	96-well plate, 1/PK	PS0502-W
	10 g	-	bottle	PS0010
	100 g	-	bottle	PS0100

Cleanert® HXN *(Mid Polar Polymers Specially for Sulfonyl Urea Extraction)*

Description

Cleanert® HXN is also made of polydivinylbenzene having surface modified with vinylpyrrolidone. This sorbent is specially designed to extract sulfonyl ureas from water and soil at ppb level. It is less polar than Cleanert® PEP and can also be used to extract, enrich and clean up samples from mid polar to high polar compounds.

Particle Characteristics

Based on polystyrene/divinylbenzene;
Average Particle Diameter: 40-60 μm;
Average Pore Size: 70 Å;
Specific Surface Area: 600 m^2/g

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® HXN	30 mg	1 mL	100	HX0301
	60 mg	1 mL	100	HX0601
	60 mg	3 mL	50	HX0603
	100 mg	3 mL	50	HX1003
	200 mg	6 mL	30	HX2006
	500 mg	6 mL	30	HX5006
	30 mg/well	2 mL	96-well plate, 1/PK	HX0302-W
	50 mg/well	2 mL	96-well plate, 1/PK	HX0502-W
	10 g	-	bottle	HX0010
	100 g	-	bottle	HX0100

Cleanert® Micro Plate

96-well micro plate can be packed with less sorbent, which allows to load a small amount of sample and elution solvent. The 96-well microplates with a special detachable design, each; loose cartridges use an internally tapered well design and make sure enough sorbent bed. Even limit packing material is embedded into each well, no breakthrough will happen. Each cartridge can be removed from the base plate, and different sorbents can be assembled in one base plate which is suitable for method development.

The micro plate optimizes the configuration of the sorbents (PEP-2, PEP, PCX, PAX, PWCX, and PWAX), packed with 30 μm particle size sorbent to enhance the efficiency of column.

Cleanert® micro plate allows low-elution volume with sensitive, robust, and reproducible results without time-consuming evaporation and reconstitution step.

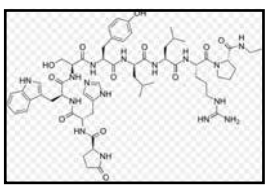
Removing Tool

Innovative Features

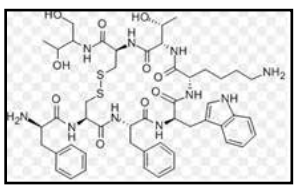
- Internally tapered well suitable for small volume of the samples;
- High sorbent bed avoids breakthrough;
- Detachable cartridges allow flexible combination;
- Low-elution volume saves time of concentration;
- Various sorbents meet the requirement of method development.

Similar Product Comparison

Detection of leuprolide and octreotide using Micro SPE plate and LC-MS/MS



Leuprolide



Octreotide

Consumables

Cleanert® PWCX 96-well micro plate (P/N: WC00501-MW);

SPE processing method

Condition	Condition the plate with 0.4 mL of 5% HCOOH in methanol ; 0.4 mL of methanol; 0.4 mL of water one by one (apply vacuum to each step).
Sample loading	Combine 100 µL of serum sample, 30 µL of IS-D , 200 µL of 100 mM ammonium acetate buffer together. And transfer the mixture to the preconditioned plate (apply vacuum if necessary).
Washing	Wash the plate with 0.4 mL water; then 0.4 mL 1.2 % NH_4OH in methanol and 0.4 mL methanol (apply vacuum to all step).
Elution	Elute twice with 0.3 mL of 5 % formic acid in methanol into each well of 96-well plate. (1.2 mL/well, apply vacuum to move out the residue solvent)
	Evaporate the eluates at 35 ºC under nitrogen and reconstitute the residue with 125 µL of mobile phase.

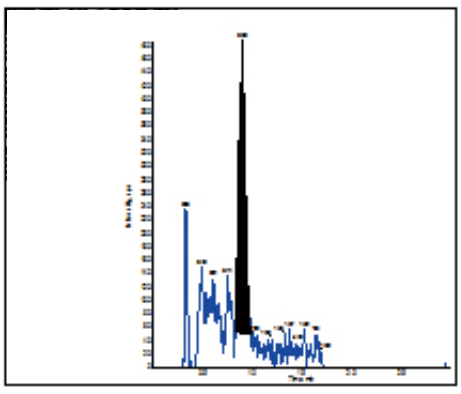
A. Recovery of Leuprolide

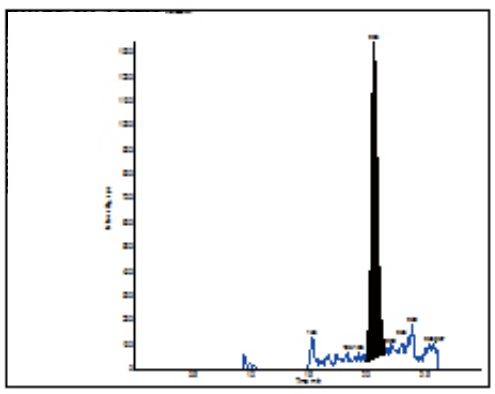
Recovery		Leuprolide Peak Area			
		QCL		QCH	
Bonna-Agela PWCX micro plate	Mean	2240	4339	310486	703048
	SD	313	230	19215	4678
	%CV	13.95	5.30	6.19	0.67
	n	3	2	3	2
	Recovery %	51.61		44.16	

B. Recovery of Octreolide

Recovery		Octreotide Peak Area			
		QCL		QCH	
Bonna-Agela PWCX micro plate	Mean	4085	5420	378178	593745
	SD	245	535	4342	3294
	%CV	11.75	10.67	1.56	0.63
	n	3	2	3	2
	Recovery %	72.53		63.11	

Chromatograms of serum samples spiked with Octreotide and Leuprolide





Detection of Metoprolol and Propranolol in Plasma using Micro Plate

Sample pretreatment

Cleanert® PEP 96-well micro plate
(5 mg/each well, PN: PE00501-MW)

↓

Condition: 200 µL of methanol and 200 µL of water

↓

Sample Loading: 100 µL of plasma

↓

Wash: 200 µL of 5% methanol water solution

↓

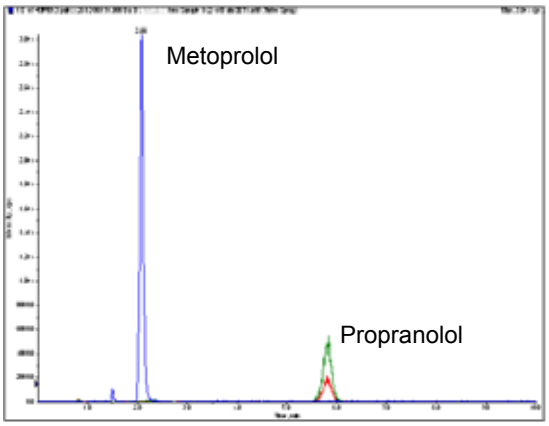
Elute: 50 µL of ACN:IPA=4:6(with 2% formic acid)

↓

Constitute the eluant to 200 µL with water and then for LC-MS/MS analysis.

HPLC condition

Column: Unisol C18 (2.1×150 mm, 5 µm, PN: UO951502)
Mobile phase A: 0.1% Formic Acid in water
Mobile phase B: 0.1% Formic Acid in ACN
A:B=78:22 for 10mins
Flow rate: 300 µL/min
Temperature: 30℃
Injection volume: 10 µL



Chromatogram of spiked plasma sample with 5 ppb metoprolol and propranolol

Analyte	Spiked concentration	Average recoveries	RSD (n=5)
Metoprolol	0.5 ng/mL	86.8 %	3.93 %
	2.5 ng/mL	92.6 %	3.12 %
	10 ng/mL	93.9 %	2.21 %
Propranolol	1 ng/mL	88.1 %	3.54 %
	5 ng/mL	90.2 %	2.03 %
	20 ng/mL	95.6 %	3.17 %

Ordering information

Product	Specification	Loose cartridge (96/PK)	Assembled plate (1/PK)
Cleanert® PEP-2	5mg / 1mL / well	PE00501-2LMW	PE00501-2MW
	10mg / 1mL / well	PE0101-2LMW	PE0101-2MW
Cleanert® PEP	5mg / 1mL / well	PE00501-LMW	PE00501-MW
	10mg / 1mL / well	PE0101-LMW	PE0101-MW
Cleanert® PAX	5mg / 1mL / well	AX00501-LMW	AX00501-MW
	10mg / 1mL / well	AX0101-LMW	AX0101-MW
Cleanert® PCX	5mg / 1mL / well	CX00501-LMW	CX00501-MW
	10mg / 1mL / well	CX0101-LMW	CX0101-MW
Cleanert® PWAX	5mg / 1mL / well	WA00501-LMW	WA00501-MW
	10mg / 1mL / well	WA0101-LMW	WA0101-MW
Cleanert® PWCX	5mg / 1mL / well	WC00501-LMW	WC00501-MW
	10mg / 1mL / well	WC0101-LMW	WC0101-MW
Base plate	/	/	96WKB
Removing Tool	/	/	RM01

Cleanert® PPT- Protein Precipitation Plate

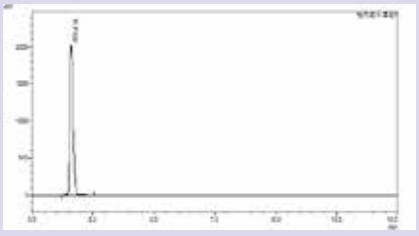
Protein precipitation is often used as a fast sample preparation method in bioanaylsis. Protein denaturation with acetonitrile in a centrifuge tube is the most used method which is laborious, time consuming, and also not compatible to automatic instruments. Cleanert® PPT plates were designed for high throughput and automated protein precipitation processing.
Bonna-Agela use high pure material and extractable residue were controlled strictly for every lot. 1mL and 2mL 96 well plates for sample loading are optional.

1mL round well plate format

2mL square well plate format

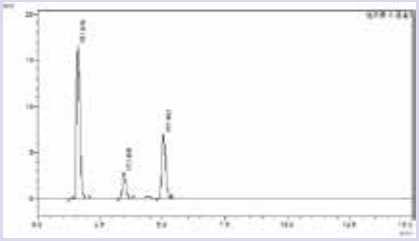
Advantages

- Protein precipitation is removed by filtration, eliminating time consuming of centrifugation and transfer steps;
- Pre-filtration design avoids clogging during operation. No acetonitrile leakage or corsstalk during mixing or incubation;
- Inert materials were used and make the plates stable in a wide range of pH from 0 to 14;
- High pure material provide low extractables residue, no interferences in UV or MS detection involved.



Background Testing

No peak area is > 1% of uracil



Recovery Testing

Recoveries of uracil, aniline, phenol and benzophenone > 95%

PPT Plate vs centrifuge

PPT Plate for 96 samples	Centrifuge for one sample
 15 mins	 45 mins
Load 600 μL of ACN and 200 μL of plasma into PPP plate Mix well Collect the eluant by vacuum	Add 1 mL of ACN and 200 μL of Plasma into centrifuge tube Vertex for 10 mins Centrifuge for 10 mins Transfer the supernatant

Typical processing method

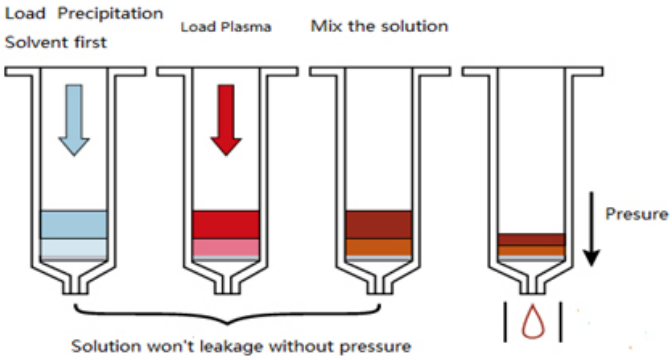
Place the Cleanert® PPT plate onto a vacuum manifold or positive pressure device;

Add precipitate solvent (such as acetonitrile and methanol) to the plate, 1:3 or 1:4 solvent to sample ratio was suggested;

Add 20-300 μL of plasma into each tube;

Mix the solvent and sample together by vortex or pipette;

Provide pressure (0.04-0.08 MPa was suggested) onto the plate to push the solvent through and then collect the elute for further analysis.



Hydrophobic frit is used to prevent leakage of organic solvent during precipitation, and avoid cross-contamination, giving enough time for adequately protein precipitation process.

Compatible with automatic and manual performance,such as vacuum manifold, positive pressure device and automatic workstation, saving operation time and labor.

Ordering Information

Product	Specification	Cat.No (2/PK)
Cleanert® PPT	1 mL	96CD1025
	2 mL	96CD2025-Q

Cleanert® FAST—Filtration Plate Series

Bonna-Agela filtration plates provide simple sample preparation of plasma, serum and other biological fluids. Optimazed membrance and frit avoid clogging and bringing in extra disturbing compounds. High throughput filtration plate method can replace manual operation, and achieve a more efficient results.

- Membranes: Glass fiber, PTFE, PP, Cellulose Acetate;
- Pore size: 0.22 μm, 0.45 μm, 1 μm, 5 μm are optional;
- 1 mL round well and 2mL square well 96well plate were available for filtration series.

1 mL Filtration plate-round well

2 mL Filtration plate- square well

Ordering Information

Product	Specification	Cat.No (2/PK)
Cleanert® Filtration Plate	2 mL; PP 0.22 μm	96GL2022
	2 mL; PP 0.45 μm	96GL2024
	2 mL;PTFE 0.22 μm	96GL0422
	2 mL; PTFE 0.45 μm	96GL0424
	2 mL;PVDF0.22 μm	96GL0322
	2 mL; PVDF 0.45 μm	96GL0324
	1 mL; PE 20 μm	96GL1025
	2 mL; PE 20 μm	96GL2025

Cleanert® MAS (Multi-function Impurity Adsorption SPE)

Cleanert® MAS is a simplified bio-sample preparation tool which offers multifunctional adsorption capabilities to remove interferences while the analytes are remained in the aquious phase. Although protein precipitation is the most common method in bioanalysis, it is the fact that this method is not efficient for eliminating the matrix effect on LCMS/MS due to the present of phospholipids. However with Cleanert® MAS the phospholipids are effectively removed follows a similar procedures.

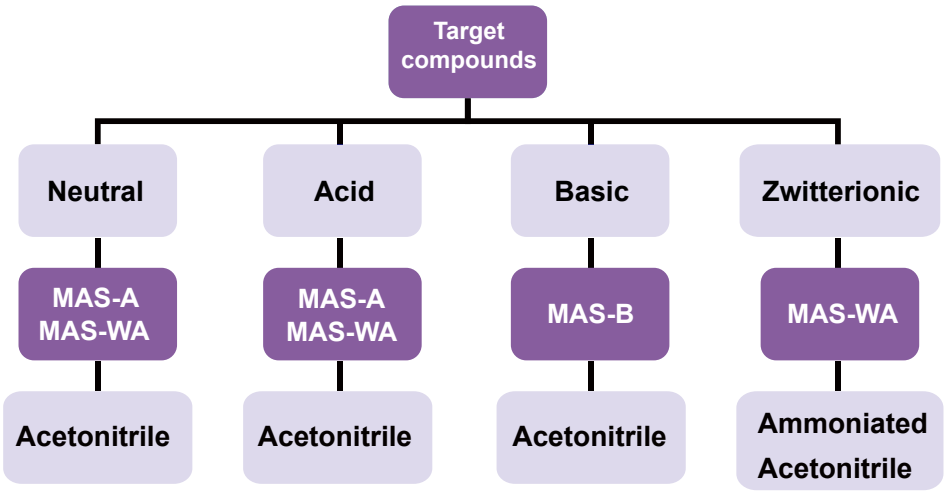
For amphoteric compounds, MAS-A should be followed by basic eluant (0.01%~1%$NH_3 \cdot H_2O$ is suggested to be added in the elution solvent), while MAS-B should be followed by acidic eluant (0.02%-2% acetic acid is suggested to be added in the elution solvent). Because the acidity/basicity of most compounds are weaker than that of phospholipids which contain choline and phosphate group, elution of analytes and retention of phospholipids can be achieved by adjusting different pH value of the solvent.

Product Format

There are two formats for Cleanert® MAS
MAS cartridge: better choice for method development
MAS plate: suitable for high throughput sample analysis requirement

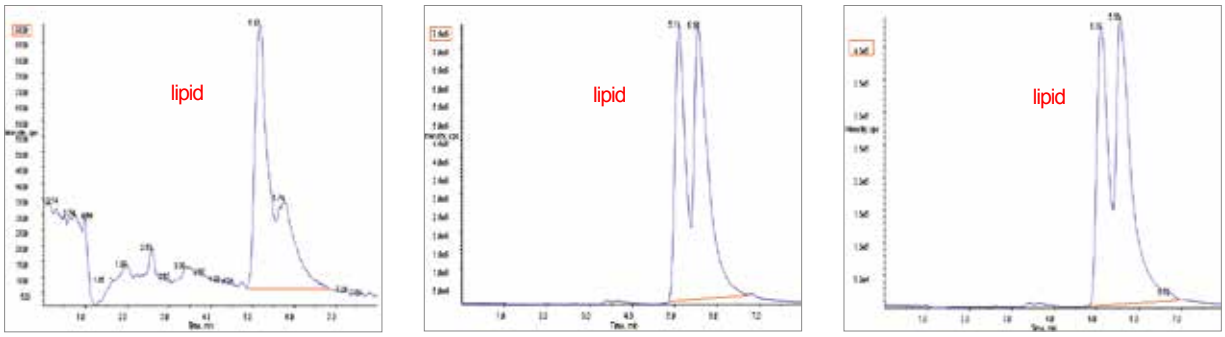
Product and Solvent Selection

Cleanert® MAS is divided into three kinds of sorbents, named MAS-A, MAS-B and MAS-WA, which can used for neutral, acid, basic and amphoteric compounds respectively. Three kinds of products can be chosen according to the chart below. In some cases, mixing 10% methanol with the eluant may improve the efficiency of protein precipitation. 9:1~1:1 is general ratio for ACN to methanol. Proper amount of water could be mixed with the eluant for the drugs with high polarity; however, the volume ratio should not be over 1/3 to make sufficient protein precipitation. Eluant mixed with a weak reagent (acetic ether, dichloromethane etc.) is suitable for sample with low polarity.



Flow chart of solvent and products selection

Removal of Phospholipids MAS vs PPT and SPE



Response : $9*10^3$ CPS
MAS

Response : $7.5*10^5$ CPS
PPT

Response : $4.3*10^5$ CPS
SPE

Fig 1 the comparison of phospholipids signal after processed by PPT, SPE and MAS method
The result showed that phospholipids signal deduced by 10-100 times using MAS plate.

Perfect performance for protein removal

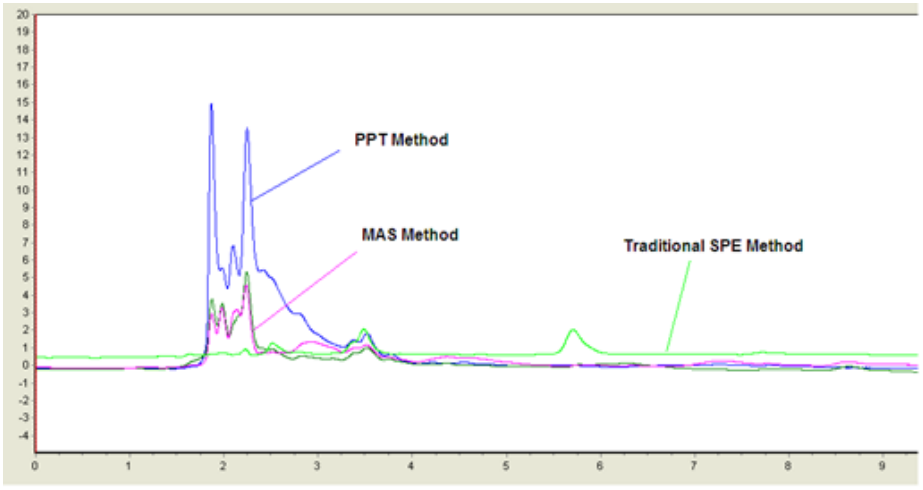


Fig 2 the comparison of protein signal after processed by PPT, SPE and MAS method

Suggested processing method

96 well Plate Format

Turn the "UP/DWON" knob to "UP" position to raise the Gas block and pull the platform out. Place the PPT Plate on a collection plate for processing. Place all these two plates onto the platform. Add 0.5-1 mL of acetonitrile (or acetonitrile with 0.01%-1% $NH_3 \cdot H_2O$ for amphoteric medicine) as precipitation solvent, and then add the sample into each well and mix well for 3 mins by pipette or vortex.

Push the platform back and turn the "UP/DOWN" knob to "DOWN" position. Switch the gas selection valve to "LOW FLOW" position and collect the eluates into collection plate. Analyze the eluate directly or concentrate the eluate and reconstitute, then for further analysis.

SPE Cartridge Format

Condition: Place a MAS cartridge onto the manifold. Add 0.5-1 mL of acetonitrile (or acetonitrile with 0.01%-1% $NH_3 \cdot H_2O$ for amphoteric medicine) to each well as the precipitation solvent;

Sample loading: Add 10-50 μL of plasma (or serum, urine, etc.) sample to each cartridge;

Mix well for 3 mins by pipette or vortex

Elution: Apply vacuum to each cartridge, and collect the eluate.
Analyze the eluate directly or Concentrate the eluate and reconstitute the residue by mobile phase solution, then for further analysis.

Ordering information

Production	Cat.No.	Spec.	Application	Package
MAS cartridge	MSC-B-0301	1 mL	Used for clean-up of basic and neutral compounds in plasma and biological samples	100
	MSC-B-0601	1 mL		100
	MSC-A-0301	1 mL	Used for clean-up of acidic compounds in plasma and biological samples	100
	MSC-A-0601	1 mL		100
	MSC-WA-0301	1 mL	Used for clean-up of weak acidic compounds in plasma and biological samples	100
	MSC-WA-0601	1 mL		100
MAS 96 well plate	MS-B-0302W	30mg / 2mL / well	Used for clean-up of basic and neutral compounds in plasma and biological samples	2
	MS-A-0302W	30mg / 2mL / well	Used for clean-up of acidic compounds in plasma and biological samples	2
	MS-WA-0302W	30mg / 2mL / well	Used for clean-up of weak acidic compounds in plasma and biological samples	2

Cleanert® SLE Products

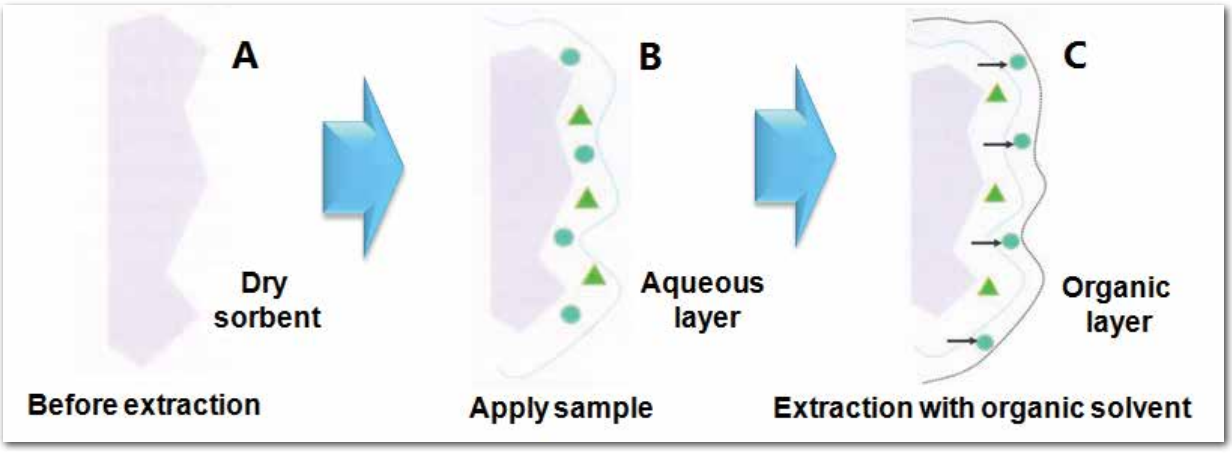
Supported Liquid Extraction Products

Cleanert® SLE (Supported Liquid Extraction) plates and cartridges contain a high quality modified diatomaceous earth with an ideal surface with large specific area and low activity. Cleanert® SLE plates and cartridges are used to extract analytes from bio-analytical, clinical, forensic, environmental and agrochemical samples, it even can replace most of the Liquid/Liquid extraction (LLE).

Supported Liquid Extraction (or Solid Supported Liquid/Liquid Extraction) can be considered as a micro liquid-liquid extraction with the same principles of traditional liquid-liquid extraction but performed on high quality modified diatomaceous earth. The target compounds are extracted from an aqueous phase into an immiscible organic phase.

Cleanert® SLE columns

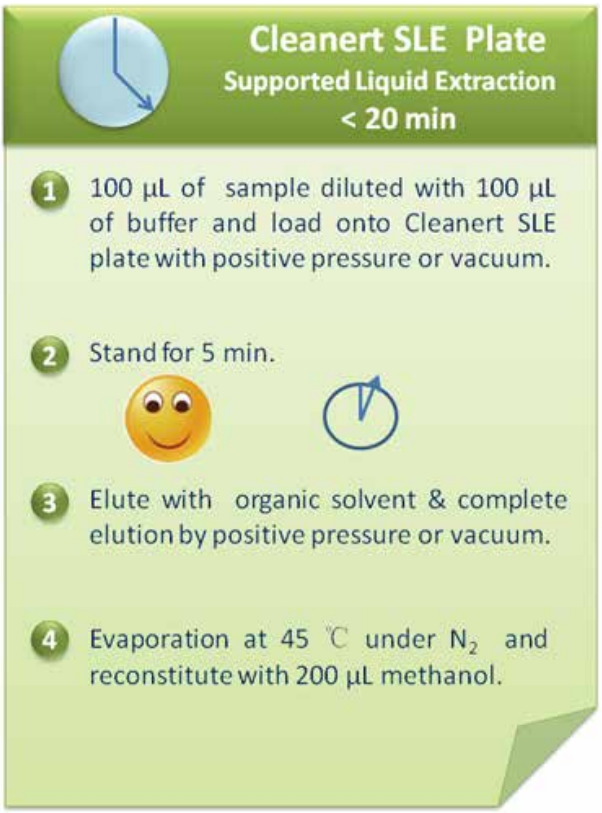
Cleanert® SLE plate



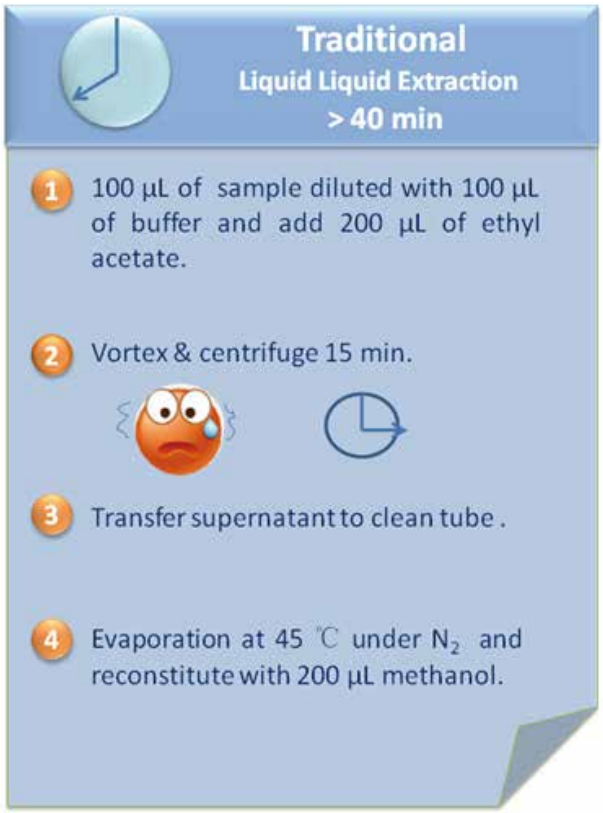
Benefits compared with LLE method

- Improving sample cleanliness and method sensitivity;
- The performance of SLE is very simple: Load-Wait-Elute, and easy to automate with exiting automatic device in Labs;
- No vigorous shaking and therefore emulsions cannot be formed, and ensure the reproducible results;
- The intimate contact between the aqueous sample and organic phases allows very efficient partitioning and improve the recoveries and reproducibility.

Fast & Simple



Slow & Tedious



Product Specification selection guide

The SLE approach can duplicate any developed LLE method with a few minor adjustment. In general, dilute the sample with buffer or water, follow the max sample loading volume to avoid over loading. For bio-samples such as urine and plasma, the sample volume can be very small (200 - 400 µL), especially when LC-MS/MS as a final technique for analysis.

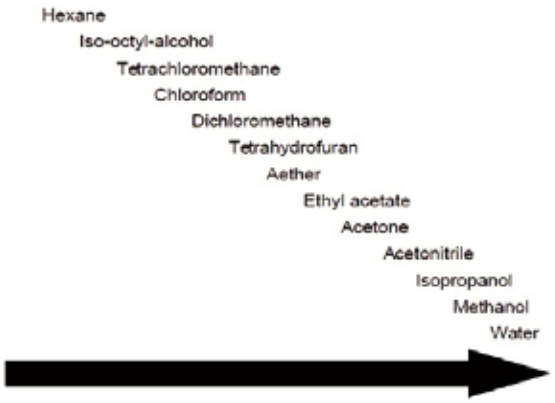
For sticky sample, SLE Plus series which using bigger particle size is suggested to improve flow rate also avoid blocking. The bigger particle size is also provide a benefit tool for filtration to get rid of particles and matrix.

Elution solvent selection guide

Normally, for very polar compounds, middle polar organic solvents should be selected such as dichloromethane or ethyl acetate. Since most of polar organic solvent such as isopropanol and methanol are 100% miscible with water, they are not suitable as a elution solvent in SLE directly. However, as a modifier, isopropanol or methanol can be added into extraction solvent to improve the extraction efficiency.

For non-polar compounds, non-polar solvents are suitable such as hexane and isooctane.

Non-polar solvents can be used as the immiscible organic solvent, the organic solvent should be spectroscopic grade or better since the collected fraction is often evaporated to dryness, and any solvent containing nonvolatile impurities will interfered the analysis of the target compounds.



The polarity chart of common solvents

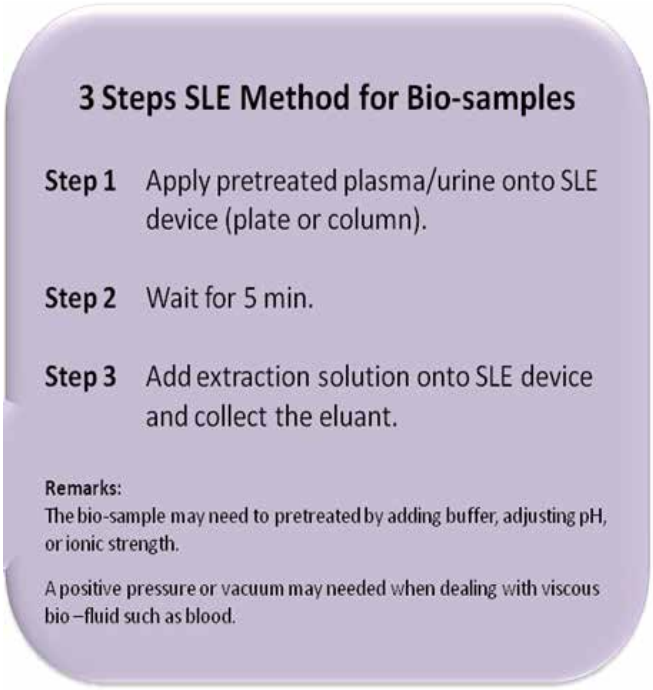
Salting-out in SLE

The principle of salting-out can be applied in SLE since it is a liquid-liquid extract. As in LLE the high concentration of salt in aqueous phase can enhance the extraction of high polar or water-soluble target compounds. As a result, the recovery of the target compound is increased. The extraction of dexamethasone is an example to demonstrate the effect of salting-out in SLE. Dexamethasone is a polar compound with Log P of 1.83 and pKa of 12.14. The recovery of dexamethasone in water diluted plasma is 53.5%. However, the recovery is improved significantly with the increase of the concentration of ammonium acetate (NH_4AC) in plasma. As demonstrated in the Table below the recovery reaches to 82.2% when the concentration of ammonium salt is 200 mM.

The effect of salting-out in recovery of dexamethasone in plasma by SLE

Sample	Concentration of NH_4AC	% Recovery
Plasma + 50 µL water	0	53.5
Plasma + 50 µL salt solution	20 mM	59.0
Plasma + 50µL salt solution	200 mM	82.2

Recommended SLE Method



Tips:

◆ Cleanert® SLE plates and cartridges usually are used under gravity, additional positive pressure or vacuum which can help sample loading step to pass aqueous sample through the upper hydrophobic frit and for a final draw of organic solvent.

◆ Thickness samples are proposed to be diluted with an equal volume of buffer or water. 1M ammonium buffer (pH 9-10) are recommended for basic compounds and 1M phosphate buffers (pH 2-3) for acidic compounds. MTBE is widely used as elution solvent. Ethyl acetate, DCM and mixed solvents are also good choices for elution solvent. For some very polar compounds, more rigorous pH control may be needed in order to receive appropriate recoveries. Elution with 2-3 times aliquots instead of one may improve extraction efficiency and recovery.

Ordering Information

Max sample loading volume	Tube format	Package	Basic surface Similar product Biotage SLE+	Plus series,for sticky sample; Neutral surface Similar product Agilent Chem Elut
1 mL	6 mL	30	HC0006SQ-9	HC0006Q-7
2 mL	12 mL	20	HC200012SQ-9	HC200012Q-7
5 mL	25 mL	15	-	HC400025Q-7
10 mL	60 mL	10	-	HC1000060Q-7
17 mL	60 mL	10	HC2000060-9	HC2000060-7
200 µL	2 mL 96-wellplate	2	HC2002SQ-9W	HC2002Q-7W
400 µL	2 mL 96-wellplate	2	HC4002SQ-9W	HC4002Q-7W
500 µL	3 mL detachable deep well plate	1	HC5003SQ-9DW	HC5003Q-7DW
600 µL	3 mL detachable deep well plate	1	HC6003SQ-9DW	HC6003Q-7DW

Cleanert® MAS-Q (QuEChERS)

Description

QuEChERS means "Quick, Easy, Cheap, Effective, Rugged and Safe", which was first introduced by USDA and scientists in 2003. The procedure of QuEChERS extraction simplified the cleanup for pesticide residues analysis. It uses minimal amount of solvent along with extraction salts to extract the analytes into organic phase for cleanup then followed by dispersive solid phase extraction. Due to the high flexibility of the procedure, sample after treated can be directly introduced to determinative device such as LC-MS/MS or GC/MS. It has become increasingly popular in the area of multi-residue pesticide analysis in food and agricultural products.

MAS-Q (Multi-mechanism Adsorption SPE-QuEChERS) is an application of modified QuEChERS method which is suitable for most of the pesticides residue analysis, drug or antibiotic residue analysis in vegetable, fruit, grain, and animal issues.

Slim bag package

- ◆ Single extraction method could be applied to different kinds of sample matrix;
- ◆ High recovery rate for multiple pesticides residue analysis;
- ◆ Universal Clean-up kits are optimized for different application requirement;
- ◆ The method is simple, fast, reproducible and labor-saving.

- The clean up kits are available in 2 mL, 15 mL for different sample volume requirement, and extraction kits are packed in anhydrous sealed packets;
- Superior centrifuge tubes and glass homogeneous ball provide you a simple and convenient experience.

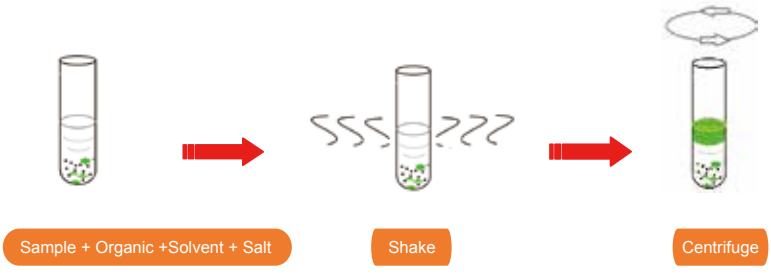
Homogenous balls

Recommended Standard Operating Procedure for MAS-Q

- Step 1: Extraction

Weight comminuted sample (10 g or 15 g) into a 50mL centrifuge tube, and then open the extraction kit package, add the salt after adding your solvent to the sample.

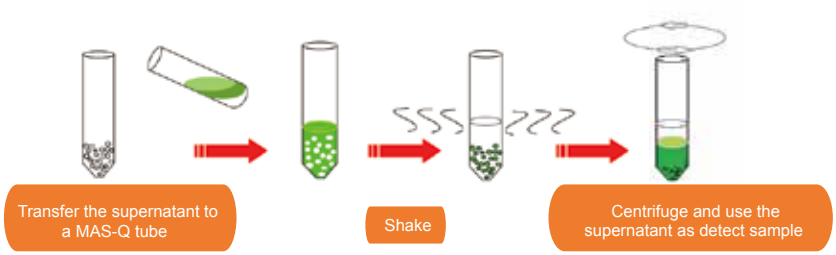
Extraction into the organic layer. Four glass homogeneous balls are suggested to add in to break the $MgSO_4$ caking caused by sample rich in water.



● Step 2: Cleanup

Take proper supernatant from step one, and add it into a selected clean up tube which is suited to the food matrix also the method you are following.

2 mL or 15 mL centrifuge tube containing different kind of SPE sorbent and $MgSO_4$ is available for different sample volume. The sorbent will adsorb the interfering matrix, and the $MgSO_4$ helps remove excess water.



Production Selection Guide

Extraction			Clean up
AOAC 2007.01			
AOAC 2007.01 -AOAC Method 6 g $MgSO_4$ (anhydrous) ; 1.5 g NaAc P/N: MS-MG5052	General fruit and vegetable		2 mL Centrifuge tube for 1 mL sample 50 mg PSA, 150 mg $MgSO_4$, P/N: MS-PA0250
			15 mL Centrifuge tube for 8 mL sample 400 mg PSA, 1200 mg $MgSO_4$, P/N: MS-PA1012
	Fruits and vegetables with fats and waxes		2 mL Centrifuge tube for 1 mL sample 50 mg PSA, 50 mg C18, 150 mg $MgSO_4$, P/N: MS-9PA0203
			15 mL Centrifuge tube for 8 mL sample 400 mg PSA, 400 mg C18, 1200mg $MgSO_4$, P/N: MS-9PA1011
	Pigmented fruits and vegetables		2 mL Centrifuge tube for 1 mL sample 50 mg PSA, 50 mg PC, 150 mg $MgSO_4$, P/N: MS-PP0250
			15 mLCentrifuge tube for 8 mL sample 400 mg PSA, 400 mg PC, 1200 mg $MgSO_4$, P/N:MS-PP1550
EN 15662			
EN 15662 -European and Mini-Multi residue Method 4 g $MgSO_4$, 1 g NaCl, 1 g NaCitrate, 0.5 g disodium citrate sesquihydrate P/N: MS-NMS5050	General fruit and vegetable		2 mLCentrifuge tube for 1 mL sample 25 mg PSA, 150 mg $MgSO_4$(anhydrous), P/N: MS-PA0251
			15 mL Centrifuge tube for 6 mL sample 150 mg PSA, 900 mg $MgSO_4$(anhydrous), P/N:MS-PA1011
Original Unbuffered Method 6 g $MgSO_4$ (anhydrous); 1.5 g NaCl P/N: MS-MG5051 4 g $MgSO_4$ (anhydrous); 1 g NaCl P/N: MS-MG5055	Fruits and vegetables with fats and waxes		2 mL Centrifuge tube for 1 mL sample 25 mg PSA, 25 mg C18, 150 mg $MgSO_4$(anhydrous) P/N: MS-9PA0204
			15 mL Centrifuge tube for 6mL sample 150 mg PSA, 150 mg C18, 900 mg $MgSO_4$(anhydrous) P/N: MS-9PA1210
	Pigmented fruits and vegetables		2 mL Centrifuge tube for 1 mL sample 25 mg PSA, 2.5 mg PC, 150 mg $MgSO_4$(anhydrous) P/N: MS-PP0251
			15 mL Centrifuge tube for 6 mL sample 150 mg PSA, 15 mg PC, 900 mg $MgSO_4$(anhydrous) P/N: MS-PP1511

Ordering Information

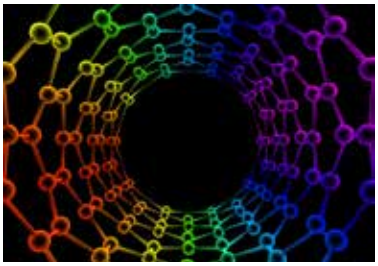
Cat.No.	Centrifuge tube	Sorbent Weight	Description	Package	Agilent Sampli Q	Water DisQuE	SUPELCO
MS-MG5052	50 mL	6g $MgSO_4$(anhydrous), 1.5 g anhydrousNaAc	QuEChERS extraction kit (with buffered) AOAC 2007.01 method for 15 g sample	50	5982-5755	186004571	55234-U
MS-MG5052-1	No tube	6 g $MgSO_4$(anhydrous), 1.5 g anhydrousNaAc	QuEChERS extraction kit (with buffered) AOAC 2007.01 method for 15 g sample	50	5982-6755	-	-
MS-NMS5050	50 mL	NaCl 1 g, $MgSO_4$(anhydrous) 4 g, 0.5 g disodium citrate sesquihydrate (TSCD), 1 g NaCitrate (DHS)	QuEChERS extraction kit (with buffered) EN 15662 method for 10 g sample	50	5982-5650	186004837	55227-U
MS-NMS5050-1	No tube	NaCl 1 g, $MgSO_4$(anhydrous) 4 g, 0.5 g disodium citrate sesquihydrate (TSCD), 1 g NaCitrate (DHS)	QuEChERS extraction kit (with buffered) EN 15662 method for 10 g sample	50	5982-6650	-	-
MS-NMS5151	50 mL tube	6 g Magnesium Sulfate, 1.5 g Sodium Chloride, 1.5 g Trisodium Citrate Dihydrate, and 0.75 g Disodium Hydrogencitrate	Extraction kit, apply to vegetables and fruit matrix, EN Method 15662	50	-	-	-
MS-MG5053	50 mL	6 g $MgSO_4$(anhydrous), 1 g NaCl	QuEChERS extraction kit (unbuffered) for 15 g sample	50	-	-	-
MS-MG5055	50 mL	4 g $MgSO_4$(anhydrous), 1g NaCl	QuEChERS extraction kit (unbuffered) for 15 g sample	50	5982-5550	-	-
MS-MG5051	50 mL	6 g $MgSO_4$(anhydrous), 1.5 g NaCl	QuEChERS Original extraction kit (unbuffered) for 15 g sample	50	5982-5555	-	-
MS-PA0251	2 mL	25 mg PSA, 150 mg $MgSO_4$(anhydrous)	EN 15662 (1 mL extract solution)	100	5982-5021	186004831	-
MS-PA0250	2 mL	50 mg PSA, 150 mg $MgSO_4$(anhydrous)	AOAC 2007.01 (1 mL extract solution)	100	5982-5022	186004572	-
MS-PA1011	15 mL	150 mg PSA, 900 mg $MgSO_4$(anhydrous)	EN 15662 (6 mL extract solution)	50	5982-5056	186004833	55228-U
MS-PA1012	15 mL	400 mg PSA, 1.2 g $MgSO_4$(anhydrous)	AOAC 2007.01 (8 mL extract solution)	50	5982-5058	-	55282-U

Part No.	Centrifuge tube	Sorbent Weight	Description	Package	Agilent Sampli Q	Water DisQuE	SUPELCO
MS-9PA0204	2 mL	25 mg C18, 25 mg PSA, 150 mg $MgSO_4$(anhydrous)	EN 15662 (1 mL extract solution)	100	5982-5121	186004832	-
MS-9PA0203	2 mL	50 mg C18, 50 mg PSA, 150 mg $MgSO_4$(anhydrous)	AOAC 2007.01 (1 mL extract solution)	100	5982-5122	186004830	-
MS-9PA1210	15 mL	150 mg C18, 150 mg PSA, 900 mg $MgSO_4$(anhydrous)	EN 15662 (6 mL extract solution)	50	5982-5156	186004834	55229-U
MS-9PA1011	15 mL	400 mg C18, 400 mg PSA, 120 mg $MgSO_4$(anhydrous)	AOAC 2007.01 (8 mL extract solution)	50	5982-5158	-	55283-U
MS-PP0251	2 mL	25 mg PSA, 2.5 mg PestiCarb, 150 mg $MgSO_4$(anhydrous)	EN 15662 (1 mL extract solution)	100	5982-5221	-	-
MS-PP1511	15 mL	150 mg PSA, 15 mg PestiCarb, 900 mg $MgSO_4$(anhydrous)	EN 15662 (6 mL extract solution)	50	5982-5256	-	-
MS-PP0252	2 mL	25 mg PSA, 7.5 mg PestiCarb, 150 mg $MgSO_4$(anhydrous)	EN 15662 (1 mL extract solution)	100	5982-5321	-	-
MS-PP1510	15 mL	150 mg PSA, 50 mg PC, 900 mg $MgSO_4$(anhydrous)	EN 15662 (6 mL extract solution)	50	5982-5356	-	-
MS-PP0250	2 mL	50 mg PSA, 50 mg PC, 150 mg $MgSO_4$(anhydrous)	AOAC 2007.01 (1 mL extract solution)	100	5982-5222	-	-
MS-PP1550	15 mL	400 mg PSA, 400 mg PC, 120 mg $MgSO_4$(anhydrous)	AOAC 2007.01 (8 mL extract solution)	50	5982-5258	-	-
MS-9PP0250	2 mL	50 mg PSA, 50 mg C18, 50 mg PC, 150 mg $MgSO_4$(anhydrous)	AOAC 2007.01 (1 mL extract solution)	100	5982-5421	-	-
MS-9PP0253	15 mL	400 mg PSA, 400 mg C18, 400 mg PC, 120 mg $MgSO_4$(anhydrous)	AOAC 2007.01 (8 mL extract solution)	50	5982-5456	-	-
MS-SPC5001	50 mL	Ion exchange and reverse phase materials	Melamin detection in complicated Samplemeat,egg,milk powder,fish and so on	50	-	-	-
MS-SPM5001	50 mL		Melamin detection in milk	50	-	-	-
HG01	-	homogeneous balls	Glass material, 4 balls are suggested to be added for improve extraction	50/bag	-	-	-
HG500	-			500/ box	-	-	-

Cleanert® NANO

—— A New Material of Carbon Nanotube

Cleanert® NANO was developed base on carbon nanotube material. After Functionalized process, the nano material has better affinity to remove colorants and fatty acids. And the surface deactivation process could control the over-adsorption within limited area, and ensure the recovery of pesticide with a benzene ring structure. The material which has a layer stacked structure could significantly increase the specific surface area and also the loading capacity.



Structure of the Cleanert® Nano

These characteristics allow much less use of the material and improve specificity and selectivity. The amount of material used per sample is about only 1/10 to 1/5 when comparing to traditional PSA, C18 or GCB SPE material.
It could be widely used in the analysis of multiple-pesticide residues in fruit, vegetable and other agro-products. Small amount of the nano material about 10-15mg is enough for most of the sample matrix, and small sorbent bed make it could be packed as a filter format cartridge and show more benefit for fast analysis and small amount of sample analysis requirement.

Syringe Filter Format

Product Formats

Optimized sorbent was packed into a syringe filter format cartridge and make it could be operated with a simple two-Step operation, conditioning, loading sample and collect the eluent.
This kind of design make it could be easily compatible with an automated workstation for high throughput requirement.

Application Note

Detection of 112 kinds of pesticide residue in black tea leaves sample using LC-MS/MS method

Sample Preparation Procedure

10 g Sample + 10 mL water and stand for 1h

↓

Add 15mL ACN firstly into the sample, and then add the extraction salts 4 g $MgSO_4$ +1 g NaCl (P/N: MS-MG5055) into the sample also four glass homogenizer

↓

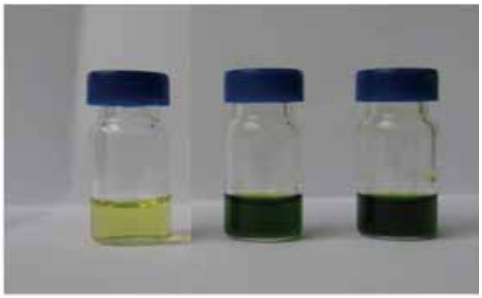
Shake by hand for 1 mins and then centrifuge at 8000 r/min

↓

Take 1 mL Supernatant, and pass the sample through the NANO cartridge containing 150 mg $MgSO_4$ + 10 mg MWCNTs (PN: IC-NN1510-C)

↓

Repeat the last step three times, and collect all the solution, filtered by Nylon filter and then for LC-MS/MS analysis



Cleanup performance comparison for leek sample: cleanert nano(left), PSA (middle) and before cleanup (right)

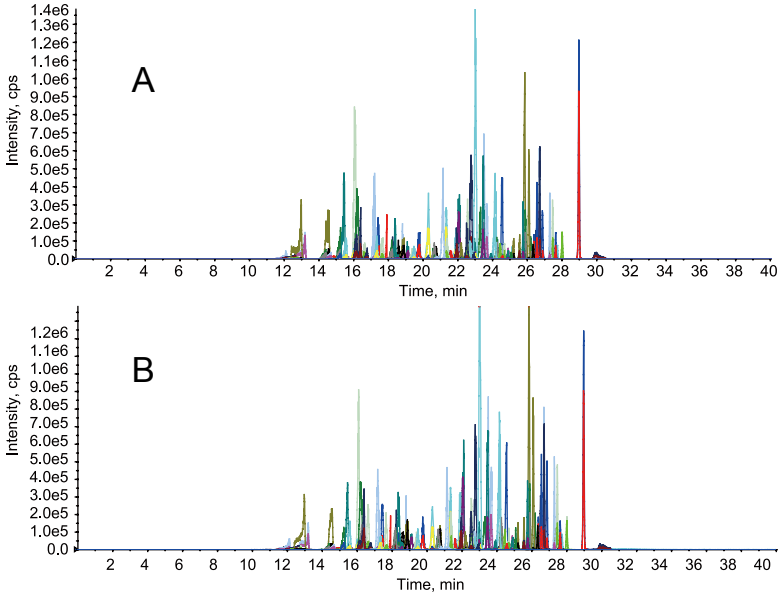


Figure 1. LC-MS/MS chromatogram of tea leaves extract. (A) 114 kinds of pesticides standard solution; (B) tea leaves extract spiked with 0.02 μg/mL 114 kinds of pesticides standard solution.

The recoveries for 114 kinds of pesticides are from 60 to 110%, and the RSD is lower than 10%.

Ordering Information

Product Name	Specification	Tubes/box	Cat.No.
Cleanert® NANO Nano tube for Complex Samples	10 mg Nano, 150 mg $MgSO_4$	50/PK	IC-NN1010-V
	15 mg Nano, 150 mg $MgSO_4$	50/PK	IC-NN1510-C

Special Products

Cleanert® PAE and DEHP*NEW !*

—— Plasticizers Detection in Food and Water

Food contamination by phthalates has caused considerable hazard to food safety. The most popular used plasticizers are phthalates series, which may cause cancer. Bonna-Aglea provides Cleanert® PAE and DEHP to analyze phthalates in different matrices including food containing fats and oil, and aqueous samples.

Suggested Processing Method

Applicative sample matrix

Liquid sample such as bottled Drinking water and Soft drink

SPE Process

Activation: 5 mL of methanol, 5 mL of water
Sample loading: 10 mL liquid sample
Washing: 5 mL water contained 5% methanol, vacuum for 20min
Elute: 5 mL of methanol. Concentrated the elution to dryness with N_2, and reconstitute with 1mL of methanol.

Analytical Method

Analysis by HPLC or GC-MS

Ordering Information

SPE Cartridge	Cleanert® DEHP; 500 mg / 6 mL; Cat.No: DEHP5006-G, 30/pk
HPLC columnn	Venusil® XBP C18-L; 4.6×250 mm, 5 μm,150 Å; Cat.No: VX952505-L

Applicative Sample

Liquid sample with high protein constituent
Milk, jam,syrup, milk powder, biscuits, cakes, jelly or candy

Sample-treatment

For liquid sample, add 2 mL of sample into a glass centrifuge tube, then 4mL of acetonitrile and methyl tert-butyl mystery (v/v=9:1), vortex for 2 min and then add the MAS-PAE sorbents into the sample, vortex for another 2 min, centrifuge for 5 min at 7000rpm. For solid sample, further dilute step is needed.
Take the supernatant , after filtration and then for analysis.

Analytical Method

GC/MS analysis

Ordering Information

SPE Cartridge	Cleanert® MAS-PAE; Cat. No: MS-PAE40, 15/pk Cleanert® MAS-PAE (sample with colorant); Cat. No: MS-PAE40-C, 15/pk
HPLC columnn	PAE Syringe filters; Cat.No: PAE041345-T; 100/pk
GC Column	DA-5MS; 30m×0.25mm×0.25μm; Cat. No: 1525-3002, 1/pk

Sample

High oil content sample such as vegetable oil, cheese or other animal tissues

Extraction

Weight 1 g of sample and extract with 10 mL hexane under ultrasonic, centrifuge for 5 min, take the supernatant. Repeat the extraction step and combine the supernatant together. Evaporate to dryness with N_2 under 40˚C , and reconstitute with 2 mL hexane and then for SPE process.

SPE Process

Activation: 5 mL of hexane
Sample loading: transfer all the sample upside
Elute: 3 mL×2 of ethyl acetate : hexane combination (50:50, v / v).
Evaporate the elution to nearly dryness with N_2, add 1 mL of acetonitrile for reextraction, vortex for 3 min, vortex for 3min, centrifugal for 5min at 4000rpm, take the supernatant for analysis.

Analytical Method

HPLC analysis

Ordering Information

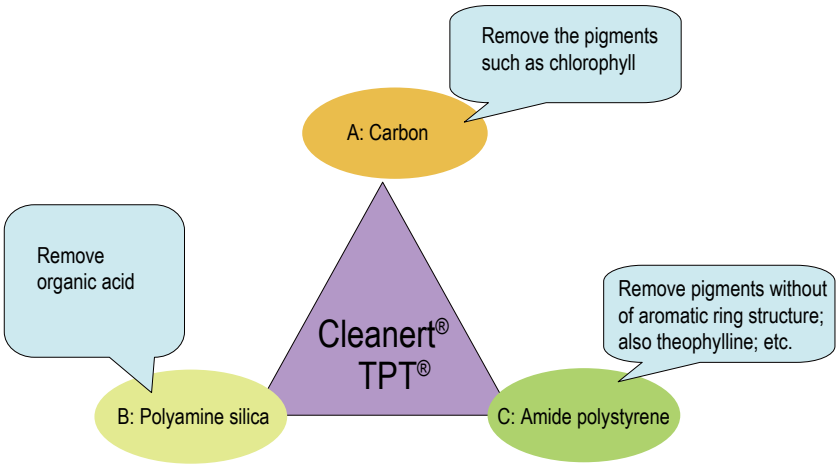
SPE Cartridge	Cleanert® PAE, Cat. No:PAE30006-G, 30/pk Cleanert® PAE (Sample with colorant); Cat.No:PAE30006-C; 30/pk
GC Column	DA-5MS, 30 m×0.25 mm×0.25 μm, Cat. No: 1525-3002, 1/pk

Cleanert® TPT® *NEW !*

—— Triple Phases SPE for Tea Leaves

Description

Cleanert® TPT® constitutes three type of materials, which employ different mechanisms to get rid of the interferences such as colorants, organic acids, polyphenols as well as nonpolar matrix. By mixing different materials together, we can achieve a better cleanup result for complicated sample matrices. Cleanert® TPT® is developed for determination of residues of 653 Multi class Pesticides and Chemical Pollutants in Tea, which has been recommended by AOAC official method and Chinese National Standard Method.



Comparison of Clean up Effectiveness

Left: sample processed by Cleanert® TPT®
Right: sample processed by PC/NH_2 SPE cartridge).

Cleanert® TPT® can reduce 90% of the colorants in tea leaves

Excellent Recoveries for Pesticide Residues

Recoveries range	Pesticide numbers within the recovery range	
	PC/NH_2	Cleanert® TPT®
<60%	3	0
60%-120%	99	103
120%-130%	0	0
>130%	1	0

After being processed by Cleanert® TPT®, 103 pesticides could be detected in valid recoveries range

AOAC 2014.09 - Determination and Confirmation of Residues of 653 Multiclass Pesticides and Chemical Pollutants in Tea GC-MS, GC-MS/MS, and LC-MS/MS First Action 2014

An efficient and sensitive method has been established for simultaneous determination of 653 pesticides in teas by GC-MS and LC-MS/MS. The method involved extraction with acetonitrile followed by GC-MS and 448 pesticides by LC-MS/MS respectively. The limits of detection by LC-MS/MS were between 0.03 and 4820 µg/kg. At the low fortification levels of 0.01-100 µg/kg. The average recoveries of 94% pesticides determined by GC-MS were between 60% and 120%. Among 775 of which had a relative standard between 60% and 120% for 91% of them. Among 76% of which had RSD below 20%. The paper also reported a novel SPE column. **Cleanert® TPT® cartridge**, which is comprised of graphitized carbon black(PestiCarb). Polyamine silica and amide polystyrene for purifying the tea samples. The results indicated that the cartridge has relatively higher cleanup capabilities for tea samples and a good repeatibility and reproducibility were also observed.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® TPT®	1 g	6 mL	30	TPT0006
	2 g	12 mL	20	TPT200010

Cleanert® TPH *NEW !*

—— Triple Phases SPE for Herb Samples

Description

Cleanert® TPH means triple phases SPE for Herb samples, and it is a composite of three types of sorbent with different mechanisms. By mixing different materials together, we achieve a better result of clean up, particularly for complicated matrices. Cleanert® TPH is mainly used for extraction and detection of pesticides from Chinese Herb Medicines such as Ramulus Mori, Honeysuckle and the Fruit of Chinese Wolfberry. It can reduce effectively the interference without adsorbing any pesticide residues.

Cleanert® TPH has been used in Chinese national standard methods, determinating of 488 Pesticide Residues and Related Chemicals Residues and 413 Pesticide Residues and Related Chemicals Residues in the Ramulus Mori, Honeysuckle and the Fruit of Chinese Wolfberry with GS-MS and LC-MS/MS respectively.

The process includes ACN extraction, loading the sample onto the pre-activated column, and the subsequent elution by ACN- toluene (3+1).

For completely application, please check the application part at the end of this catalog.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® TPH	1 g	6 mL	30	TPH0006
	2 g	12 mL	20	TPH200010

Cleanert® BAP *NEW !*

—— Benzopyrene Detection in Edible Oil

Description

Cleanert® BAP is a chromatographic column pre-packed with Alumina material aiming to separate benzopyrene and triglyceride in oil.

Suggested Processing Method (BAP)

Activation: Add about 30 mL n-hexane to activate Cleanert® BAP catridge. (Note: during n-hexane drop-out process, keep adding n-hexane to avoid n-hexane liquid level lower than the upper sieve.)

Sample loading: Add the dissolved oil sample (0.3 g) to the pre-activated Cleaner® BAP catridge. Notice to avoid the upper sieve dried during the operation.

Elution: Add 80 mL n-hexane to the column, and use a 150 mL rotary evaporation bottle to collect the elution, until 80 mL n-hexane drop out completely by gravity. In order to ensure recovery, the elution volume could be increased up to 120 mL.

Concentrate the eluant under 45℃ , and add 300 μL n-hexane to reconstitute the residue, then for further analysis.

Suggested Processing Method (Bap-3)

Activation: Add 5 mL dichloromethane and n-hexane separately;

Sample loading: Add the dissolved oil sample(0.5g) to the pre-activated cartridge;

Washing: 10mL n-hexane

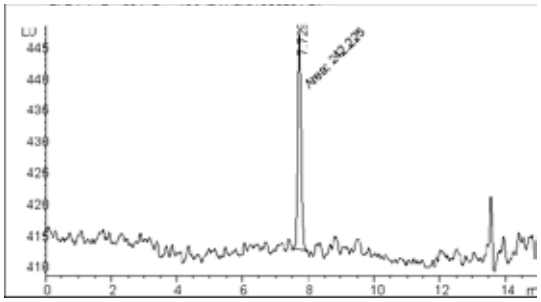
Elution: 5 mL dichloromethane

Concentrate the eluant under 45℃ , and add 1mL ACN to reconstitute the residue, filter the sample with 0.22 PTFE syringe filter and then for further analysis.

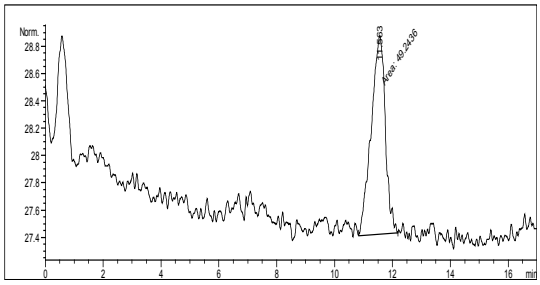
	Sorbent	Elution Volume	Max Sample Volume
BAP-3	Polymer	5 mL dichloromethane	All kind of oil sample, 0.5 g
BAP	Alumina	80 mL Hexane (concentration is needed)	0.3 g Not work for Oil with more polar component

Chromatographic condition

Column: Venusil® PAH, 5.0 μm, 4.6 mm × 250 mm (P/N: VP952505-L)
Mobile phase: ACN : Water = 95 : 5;
Flow rate: 1.0 mL/min;
Injection volume: 20 μL
Fluorescence detector: Emission 406 nm, Excitation 384 nm.



Standard solution



Edible oil sample spiked with 10μg/Kg benzopyrene

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® BAP	22 g	60 mL	10	BAP2260-0
Cleanert® BAP-3	500 mg	6 mL	30	BAP5006

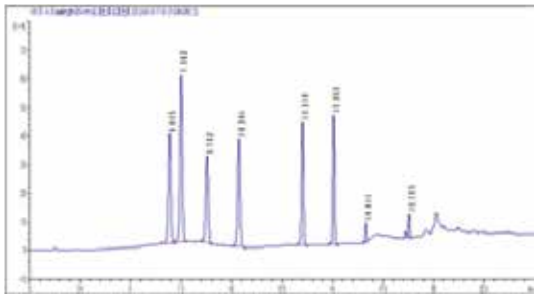
Cleanert® PA

Description

Cleanert® PA Polyamide cartridge is designed to be used for food coloring detection in different matrix, such as food and wine. Liquid sample can be loaded directly and some kinds of sample matrix are need to be degas or heated to get rid of alcohol, and also pH adjustment can be done if needed.

SPE Process

Activation: 6 mL methanol and then 6 mL water
Sample loading: 10 mL
Washing: 6 mL water (adjust the pH=4 using citric acid solution),
6 mL methanol: Formic acid =6:4,
6 mL water(adjust the pH=6 using citric acid solution);
Elution: 6 mL methanol with 2% ammonia solution;
Concentrate the eluate under 50°C N_2, reconstitute it with 1 mL water and filtered by 0.45 μm syringe filter, then for analysis.

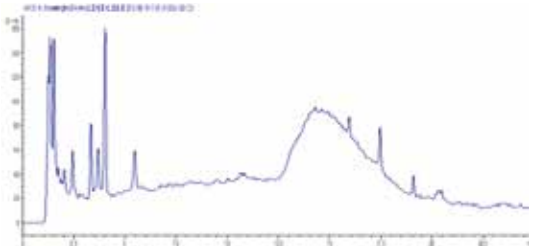


Standard solution of 8 kinds of pigment (Lemon yellow; new red; Amaranth; Indigo; Carminum; Sunset Yellow; Allura Red; Brilliant Blue)

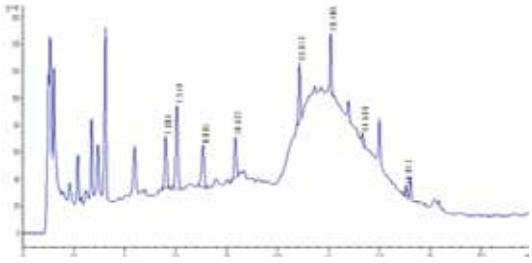
HPLC Conditions

HPLC column: Venusil™ XBP C18, 5 μm, 100 Å, 4.6×150mm;
Flow rate:1.0 mL/min
Injection Volume: 20 μL
UV detector: 254 nm
Mobile phase: A (0.02mol/L ammonium acetate solution)
B Methanol
Gradient

Time (mins)	A%	B%
0	95	5
10	80	20
18	40	60
25	40	60
25.01	95	5
40	95	5



Blank sample of red wine



Blank sample spiked by 5ppm standard solution

Cleanert® SUL-5
(Specific Columns for Sulfonamides)

Description

Cleanert® SUL-5 (specific columns for sulfonamides) is specially designed for the extraction of five sulfonamides (SM2,SMM,SMZ,SDM.SQ) in pork.

Please find the detailed experimental information at the application part in this catalog.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® SUL-5	2 g	12 mL	20	SUL-5

Cleanert® DNPH-Silica

Cleanert® DNPH-Silica prepared by acidified dinitrophenylhydrazine reagent coated on silica is used for collection of air samples. It was one of most sensitive and specific method for analyzing aldehydes and ketones which is based on their reaction with 2,4-dinitrophenylhydrazine (DNPH) and subsequent analysis of the hydrazone derivatives by HPLC. DNPH-Silica can be used in EPA Method TO–11A; ASTM D5197 for carbonyl compounds in air and JPMOE Official Methods for aldehydes: odor in out door air and in exhaust gas.

3 mL DNPH cartridge

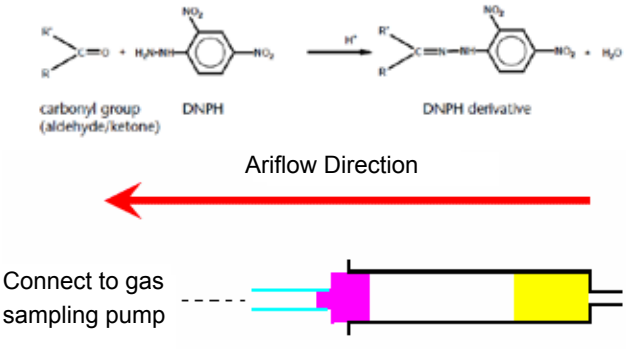
Left: 200 mg/1 mL
Right: 350 mg/1 mL

Specification

- Ave Background value: ≤0.1 μg (calculated by formaldehyde)
- Max sampling quantity: ≥75 μg (calculated by formaldehyde)
- Max Sampling Pressure: 0.15 MPa
- Recoveries: >90%

Operation

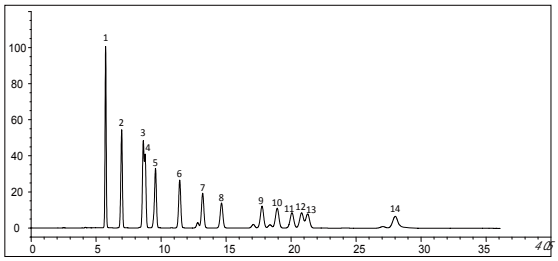
Using a vacuum pump, the air sample is drawn through the new Cleanert® DNPH-silica cartridge. The aldehydes and ketones react with the DNPH and form the hydrazone derivative, which is retained on the cartridge. After sampling, the hydrazones are eluted from the cartridge with acetonitrile and analyzed by HPLC.



Ozone has been shown to interfere with the analysis of carbonyl compounds in air samples. Cleanert® Ozone remover cartridge contains potassium iodide could be used connected with Cleanert® DNPH to remove ozone interferences.

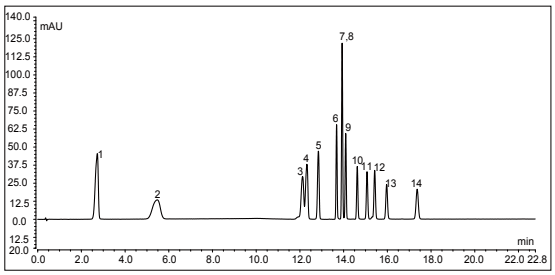
Cleanert® DNPH-Silica Cleanert® Ozone remover

Separation of fourteen 2,4-dinitrophenylhydrazone derivatives formaldehyde, acetaldehyde, acraldehyde, propylaldehyde, crotonaldehyde, butanone, butaldehyde, methacrolein, benzaldehyde, amyl aldehyde, toluyl aldehyde, cyclohexanone, caproaldehyde



Sample: 14 DNPHs
Column: Venusil® XBP C18, 4.6×250 mm, 5 μm
P/N: VX952505-0
Detection: UV 360 nm
Flow Rate: 1 mL/min
Mobile Phase: ACN:Water=60:40

Using Bonshell C18 colume, high speed analysis has been developed to provide excellent quantitation capability.



Sample: 14 DNPHs
Column: Bonshell C18, 4.6×100 mm, 2.7 μm, 90 Å,
Detection: UV 360 nm
Flow Rate: 1.2 mL/min

Mobile phase:

Time	Water%	ACN%
0	60	40
5	70	30
9	75	25
13	40	60

Ordering Information

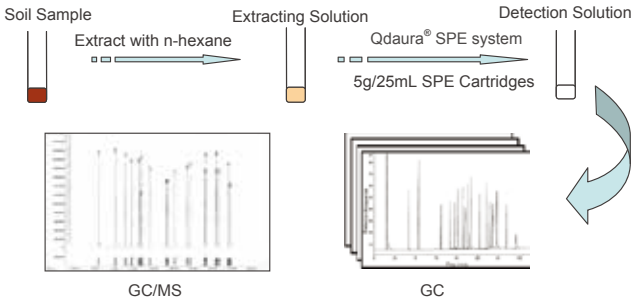
Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® DNPH	200 mg	1 mL	50/PK	IC-DN2001
Cleanert® DNPH	200 mg	3 mL	50/PK	DN2003
Cleanert® DNPH	350 mg	1 mL	50/PK	IC-DN3501N
Cleanert® Ozone remover	1.45 g	2.5 mL	50/PK	KI1450

Cleanert® EPH *NEW !*

—— for Extractable Petroleum Hydrocarbons

Description

Cleanert® EPH uses a special silica material to separate aliphatic hydrocarbon from aromatics in environmental samples. The column was used in New Jersey Department of Environmental Protection Site Remediation Program, extractable petroleum hydrocarbons methodology. The method can also be adopted onto a fully automatic SPE workstation.



Please find the detailed experimental information in the application part.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® EPH	5 g	25 mL	15	SI500025-30

Cleanert® ACA *NEW !*

——Polar Pollutant Analysis in Water Analysis

Description

Cleanert® ACA use coconut charcoal material to concentrate polar substances in water sample, such as acrylamide which could not been adsorbed by C18 or other RP phase material. It could be used in EPA 521 and EPA 522.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® ACA	500 mg	6 mL	30	ACA5006
	200 mg	6 mL	30	ACA2006

Cleanert® SLE-OD

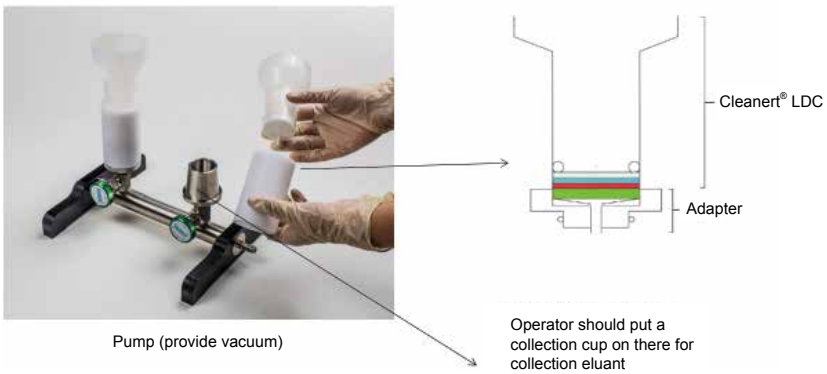
Bonna-Agela provide commercial diatomaceous column which mentioned in EN 14362-1:2012(E), and 22 kinds of aromatic amine could be detected in textile samples.

Ordering Information

Material	Max sample loading volume	Tubes/box	Buffered material improve the recoveries	Cat.No.
Cleanert® SLE-OD	20 mL	10/PK	Base on method EN 14362-1:2012(E)	GB/T17592-2006
	20 mL	100/PK		GB/T 17592-2006-M
	20 mL	100/PK		EN2000060-9

Cleanert® LDC *NEW !*

—— Large Disk for Water Pollutant Analysis



Cleanert® LDC and SPE-D6 Large sample loading vacuum device

Description

Cleanert® LDC is a specially designed disk format with a much larger cross-section area to allow a large flow rate through the column, which is mostly useful to concentrate trace pollutants from a large volume of aqueous samples.

- The special cartridge design is suitable for water concentration, and it can replace SPE disk in most of the analysis.
- Cleanert® LDC uses AQ C18 sorbent to concentrate trace chemical substances in method EPA 525, show good recovery and stability for hundreds of compounds.

Ordering Information

Material	Vol	Tubes/box	Cat.No.
Cleanert® LDC AQ C18	100 mL	4	L182000100

Cleanert® LRC *NEW !*

—— Large Receiver Column

Description

Cleanert® LRC is designed to load more solvent in SPE process, specially for applications using a small amount of sorbents but a large volume of eluents. All kinds of SPE material can be customized using LRC tube; Suggested specifications for Polymers are from 30 mg to 150 mg; Silica materials are from 100 mg to 300 mg;

Ordering Information

Material	Vol	Tubes/box	Cat.No.
Cleanert® LRC C18	200 mg/10 mL	20	L1820010
Cleanert® LRC PEP-2	60 mg/10 mL	20	LPE0610-2

Cleanert® SPE in Glass Tubes *NEW !*

Description

Cleanert® glass column series can avoid the residue interference from the plastic tubes and are recommended to be used for trace analysis of food and environmental samples. Leave space PTFE frit are optional; All kinds of SPE material can be customized using glass tube;

Ordering Information

Material	Vol	Tubes/box	Cat.No.
Cleanert® C18 glass	200mg / 3mL	50	S182003-G
Cleanert® C18 glass	500mg / 6mL	30	S185006-G
Cleanert® PEP glass	60mg / 3mL	50	PE0603-G
Cleanert® PEP glass	150mg / 6mL	30	PE1506-G
Cleanert® PCX glass	60mg / 3mL	50	CX0603-G

Conventional Products

Bonded Silica SPE

Cleanert® AQ C18

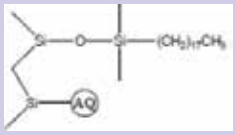
Description

AQ C18 series using a polar chemical group to modify the spherical C18 surface and enhance the retention for polar substance. The bonded chemical group also make RP surface more hydrophilic, and provide good recovery for both polar and non-polar compounds, especially for water samples.

Particle Characteristics

Based on Spherical Silica; C%: 18-19%;
Average Particle Diameter: 50 μm;
Average Pore Size: 60 Å;
Specific Surface Area: 600 m^2/g.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® AQ C18 (Hydrophilic C18) 	100 mg	1 mL	100	S181001-AQ
	200 mg	3 mL	50	S182003-AQ
	500 mg	3 mL	50	S185003-AQ
	500 mg	6 mL	30	S185006-AQ
	1000 mg	6 mL	30	S180006-AQ
	2000 mg	12 mL	20	S18200012-AQ
	50 mg/well	2 mL	96-well plate, 1/pk	S180502AQ-W
	100 mg/well	2 mL	96-well plate, 1/pk	S181002AQ-W
	10 g	-	bottle	S180010-AQ
	100 g	-	bottle	S180100-AQ

Cleanert® S C18 (End-capped)

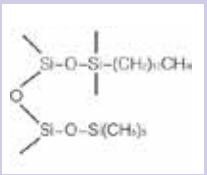
Description

Cleanert® S C18 columns and plates are packed with octadecylsilane bonded silica sorbents. The sorbent is double end-capped and has a high bonding density (%C > 17). These products can be used for desalting biomolecules, such as proteins and DNAs.

Particle Characteristics

Based on Spherical Silica; C%: 18-19%;
Average Particle Diameter: 50 μm;
Average Pore Size: 60 Å;
Specific Surface Area: 600 m^2/g.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® S C18 (Sperical Endcapped) 	100 mg	1 mL	100	S181001
	200 mg	3 mL	50	S182003
	500 mg	3 mL	50	S185003
	500 mg	6 mL	30	S185006
	1000 mg	6 mL	30	S180006
	2000 mg	12 mL	20	S18200012
	50 mg/well	2 mL	96-well plate, 1/pk	S180502-W
	100 mg/well	2 mL	96-well plate, 1/pk	S181002-W
	10 g	-	bottle	S180010
	100 g	-	bottle	S180100

Cleanert® S C18-N (Non-end-capped)

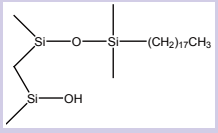
Description

Cleanert® S C18-N is a type of C18 sorbent bonded to silica without end-capping modification, providing extra silanol residuals around the root of alkyl chain on silica surface.The extra silanol silica of the sorbent provides additional polar interactions associated with surface silanol groups which enhance the retention of basic compounds.

Particle Characteristics

Based on spherical Silica; C%: 17-18%;
Average Particle Diameter: 50 μm;
Average Pore Size: 60 Å;
Specific Surface Area: 600 m^2/g (Spherical Silica);.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® S C18-N (Spherical Non-end-capped) 	100 mg	1 mL	100	S181001-N
	200 mg	3 mL	50	S182003-N
	500 mg	3 mL	50	S185003-N
	500 mg	6 mL	30	S185006-N
	1000 mg	6 mL	30	S180006-N
	2000 mg	12 mL	20	S18200012-N
	50 mg/well	2 mL	96-well plate, 2/pk	S180502-N-W
	100 mg/well	2 mL	96-well plate, 2/pk	S181002-N-W
	10 g	bottle	-	S180010-N
	100 g	bottle	-	S180100-N

Cleanert® S C8 (Octyl)

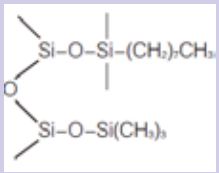
Description

Cleanert® S C8 products has less retentive than Cleanert® S C18, which accelerates the elution of more hydrophobic substance. Cleanert® S C8 is successfully used for the extraction of both water-soluble and fat-soluble vitamins from serum, as well as the desalting of biological macromolecules.

Particle Characteristics

Based on spherical Silica; C%: 9-10%;
Average Particle Diameter: 50 μm;
Average Pore Size: 60 Å;
Specific Surface Area: 600 m^2/g;.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® S C8 	100 mg	1 mL	100	S081001
	200 mg	3 mL	50	S082003
	500 mg	3 mL	50	S085003
	500 mg	6 mL	30	S085006
	1000 mg	6 mL	30	S080006
	50 mg/well	2 mL	96-well plate	S080502-W
	100 mg/well	2 mL	96-well plate	S081002-W
	10 g	bottle	-	S080010
	100 g	bottle	-	S080100

Cleanert® CN (Cyanopropyl)

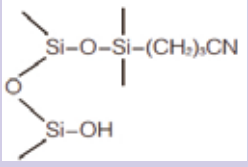
Description

Cleanert® CN(Cyano) SPE is silica based sorbent bonded with cyanopropyl functional groups. This polar sorbent exhibits both polar and non-polar interactions. It can be used for extraction of both polar and non-polar molecules in either normal phase or reversed phase mode.

Particle Characteristics

Based on spherical Silica; C%: 5-6%;
Average Particle Diameter: 50 μm;
Average Pore Size: 60 Å;
Specific Surface Area: 600 m^2/g (Spherical Silica);.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® CN 	100 mg	1 mL	100	CN1001
	200 mg	3 mL	50	CN2003
	500 mg	3 mL	50	CN5003
	500 mg	6 mL	30	CN5006
	1 g	6 mL	30	CN0006
	50 mg/well	2 mL	96-well plate, 1/PK	CN0502-W
	100 mg/well	2 mL	96-well plate, 1/PK	CN1002-W
	10 g	-	bottle	CN0010
	100 g	-	bottle	CN0100

Cleanert® NH_2 (Aminopropyl)

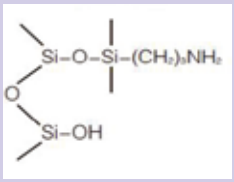
Description

Cleanert® NH_2 products are silica based sorbent bonded with aminopropyl funtional group. This sorbent can be used in either normal phase or reversed phase mode. It retains the analytes either by a polar adsorption (from non-polar solution) or by weak anion exchange (from aqueous solution). pKa=9.8.

Particle Characteristics

Based on Irregular Silica;
Average Particle Diameter: 40-60 μm;
Average Pore Size: 60 Å;
Specific Surface Area: 480 m^2/g.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® NH_2 	100 mg	1 mL	100	NH1001
	200 mg	3 mL	50	NH2003
	500 mg	3 mL	50	NH5003
	500 mg	6 mL	30	NH5006
	1 g	6 mL	30	NH0006
	50 mg/well	2 mL	96-well plate, 1/PK	NH0502-W
	100 mg/well	2 mL	96-well plate, 1/PK	NH1002-W
	10 g	-	bottle	NH0010
	100 g	-	bottle	NH0100

Cleanert® PSA {(N-aminoethyl) Aminopropyl}

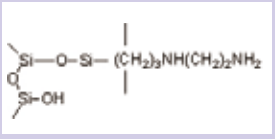
Description

Cleanert® PSA SPE is similar to Cleanert® NH_2. It has two amino groups with pKa = 10.1 and 10.9, respectively. This sorbent is an anion exchanger slightly stronger than Cleanert® NH_2. It can be used for the extraction of metal ions by chelating interactions. It is also commonly used to remove organic acids, pigments and metal ions from organic samples such as vegetables and fruits.

Particle Characteristics

Based on Irregular Silica;
Average Particle Diameter: 40-60 μm;
Average Pore Size: 60 Å;
Specific Surface Area: 480 m^2/g.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® PSA 	100 mg	1 mL	100	PA1001
	200 mg	3 mL	50	PA2003
	500 mg	3 mL	50	PA5003
	500 mg	6 mL	30	PA5006
	1 g	6 mL	30	PA0006
	50 mg/well	2 mL	96-well plate, 1/PK	PA0502-W
	100 mg/well	2 mL	96-well plate, 1/PK	PA1002-W
	10 g	-	bottle	PA0010
	100 g	-	bottle	PA0100

Cleanert® SAX (Strong Anion Exchanger)

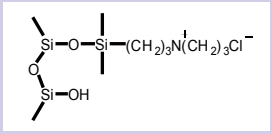
Description

Cleanert® SAX SPE products are packed with silica based sorbent bonded with a quaternary amine. This strong anion exchanger is used to extract compounds capable of carrying a negative charge from both aqueous and non-aqueous solutions. They are ideally suitable to extraction of weak acids and desalting of biological macromolecules.

Particle Characteristics

Based on Spherical Silica; C%: 9-10%;
Average Particle Diameter: 50 μm;
Average Pore Size: 60 Å;
Specific Surface Area: 600 m^2/g;
The Ion Exchange Degree: 0.5 meq/g.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® SAX 	100 mg	1 mL	100	SA1001
	200 mg	3 mL	50	SA2003
	500 mg	3 mL	50	SA5003
	500 mg	6 mL	30	SA5006
	1 g	6 mL	30	SA0006
	50 mg/well	2 mL	96-well plate, 1/PK	SA0502-W
	100 mg/well	2 mL	96-well plate, 1/PK	SA1002-W
	10 g	-	bottle	SA0010
	100 g	-	bottle	SA0100

Cleanert® COOH (Weak Cation Exchanger)

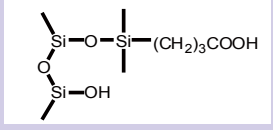
Description

Cleanert® COOH SPE products consist of a propyl carboxylic acid on the inner silica surface. The pKa of the carboxylic acid group is approximately 3.8. It is a useful sorbent for quaternary ammonium salt and other strong cations.

Particle Characteristics

Based on Spherical Silica; C%: 5-6%;
Average Particle Diameter: 50 μm;
Average Pore Size: 60 Å;
Specific Surface Area: 600 m^2/g.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® COOH 	100 mg	1 mL	100	CH1001
	200 mg	3 mL	50	CH2003
	500 mg	3 mL	50	CH5003
	500 mg	6 mL	30	CH5006
	1 g	6 mL	30	CH0006
	50 mg/well	2 mL	96-well plate, 1/PK	CH0502-W
	100 mg/well	2 mL	96-well plate 1/PK	CH1002-W
	10 g	-	bottle	CH0010
	100 g	-	bottle	CH0100

Cleanert® PRS (Propane Sulfonic Acid)

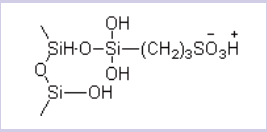
Description

Cleanert® PRS SPE sorbent is a silica gel based strong cation exchanger. This sorbent, consisting of a propane sulfonic acid, has slightly less exchange capability than SCX. It can be applied to the extraction of weak cations, such as pyridine, with high recovery.

Particle Characteristics

Based on Spherical Silica;
Average Particle Diameter: 50 μm;
Average Pore Size: 60 Å;
Specific Surface Area: 600 m^2/g
The Ion Exchange Degree: 0.3 meq/g.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® PRS 	100 mg	1 mL	100	PR1001
	200 mg	3 mL	50	PR2003
	500 mg	3 mL	50	PR5003
	500 mg	6 mL	30	PR5006
	1 g	6 mL	30	PR0006
	50 mg/well	2 mL	96-well plate 1/PK	PR0502-W
	100 mg/well	2 mL	96-well plate 1/PK	PR1002-W
	10 g	-	bottle	PR0010
	100 g	-	bottle	PR0100

Cleanert® SCX (Strong Cation Exchanger)

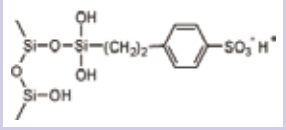
Description

Cleanert® SCX sorbent is a strong cation exchanger based on silica gel, with benzene sulfonic acid. The sorbent is used to extract positively charged basic compounds or remove the salt from biological samples. It can also be mixed with C18 sorbent to extract the organic bases.

Particle Characteristics

Based on Spherical Silica;
Average Particle Diameter: 50 μm;
Average Pore Size: 60 Å;
Specific Surface Area: 600 m^2/g;
The Ion Exchange Degree: 0.5 meq/g.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® SCX 	100 mg	1 mL	100	SC1001
	200 mg	3 mL	50	SC2003
	500 mg	3 mL	50	SC5003
	500 mg	6 mL	30	SC5006
	1 g	6 mL	30	SC0006
	50 mg/well	2 mL	96-well plate 1/PK	SC0502-W
	100 mg/well	2 mL	96-well plate 1/PK	SC1002-W
	10 g	-	bottle	SC0010
	100 g	-	bottle	SC0100

Cleanert® Silica

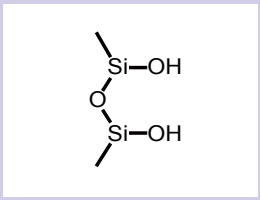
Description

Cleanert® Silica use unbonded, activated irregular silica as sorbent. This sorbent exhibits high polar interactions and is used to retain polar interference and to pass through weak-polar or non-polar compounds of interest such as oil samples. In addition, the silanol groups are ionizable at intermediate pH, so it can be used as a weak cation exchanger.

Particle Characteristics

Based on Irregular Silica;
Average Particle Diameter: 50 μm;
Average Pore Size: 60 Å;
Specific Surface Area: 480 m^2/g.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® Silica 	100 mg	1 mL	100	SI1001
	200 mg	3 mL	50	SI2003
	500 mg	3 mL	50	SI5003
	500 mg	6 mL	30	SI5006
	1 g	6 mL	30	SI0006
	50 mg/well	2 mL	96-well plate 1/PK	SI0502-W
	100 mg/well	2 mL	96-well plate 1/PK	SI1002-W
	10 g	-	bottle	SI0010
	100 g	-	-bottle	SI0100

Cleanert® Diol

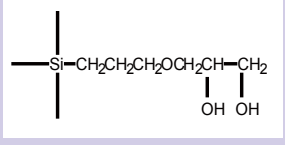
Description

Cleanert® Diol is a silica based material bonded with dihydroxy chemical group. It is used to extract polar analytes from non-polar solutions. It is a neutral sorbent and extracts compounds by forming hydrogen bonding or polar-polar interaction. As an example, it can be used to extract THC.

Particle Characteristics

Based on Spherical Silica; C%: 5-6%;
Average Particle Diameter: 50 μm;
Average Pore Size: 60 Å;
Specific Surface Area: 600 m^2/g.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® Diol 	100 mg	1 mL	100	DI1001
	200 mg	3 mL	50	DI2003
	500 mg	3 mL	50	DI5003
	500 mg	6 mL	30	DI5006
	1 g	6 mL	30	DI0006
	50 mg/well	2 mL	96-well plate, 1/PK	DI0502-W
	100 mg/well	2 mL	96-well plate, 1/PK	DI1002-W
	10 g	-	bottle	DI0010
	100 g	-	bottle	DI0100

Non-silica Adsorption Phase Cartridges

Cleanert® Florisil (Magnesia Silica)

Description

Cleanert® Florisil is a highly selective adsorbent, which contains silica (84%), magnesium oxide (15.5%), and sodium sulfate (0.5%). It was used for AOAC, EPA and other methods designed for the separation of pesticide residues, internal secretion, oil, PCBs, PAHs, nitrogen compounds and antibiotic substances in hydrocarbons.

Particle Characteristics

Adsorption Sorbents;
Average Particle Diameter: 60-100 mesh (100-200 mesh optional);
Average Pore Size: 80 Å;
Specific Surface Area: 290 m^2/g.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® Florisil	100 mg	1 mL	100	FS1001
	200 mg	3 mL	50	FS2003
	500 mg	3 mL	50	FS5003
	500 mg	6 mL	30	FS5006
	1 g	6 mL	30	FS0006
	2 g	6 mL	30	FS20006
	50 mg/well	2 mL	96-well plate, 1/PK	FS0502-W
	100 mg/well	2 mL	96-well plate, 1/PK	FS1002-W
	10 g	-	bottle	FS0010
	100 g	-	bottle	FS0100

Cleanert® PestiCarb (Graphitized Carbon Black)

Description

Cleanert® PestiCarb using graphitized carbon material has been used for sample cleanup in pesticide residues in plants or animal tissues. This sorbent can effectively reduce the background noise and increase sensitivity.

Particle Characteristics

Adsorption Sorbents;
Average Particle Size: 120~400 mesh.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® PestiCarb	100 mg	1 mL	100	PC1001
	200 mg	3 mL	50	PC2003
	500 mg	3 mL	50	PC5003
	500 mg	6 mL	30	PC5006
	1 g	6 mL	30	PC0006
	50 mg/well	2 mL	96-well plate, 1/PK	PC0502-W
	100 mg/well	2 mL	96-well plate, 1/PK	PC1002-W
	10 g	-	bottle	PC0010
	100 g	-	bottle	PC0100

Cleanert® Alumina N (Aluminium Oxide; Neutral)

Description

Cleanert® Alumina N sorbent (pH = 7.5) can adsorb molecules by interaction with the aluminum metal center. The neutralized surface allows interaction with compounds whose heteroatoms are electronegative (e. g. N, S, P) or with an electron-rich highly aromatic structure.This material is generally used in the detection of pigment such as Sudan and malachite green.

Particle Characteristics

Adsorption Sorbents;
Average Particle Size: 100-200 mesh;
Average Pore Size: 80 Å.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® Alumina N	100 mg	1 mL	100	AL1001-N
	200 mg	3 mL	50	AL2003-N
	500 mg	3 mL	50	AL5003-N
	500 mg	6 mL	30	AL5006-N
	1 g	6 mL	30	AL0006-N
	50 mg/well	2 mL	96-well plate, 1/PK	AL0502-N-W
	100 mg/well	2 mL	96-well plate, 1/PK	AL1002-N-W
	10 g	-	bottle	AL0010-N
	100 g	-	bottle	AL0100-N

Cleanert® Alumina A (Aluminium Oxide; Acidic)

Description

Cleanert® Alumina A sorbent (pH = 4.5) can be used as a strong polar absorbent or a mild cation exchanger. This sorbent is processed with a special deactivation procedure which ensures high analytes recovery.

Particle Characteristics

Adsorption Sorbents;
Average Particle Size: 100-200 mesh;
Average Pore Size: 80 Å.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® Alumina A	100 mg	1 mL	100	AL1001-A
	200 mg	3 mL	50	AL2003-A
	500 mg	3 mL	50	AL5003-A
	500 mg	6 mL	30	AL5006-A
	1 g	6 mL	30	AL0006-A
	50 mg/well	2 mL	96-well plate, 1/PK	AL0502-A-W
	100 mg/well	2 mL	96-well plate, 1/PK	AL1002-A-W
	10 g	-	bottle	AL0010-A
	100 g	-	bottle	AL0100-A

Cleanert® Alumina B (Aluminium Oxide; Basic)

Description

Cleanert® Alumina B products (pH = 10.0) can be used to remove organic acids and phenols in sample matrix. They have been pre-treated by special deactivation to ensure high analytes recovery.

Particle Characteristics

Adsorption Sorbents;
Average Particle Size: 100-200 mesh;
Average Pore Size: 80 Å.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® Alumina B	100 mg	1 mL	100	AL1001-B
	200 mg	3 mL	50	AL2003-B
	500 mg	3 mL	50	AL5003-B
	500 mg	6 mL	30	AL5006-B
	1 g	6 mL	30	AL0006-B
	50 mg/well	2 mL	96-well plate, 1/PK	AL0502-B-W
	100 mg/well	2 mL	96-well plate, 1/PK	AL1002-B-W
	10 g	-	bottle	AL0010-B
	100 g	-	bottle	AL0100-B

Mixed and Layered Phases

Cleanert® Double-layer series

Cleanert® PestiCarb/NH_2

Description

Cleanert® PestiCarb/NH_2 SPE column is packed with 500 mg PestiCarb and 500 mg NH_2. It has been widely used in analysis of pesticide residues, esp. for the Japanese Positive List System. JAP-178 GC/MS and LC/MS detection method for pesticides.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® PestiCarb/NH_2	500 mg/500 mg	6 mL	30	PN0006
	300 mg/500 mg	6 mL	30	PN8006

Cleanert® Pesticarb/PSA

Description

Cleanert® Pesticarb/PSA is a dual layer SPE tube packed with 500 mg PestiCarb and 500 mg PSA. It was widely accepted by customers for analysis of pesticides residue, such as the Japanese Positive List System JAP-178 GC/MS and LC/MS detection method for pesticides.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® PestiCarb/PSA	500 mg/500 mg	6 mL	30	PP0006
	300 mg/500 mg	6 mL	30	PP8006

Cleanert® SAX/PSA

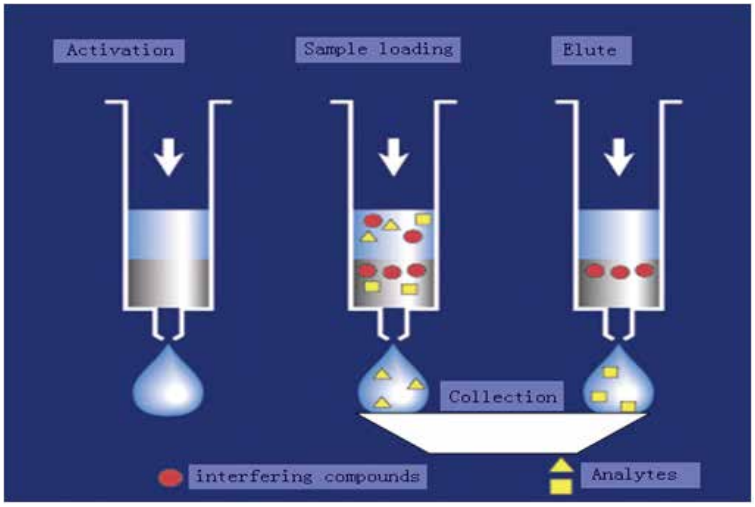
Description

Cleanert® SAX/PSA is a dual layer SPE tube packed with 500 mg SAX(quaternary amine) and 500 mg PSA. It was widely accepted by customers for analysis of pesticides residue, such as the Japanese Positive List System JAP-178 GC/MS and LC/MS detection method for pesticides.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® SAX/PSA	500 mg/500 mg	6 mL	30	SP0006

Suggested processing method for double layer cartridge



Tips:
Instead of obtain the target compounds, another kind of cartridge is base on the mechanism that to keep the matrix on the sorbent, and let the target compounds pass through the cartridge. This kind of cartridge often apply to multi-residues analysis, or at the situation that the target compounds couldn't be adsorb on the SPE material.

Cleanert® C8/SCX

Description

Clearnert® C8/SCX is a mixed-mode SPE based on silica of C8 and strong cation-exchange. It is usually used for the extraction of basic drugs from urine or blood.It has been widely used in drug abuse and forensic analysis.

Particle Characteristics

Average Particle Diameter: 50 μm;
Average Pore Size: 60 Å;
Specific Surface Area: 600 m^2/g.

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleaner® C8/SCX	50 mg	1 mL	100	CS0501
	130 mg	3 mL	50	CS1303
	300 mg	3 mL	50	CS3003
	500 mg	6 mL	30	CS5006
	1 g	6 mL	30	CS0006
	50 mg/well	2 mL	96-well plate, 1/PK	CS0502-W
	100 mg/well	2 mL	96-well plate, 1/PK	CS1002-W
	10 g	-	bottle	CS0010
	100 g	-	bottle	CS0100

Cleanert® IC:

Sample Clean-up Cartridges for Ion Chromatography

Description

Cleanert® IC series are used for removing matrix interferences such as phenolics, metalions, cations, anions, or hydrophobic substances encountered in many ion chromatography applications. They can absorb metal ions and reduce interferential ion in the sample, thus prolong the lifetimes of the analytical columns. They also can be used for some trace-level determinations.

The IC cartridges are designed as luer inlet and outlet for easy operation. They also can connect several cartridges together for complex matrix. The sample solution can be passed through the IC cartridge by syringe, and also by vacuum manifold connected with an empty SPE cartridge as funnel.

Type	Ion-exchange capability	Application	Spec.	Cat. No.
IC-ODS	—	To remove the hydrophobe from biological samples.	1 cc, 50/pk 2.5 cc, 50/pk	IC-1810 IC-1825
IC-RP	—	To remove substances such as aromatic dyes, some aromatic carboxylic acids, hydrocarbons, and surfactants from sample matrices.	1 cc, 50/pk 2.5 cc, 50/pk	IC-RP10 IC-RP25
IC-P	—	To remove the phenolic fraction of humic acids, tannic acids, lignins, anthocyanins, and azodyes from samples.	1 cc, 50/pk 2.5 cc, 50/pk	IC-P10 IC-P25
IC-A	0.7 meq/1 cc	To remove anion contaminant and neutralize the strongly acidic sample solution.	1 cc, 50/pk 2.5 cc, 50/pk	IC-A10 IC-A25
IC-H	2.0-2.2 meq/1 cc	To remove high levels of alkaline earths and transition metals from sample matrices and in the neutralization of highly alkaline samples such as sodium hydroxide or sodium carbonate.	1 cc, 50/pk 2.5 cc, 50/pk	IC-H10 IC-H25
IC-Na	2.0-2.2 meq/1 cc	To remove high levels of alkaline earths and transition metals from sample matrices without acidifying the sample. This ensures good recovery of acid labile analytes such as nitrite.	1 cc, 50/pk 2.5 cc, 50/pk	IC-Na10 IC-Na25
IC-Ag	2.0-2.2 meq/1 cc	To remove chloride, bromide, and iodide from sample matrices. An IC- H cartridge should be used after the IC- Ag cartridge to remove dissolved Ag.	1 cc, 50/pk 2.5 cc, 50/pk	IC-Ag10 IC-Ag25
IC-Ba	2.0-2.2 meq/1 cc	To remove $SO4^{2-}$, the cartridge should be activated with solution contain Cl- when the concertration of anion in the sample is too low.	1 cc, 50/pk 2.5 cc, 50/pk	IC-Ba10 IC-Ba25
IC-M	0.4 meq/1 cc	To remove transition metals and matrix elimination of alkali and alkaline earth metals.	1 cc, 50/pk 2.5 cc, 50/pk	IC-M10 IC-M25
IC-Ag/H	—	A layered cartridge containing IC-Ag and IC-H,	1 cc, 50/pk 2.5 cc, 50/pk	IC-Ag/H10 IC-Ag/H25
IC-Ag/Na	—	A layered cartridge containing IC-Ag and IC-Na,	1 cc, 50/pk 2.5 cc, 50/pk	IC-Ag/Na10 IC-Ag/Na25
IC-Ba/Ag/H	—	A layered cartridge containing IC-Ba, IC-Ag and IC-H,	1 cc, 50/pk 2.5 cc, 50/pk	IC-Ba/Ag/H10 IC- Ba/Ag/H 25
IC-Ba/Ag/Na	—	A layered cartridge containing IC-Ba, IC-Ag and IC-Na,	1 cc, 50/pk 2.5 cc, 50/pk	IC-Ba/Ag/Na10 IC-Ba/Ag/Na25

SPE Apparatus and Accessories

SPE Vacuum Manifolds

Description

SPE Vacuum manifolds for SPE sample preparation, filtration and elution are available in 12 and 24 port configurations. These manifolds permit consistent extraction and filtration results. Multiple sample processing with these manifolds consists of a clear glass chamber and lid, to which a vacuum is applied to draw solvents and sample through a SPE column, cartridge, or disk. The lid is CNC machined, solvent resistant, low extractable virgin polypropylene. The lid is autoclavable, and will not wrap. The female Luer inlets and male Luer outlets are molded of pure polypropylene. Adjustable racks placed in the glass chamber will accommodate a variety of sample collection vessels, including test tubes, autosampler vials, volumetric flasks, and Erlenmeyer flasks. Eluents are deposited directly into the collection vessel of choice via polypropylene, or optional stainless steel or Teflon needles.

Cat No.:VM12(front), VM24(hind)

Ordering Information

Product Name	Specification, Package	Cat. No.
Vaccum manifold	12 positions	VM12
	24 positions	VM24
Stopcocks	12 pieces/PK	A81213
Needles	12 pieces/PK	A80100
Extended needles	12 pieces/PK	A80100-L
Female luer	12 pieces/PK	A80104
Male luer	12 pieces/PK	A80105

Luer

Stopcock

Extend Needle

Large Receiver SPE Vacuum Manifold

Description

This device is specially designed to collect large volumes of eluent in pesticide residue detection. It accommodates six samples in parallel and it also provides connection to a vacuum pump for expedited operation. Standard size spin steaming bottle can be connected to the manifold for large amount eluent collection. The whole parts is made of glass and could effectively involved contamination of plasticizer.

Cat No.:VM06

SPE-10 Automatic Sample Preparation System

Cat No.:SPE-10

Max sample loading volume 1-5000 mL, fits different application requirement

Five kinds of solvent available

Modularly design, each module could process 6 samples gradually. One control module could control max 4 extraction modules, and 24 samples could be processing in line

Multistep eluent collection, meets flexible application requirement

Flow speed area: 0.1-30 mL/min
Accuracy : ±2%

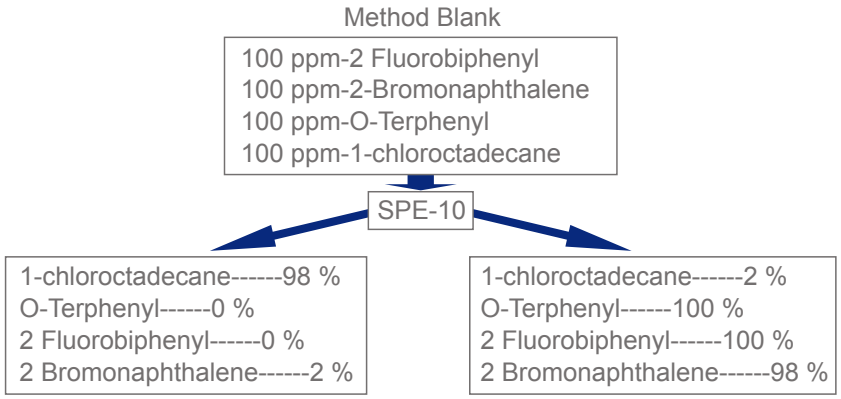
Compatible with 1-12mL SPE cartridge

Specifications

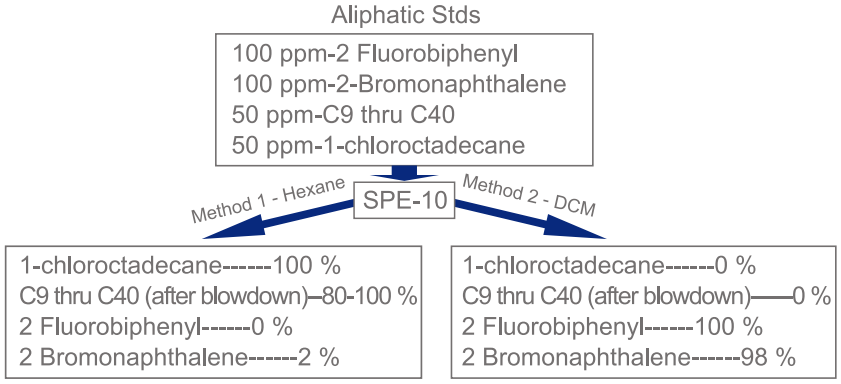
Channel	Single (extended to 4 channels)	Flow rate range	0.1-30 mL/min
Columns	6-24	Flow precision	±2 %
Sample loading	1-5000 mL	Pressure tolerance	50 psi
Collection volume	0.1-60 mL	Operation	PC and panel
Solvent	5	Pipeline material	Ceramics,PTFE and PEEK

SPE-10 Procedure for EPH Method Cartridge: Cleanert® EPH, Cat.No: SI500025-30

SPE-10 for EPH Results



SPE-10 for EPH Results



Method (1 for aliphatic fraction, 2 for aromatic fraction)

No	Method	Solvent	Flow Rate	Volume	
1	Condition1	Hexane	2	5	Dispenses 5 mL of hexane in cartridge
2	Condition1	Air	2	10	Dries Cartridge with 10 mL of air
3	Condition1	Hexane	2	10	Dispenses 10 mL of hexane in cartridge to waste
4	Condition1	Air	2	10	Dries Cartridge with 10 mL of air
5	Sample Loading (Collected)	Air	2	1	Adds 1 mL of sample to cartridge
6	Collect 1A	Hexane	2	20	Dispenses and collects 20 mL of hexane
7	Collect 1A	Air	2	10	Dries Cartridge with 20 mL of air and collects remaining fraction on the cartridge
8	Collect 2A	Methylene Chloride	2	20	Dispenses and collects 20 mL of DCM
9	Collect 2A	Air	2	20	Dries Cartridge with 20 mL of air and collects remaining fraction on the cartridge

Qdaura® Automated SPE System

The Qdaura Automated SPE system is specifically designed for high throughput sample preparation. The system automates routine SPE procedures from conditioning, sample loading, washing, dry and elution. The Qdaura utilizes positive pressure to ensure more reproducible flow rates, more reproducible recoveries, less sample to sample contamination, and higher precision. The built-in tasks optimized the steps during extraction to eliminate cross-contamination. The system can treat 1-4 samples parallel and up to 24 samples can be performed with in a run. The Qdaura could been used in the area of agricultural, food testing and antibiotic residue detection.

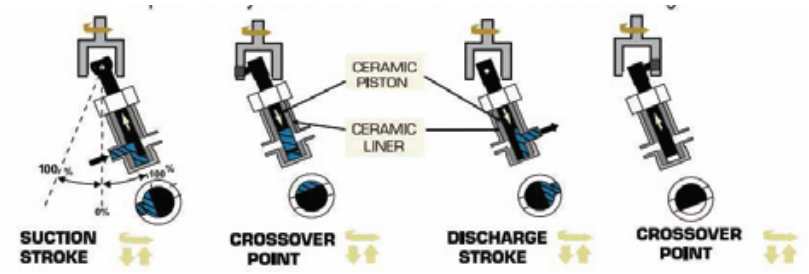
Specifications

Item	Qdaura
Sample Capacity	24
Channels	4
Solvents	5
Sample Loading Volume	0-30 mL
Flow Rate	0.05-30 mL/min
Pump	Valveless metering pump
Fractionation	2
Software	Integration Design

User Friendly Software

- Real-time display the current status of each channel
- Batch method editor
- 200 Methods can be stored

Fluid Delivery: Positive Pressure Metering Pump



96-Well Plate Vacuum Manifold

Cat. No: VM96

VM96 Features

- The degree of vacuum can be controlled by adjusting the needle value
- Vacuum gauge shows the degree of vacuum
- Corrosion-resistant design of the whole system
- Elevate the module by adding collection plate

VM96 Operation Procedures

- Use vacuum tubing to connect the suction port of VM96 and the inlet pot of the vacuum pump;
- Remove the top cover of VM96, and put 96-well collection plate into the cavity of the bottom cover of VM96;
- Put back the top cover;
- Put the 96-well SPE plate exactly on the top cover of VM96;
- Make sure the gas valve at vacuum state (perpendicular). Start the vacuum pump, adjust the vacuum valve to suitable degree of vacuum (i.e. SPE plate flow rate);
- After elution, rotate the gas valve 90 degree to non-vacuum state. Then remove the 96-well SPE plate.

Note: This device is a precision vacuum device, keep in mind is not removable to avoid collisions, so as to avoid a vacuum leak warranty.

* Note: Collection plate spacer could be used for micro collection plate to avoid cross contamination.

VM96 Specifications

Type	VM96
Dimensions	220 × 185 × 85 mm
SPE Plate Format	Std.96-well dimensions
Net weight	2.7 KG

SPE-M96 Positive Pressure SPE Device

MULTI-SPE M96 is a positive pressure manifold, specially designed for high-throughput sample preparation in research and testing laboratories. It could apply evenly gas pressure on each well to press liquid through 96-well SPE plates smoothly. It largely reduces the variation on the flow-rate between each well of 96-well plates, comparing with vacuum SPE devices, thus improving sample-to-sample consistency. For some viscous samples, vacuum may not provide enough pressure and will cause clogging issue. However, MULTI-SPE M96 can provide maximal 60 psi positive pressure to help the sample pass through 96-well plates with a adjustable flow rate.

Cat No.: SPE-M96

Features

- Low variation on the flow-rates between wells of 96-well plates which could improve the extraction consistency.
- High-throughput by processing maximal 96 samples simultaneously.
- Separated tuning to get precisely pressure control.
- The SPE stage indicator could be manually switch ed to the indicator position to remember the current stage of your operation.
- The whole SPE process can be completed in an inert (N_2) environment by choosing N2 gas source.

Compatibility	96-well plates
Loading volume	Depends on the well plate volume (sample/solvent)
Collection volume	1mL or 2mL (depending on collection plate types)
Pressure Control	Max. 60psi
SPE step indication	Manual SPE step indicator(Condition\Load\Wash\Dry\Elute)
Gas	Nitrogen or air

SPE-M48 Positive Pressure SPE Device

A Positive Pressure SPE Manifold Designed for Drug R&D Labs

MULTI-SPE M48 is a manual SPE manifold with 48 positions special designed for high throughput R&D laboratories of pharmaceutical industry.

Positive pressure eliminates the differences of the flow-rate between the cartridges that encountered frequently on vacuum SPE devices. Therefore, MULTISPE M48 will provide highly uniform extraction which may improve the reproducibility of the recoveries.

When processing viscous samples such as blood, vacuum manifold often cannot provide adequate pressure since the maximum pressure is limited to atmospheric (< -15 psi). In contrast to vacuum manifold, the Multi-SPE M48 provide maximal 60 psi pressure. Gas pressure up to 60 psi., avoiding clogging issue when loading sticky or viscous samples.

A step indicator is installed on the front panel of MULTI-SPE M48 so that the user can mark SPE process step by step.

Cat No.: SPE-M48

Features and Advantages

- Low variation on the flow-rates between the cartridges, improving the extraction consistency.
- High-throughput by processing maximal 48 samples simultaneously.
- Good flexibility: can control on/off for gas by each 12 positions to save gas; can run any number of samples between 1-48.
- Separated tuning to get precisely pressure control.
- The SPE stage indicator could be manually be switch ed to the indicator position to remember the current stage of your operation.
- The whole SPE process can be completed in an inert (N2) environment when N2 gas source is provided.

Specifications

Format of SPE	1 mL/3 mL or 6 mL cartridge
Max SPE positions	48 (can choose any number less than 48)
Max Loading volume	Depends on the SPE cartridge volume (sample/solvent)
Max Collection volume	2 mL or 5 mL (depending on test tube racks)
Pressure Control	Max. 60psi
SPE step indication	Manual SPE step indicator(Condition\Load\Wash\Dry\Elute)
Gas	Nitrogen or air

SPE-M12 Positive Pressure SPE Device

Features and Advantages

- Compatible with most of the commercial SPE columns;
- Maximal 12 samples solid phase extraction can be processed parallelly;
- Can perform different combination of application operation;
- Double gas path design, one path to fine tune the liquid flow rate the other is used for drying and removing water residue;
- 12 regulating valves can independently control the flow rate of each SPE column.

Cat No.: SPE-M12

Specifications

Format of SPE devices	1 mL, 3 mL, 6 mL, 10 mL, 15 mL, and 30 mL cartridges; Glass columns
Extraction mode	Single cartridge, Stacked cartridges
SPE positions	12
Loading capacity	Depends on the SPE cartridge volume. Maximal 30mL sample loading cartridge could be added to SPE cartridge for big volume loading requirement
Individual flow control	Adjust flow-rate of each 12 cartridges individually
Max. collection volume	50 mL
Pressure control	Max. 60psi
SPE step indication	Manual SPE step indicator (Condition\Load\Wash\Dry\Elute)
Gas	Nitrogen or air

SPE-M08 Positive Pressure SPE Device

A Positive Pressure SPE Manifold Designed for Analytical Laboratories

MULTI-SPE M08 is a multi-function manual SPE device designed for routine analytical laboratories with patented technology. This is the first positive pressure SPE manifold that can handle most of the SPE application requirements.

Positive pressure is applied to push the sample or solution through SPE cartridges smoothly. It eliminates the differences of the flow-rate between the cartridges that encountered frequently on vacuum SPE devices.

Varying from other positive pressure SPE devices, MULTI-SPE M08 is suitable not only for single- cartridge mode but also stacked cartridges mode applications. Another empty sample loading cartridge can be added on the top of the SPE cartridges to meet the bigger sample loading volume requirement. 30 to 50 mL samples can be stored in the sample loading cartridge. Even higher sample loading requirement such as up to 50mL could be achieved by sample loading pipeline which is optional. There are several collection racks are available to fit various collection tubes or flasks. The maximum collection volume is 100 mL for each fraction.

A step indicator is installed on the front panel of MULTI-SPE M48 so that the user can mark SPE process step by step.

Cat No.: SPE-M08

Simple and Flexible

MULTI-SPE M08 can fit 1 mL, 3 mL, 6 mL, 10 mL, 15 mL, 30 mL and 60 mL SPE cartridges, as well as 47 mm SPE disks. Maximal 8 samples can be extracted simultaneously. The flow-rate can be adjusted slightly by a Low Flow pressure gauge , and high flow pressure gauge could provide high airflow for cartridge drying. Furthermore, the flow-rate of each cartridge can be finely adjusted individually by needle valves.

MULTI-SPE M08 is suitable for various analytical laboratories; such as food safety, environmental sample analysis, antibiotic residue detection, forensic analysis or other biological sample analysis.

Specifications

Format of SPE devices	1 mL, 3 mL, 6 mL, 10 mL, 15 mL, 30 mL, and 60 mL cartridges; 47 mm disks glass columns
Extraction mode	Single cartridge, tandem cartridges
SPE positions	8
Loading capacity	30-50 mL for standard configuration (Large volume sample loading pipeline is optional.)
Individual flow control	Adjust flow-rate of each cartridge individually
Max. collection volume	100 mL (with 100 mL flask)
Pressure	Max. 60 psi.
SPE step indication	Manual SPE step indicator (Condition\Load\Wash\Dry\Elute)
Gas	Nitrogen or air

NV-96G Nitrogen Evaporator for 96 Well Plates

Stable · Effective · Simple Operation

NV-96G is designed for small volume elute concentration in drug screening, hormone assay, biochemical analysis and drug metabolism. This concentrator is compatible with most of commercial 96 well collection plate without 2nd transfer. The holder panel for collection plate could be elevated or brought down automatically to keep the needles close to the liquid interface. This evaporator is widely used in clinical analysis and DMPK analysis. It utilize a metal block for drying which can provide uniform heating with zero cross contamination.

Cat No.: NV-96G

Features

- Online temperature controlling;
- Compatible with most of the 96-well collection plates;
- Adjustable height for blower holder;
- Digital display and touch screen, simple and friendly operation;
- Double heating mode (through bottom plate also N2 airflow.).

Specifications

Heating Module	96-well collection plate
Flow	Adjustable
Flow Rate	0-10L/min (0-100 psi)
Pressure range	≤100 psi
Alarm	Humidity Alarm
Power	240 W
Temp range	ambient temperature+5-80 °C
Temp Accuracy	±0.5 °C
Temp errors	±1 °C
Temp stability	±0.5 °C
Working power	220 V/50 Hz; 110 v/50 HZ

NV-8G & NV-12G Nitrogen Evaporator

NV-8G Nitrogen Evaporator is widely used in all kinds of chemical labs. The compact design make it easy to put into fuming hood. Maximal 8 samples for NV-8G and 15 samples for NV-15G could be processed simultaneously, and each channel could be closed when less sample is processing. And the heating block is compatible with common tubes and 12mL vials. The venting needles could be lifted or brought down to adjust the height, and keep the airflow close to the liquid interface.

Cat No.: NV-8G

Cat No.: NV-15G

Specifications

NV-8G

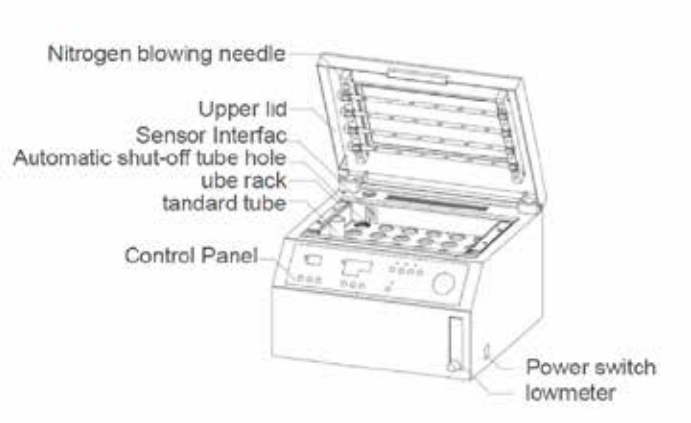
Heating module	8 hole	Hole depth of heating block	60 mm
Adjustable of flow	Adjustable	Diameter of heating block	19 mm
Gas flow	0-10 L/min(0-100 psi)	Working power supply	220 V/50 Hz
Temperature range	>Room temperature 5-80°C	Maximum power	240 W
Temperature error	Plus or minus 0.3°C	Instrument weight	3.5 kg
Work environment	Temperature 0-30 ℃ Relative humidity ≤85 %RH		

NV-15G

Heating module	15 hole	Well depth	60 mm
Adjustable of flow	Adjustable	Well Diameter	19 mm
Gas flow	0-10 L/min(0-100 psi)	Working power supply	220 V/50 Hz
Temperature range	>Room temperature 5-80 ℃	Maximum power	240 W
Temperature error	Plus or minus 0.3 ℃	Instrument weight	4.7 kg
Work environment	Temperature 0-30 ℃ Relative humidity ≤85 %RH		

NV24A-11 Nitrogen Evaperator

Cat No.: NV24A-11



NV24A-II Nitrogen Evaperator has the advantages of large processing capacity, environmental protection, completely automated operational. It has been used in the fields of environmental sample analysis, food safety analysis, antibiotic residue analysis and forensic analysis. It was a best choice for applications involved with heat sensitive or volatilizable compounds.

Features

- Max 24 samples could be processed simultaneously;
- Airflow and pressure are adjustable and displayed even during the concentration process;
- Advanced optical fiber sensor can automatically stop the system when there is only 0.5 mL and 1 mL solvent is left;
- Use PTC heating elements which can keep uniform heating such as the water bath concentration process;
- Internal recycle system which can effectively eliminate harmful gases releasing.

Specifications

Sample amount	Maximal 24 samples
Water bath temperature range	Room temperature +5°C to 80°C
Temperature control accuracy	1°C
Quantitative concentration volume	0.5 mL or 1 mL or blow-dry
Evaporation rate	2-8 mL/min
Air requirements	30-80 psi
Purge air pressure	10-20 psi
Heating power	800 W
Weight	17 kg

Accessories

Empty Columns and Accessories

Ordering Information

Product name	Specification, Package	Cat. No.	Picture
Empty Cartridge	1 mL, 100/PK	AZ001-H	
	3 mL, 100/PK	AZ003	
	6 mL, 100/PK	AZ006	
	6 mL, flangeless, 100/PK	AZ006-N	
	10 mL, LRC empty tube, 100/PK	LAZ010	
	12 mL,100/PK	AZ012	
	25 mL, 50/PK	AZ030	
	60 mL, 50/PK	AZ060	
	150 mL, 25/PK	AZ150	
	100 mL, LDC empty tube, 10/PK	LDC200	
PE Frit(10 μm)	Compatible with 1 mL cartridge, 100/PK	AS001-A	
	Compatible with 3 mL cartridge, 100/PK	AS003-A	
	Compatible with 6 mL cartridge, 100/PK	AS006-A	
	Compatible with 10 mL LRC tube, 100/PK	LAS010-A	
	Compatible with 12 mL cartridge, 100/PK	AS012-A	
	Compatible with 25 mL cartridge, 50/PK	AS030-A	
	Compatible with 60 mL cartridge, 50/PK	AS060-A	
	Compatible with 150 mL cartridge, 25/PK	AS150-A	
Adapter	Compatible with 3 mL, 6 mL, and 12 mL cartridge, 12/PK	A80115	
	Compatible with 25mL and 60mL cartridge, 6/PK	ZJT-1	
Empty end-capped cartridge	1 mL End-capped cartridge	AZ-IC-1	
	1 mL End-capped cartridge (with frit)	AZ-IC-1T	
	2.5 mL End-capped cartridge	AZ-IC-2.5	
	2.5 mL End-capped cartridge (with frit)	AZ-IC-2.5T	

SPE empty tube

SPE empty tube (flangless)

LRC empty tube

LDC empty tube

Frits

Adaptor

End-capped cartridge

Large Volume Sampling cartridge

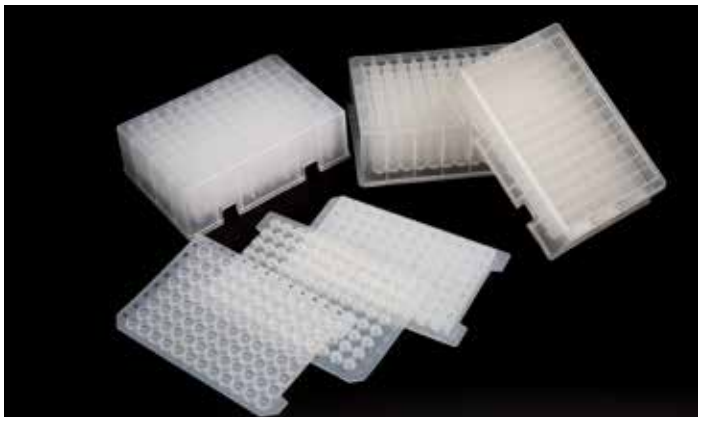
Ordering Information

Product name	Specification	Cat. No.
Large Loading Column (30 mL)	1 piece/package	A82030
Large Loading Column (60 mL)	1 piece/package	A82060
Water Loading Pipeline	1 piece/package	A80116

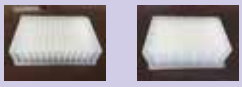
a: Water Loading Pipeline

b: Large loading column

Collection Plate and Silica Mat



Ordering Information

Product	Specification		Package/PK	Picture	Cat. No.
96-well collection plate	Square well, round bottom, 2.2mL/well	8×12well	24		96SP2036-2
	Square well, round bottom, 1.6mL/well	8×12well	24		96SP1036
	Round well, round bottom, 1mL/well	8×12well	24		96SP1036-Y
	Round well, round bottom, 2mL/well	8×12well	24		96SP2036-Y
	Round well, round bottom, 400uL/well	8×12well	10		96SP0236-U
	Round well, V-shape bottom, 360uL/well	8×12well	10		96SP0236-V
8well solution storage plate	8well solution storage plate, 20mL/well	8 well	10		08SP2036
12well solution storage plate	12well solution storage plate, 14mL/well	12 well	10		12SP1436
48well collection plate	48well collection plate, 4.6mL/well	4x12 well	10		48SP4036

Ordering Information

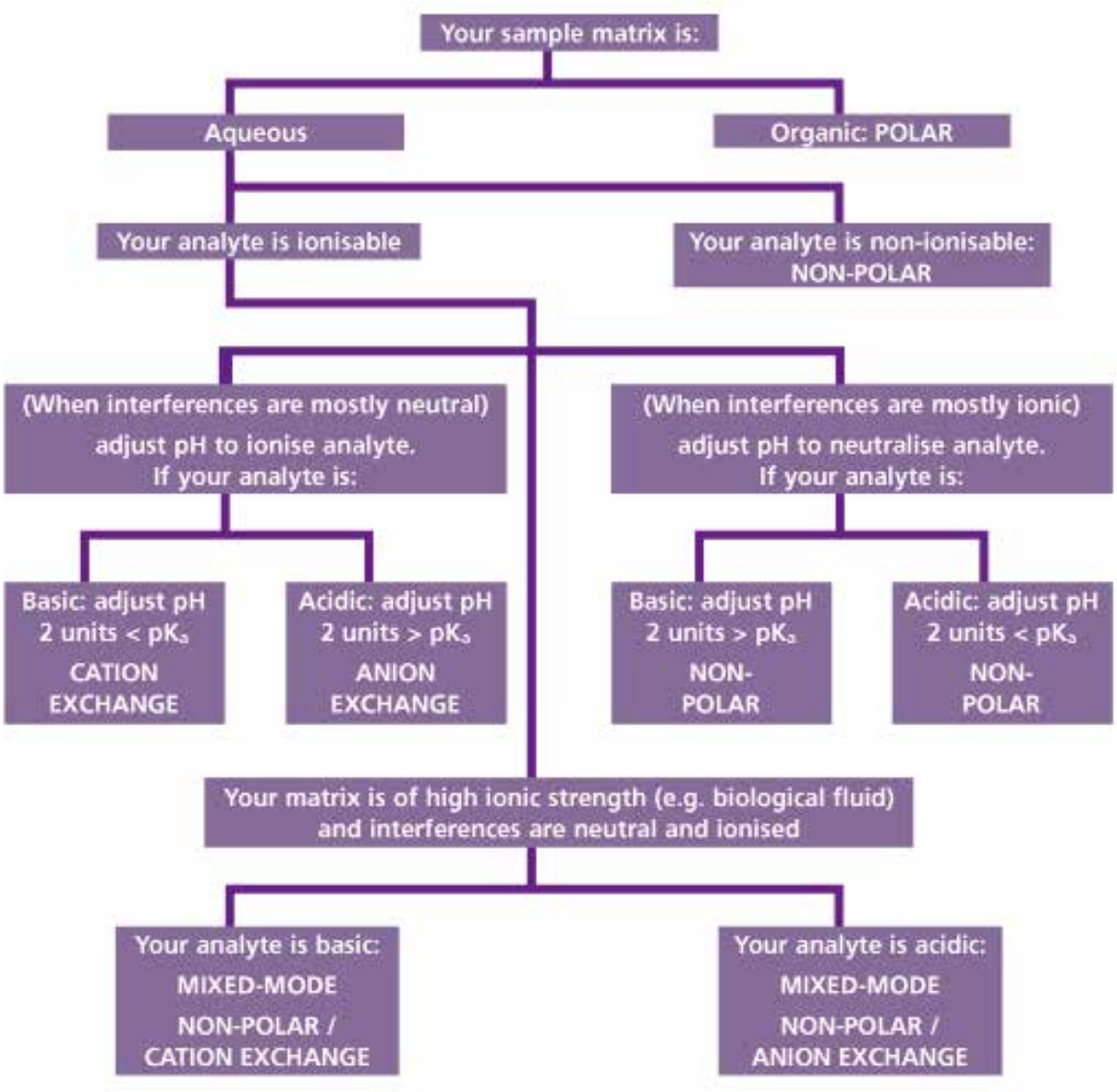
Product	Specification		Package/PK	Picture	Cat. No.
96-well collection plate	Waste collection plate		10		96WSP
Silica mat for 96 well plate	96 well silicone mat, square well	8×12well	10		96GP2036-1
	96 well silicone mat, square well, pierceable	8×12well	10		96GP2036-2
	96 well silicone mat, round well, pierceable	8×12well	10		96GP2036-M
8 collection tube	8well collection tube, round bottom, PP	8well	120		A8001

Method Development of SPE Procedures

Many factors impact SPE procedures. For SPE method deveopment, however, here are some selection guides.

The Selection of Sorbent Retention Mechanism

The guide on this page briefly outlines the decision making process required to choose a suitable extraction mechanism.



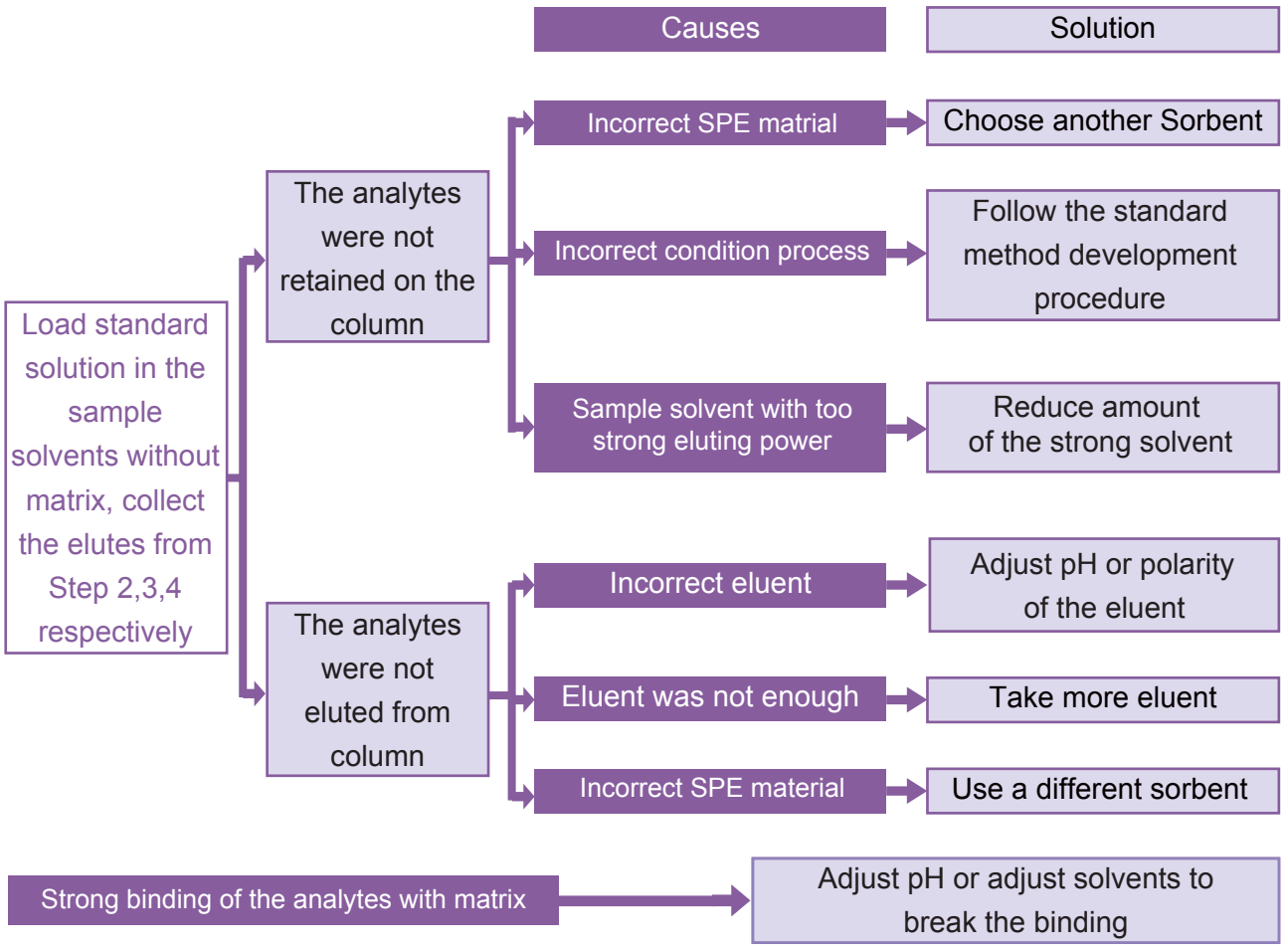
SPE Product Cross Reference Table

This guide directs the selection of an appropriate sorbent or chemistry for a SPE procedure.

	Agela	Waters	Supelco	Aglient	Agilent
C18 (End-capped)	Cleanert® C18	Sep-pak C18	ENVI-18	—	Bond Elut C18
C18 (Non end-capped)	Cleanert® C18-N	—	—	AccuBOND C18	Bond Elut C18-OH
C8	Cleanert® C8	Sep-pak C8	ENVI-8	AccuBOND C8	Bond Elut C8
Cyano group	Cleanert® CN	Sep-pak CN	LC-CN	AccuBOND CN	Bond Elut CN
Amino group	Cleanert® NH_2	Sep-pak NH_2	LC- NH_2	AccuBOND NH_2	Bond Elut NH_2
Propyl ethylene diamine	Cleanert® PSA	—	—	—	Bond Elut PSA
Quaternary ammonium salt (Strong anion-exchange cartridge)	Cleanert® SAX	—	LC- SAX	AccuBOND SAX	Bond Elut SAX
Carboxyl group (Weak cation-exchange cartridge)	Cleanert® COOH	—	LC- WCX	—	Bond Elut CBA
Propylsulfonic acid	Cleanert® PRS	—	—	—	Bond Elut PRS
Benzene sulfonic acid (Strong cation-exchange cartridge)	Cleanert® SCX	—	LC- SCX	AccuBOND SCX	Bond Elut SCX
Silica gel	Cleanert® Silica	Sep-pak Silica	LC- Silica	AccuBOND Silica	Bond Elut Silica
Diol	Cleanert® Diol	Sep-pak Diol	LC-Diol	AccuBOND Diol	Bond Elut Diol
Polystyrene/divinyl-benzene	Cleanert® PS	—	ENVI-Chrom P	AccuBOND ENV PS-DVB	—
Polar polymer cartridge	Cleanert® PEP-2	Oasis HLB	—	—	Bond Elut® Plexa
Mixed anion-exchange cartridge	Cleanert® PAX	Oasis MAX	—	—	—
Mixed cation-exchange cartridge	Cleanert® PCX	Oasis MCX	—	—	—
Cartridge specialized for sulfonylureas	Cleanert® HXN	—	—	—	—
Magnesium silicate (Florisil)	Cleanert® Florisil	Sep-pak Florisil	LC Florisil	—	Bond Elut FL
Graphitized carbon	Cleanert® PestiCarb	—	ENVI Carb	—	—
Neutral alumina	Cleanert® Alumina N	Sep-pak Alumina N	LC- Alumina N	AccuBOND Alumina N	Bond Elut Alumina N
Acidic alumina	Cleanert® Alumina A	Sep-pak Alumina A	LC- Alumina A	AccuBOND Alumina A	Bond Elut Alumina A
Basic alumina	Cleanert® Alumina B	Sep-pak Alumina B	LC- Alumina B	AccuBOND Alumina B	Bond Elut Alumina B
Mixed graphitized carbon and amino group cartridge	Cleanert® PestiCarb/NH_2	Sep-pak Carb/ NH_2	ENVI Carb/ NH_2	—	—
Cartridge specialized for sulfanilamides	Cleanert® SUL-5	—	—	—	—
DNPH-Silica cartridge (specialized for pretreatment of aldehydes and ketones in air)	Cleanert® DNPH-Silica	Sep-pak DNPH-Silica	—	—	—
Solid supported liquid/liquid Extraction columns	Cleanert® SLE	—	—	—	Chem Elut SLE

Trouble Shooting

Low Recovery



High Deviation

SPE was dry during condition step (Only for C18, C18-N, C8)	Re-activated the cartridge or change another SPE cartridge
SPE cartridge was over loading	Reduce the sample amount or increase the sorbent amount
Flow rate was too high	Reduce the flow rate below 5 mL/min
The solution used in four steps was not miscible to each other	Dry the column when change different solution
The washing solution was too strong	Change the polarity of the washing solution
The loading solution was too strong	Change the polarity and pH of the loading solution

Clogging

The sample contains colloidal particles	Centrifuge or filtration before SPE
The sample was sticky	Dilute the sample

SPE Method Development Protocol

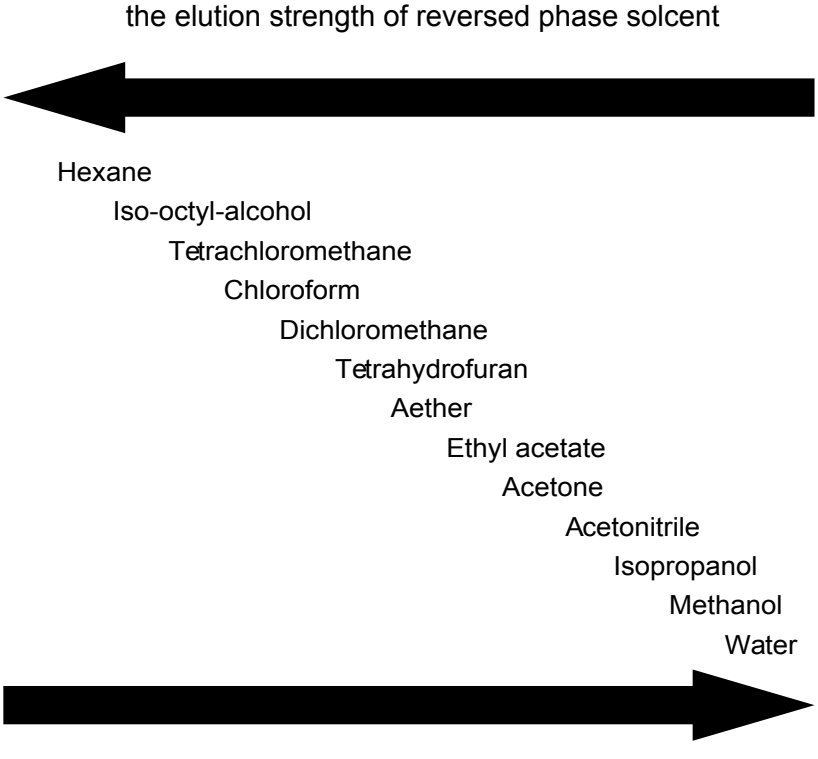
Properties	Sorbent Phase Type			
	Normal Phase	Reversed-Phase	Lon Exchange Phase or Mix-phase	
Typical Sorbents	Silica Florisil	PEP series	PAX	PCX
	Amine (NH_2)	C18 C18-N	SAX	SCX
	Cyano (CN) Diol	C8	PWAX	PWCX
Sorbent Polarity	High	Low	High	
Matrix Properties	Organic	Organic or Aqueous	Organic or Aqueous	
Analyte Properties	Slightly to Moderately Polar	Non-Polar or Polar	Acidic	Basic
Retention	Polar Non-Polar	Non-Polar polar	Ionic Strength	
Step 1: Condition	Sample solvent or other organic solvents	Water-miscible organic solvents followed by Water	Water-miscible organic solvents followed by aqueous solution with pH adjusted	
Step 2: Sample Loading	Load the sample or with dilution in low polarity solvents	Load the sample or with dilution in high polarity solvents	Load sample or with dilution in high polarity solvents with pH adjusted	
Step 3: Washing	Washing with low-polar solvents	Washing with mixture of aqueous solution or buffer with a small amount polar solvent	Washing with Polar organic solvents followed by aqueous solvents with pH adjustment to maintain analytes ionized	
Step 4: Elution	Eluting with mixture of non-polar and polar solvents	Eluting with non-polaror polar organic solvents	Eluting with polar solvents with pH adjustment	

Size, Capacity and Elution Volume in SPE Process

For the normal phase and reversed phase SPE cartridges, the weight of sample can not exceed the 5% of sorbent weight. For the ion-exchange mode, the capacity of the ion-exchange must be considered.
The table below is the capability and eluted parameter of SPE:

Specification	Quality of Loading Sample	The Minimum Volume of Elution
50 mg/1 mL	2.5 mg	125 µL
100 mg/1 mL	5 mg	250 µL
200 mg/3 mL	10 mg	500 µL
500 mg/6 mL	25 mg	1.2 mL
1 g/6 mL	50 mg	2.4 mL

The Selection of Ideal Elution Solvent



Food Safety Applications

Veterinary Drug Residues Detection

Detection of Four β- Agonist Drugs Residues (Clenbuterol Hydrochloride, Salbutamol, Cimaterol and Ractopamine etc.) in Animal Tissues (Cleanert® PCX, P/N: CX1506)

EMF10001

Material

(1) SPE Cartridge: Cleanert® PCX (150mg / 6mL);
(2) Four kinds of β- agonist standard solution: Clenbuterol hydrochloride, Salbutamol, Cimaterol and Ractopamine;

Experiment

SPE Procedure

- Condition the PCX cartridge with 5 mL methanol, 5 mL of deionized water and 5 mL of 30 mM HCl solution sequentially.
- Load sample concentrate onto the cartridge.
- Wash the loaded cartridge with 5 mL water followed by 5 mL methanol, and discard the eluate.
- Dry the cartridge by passing through nitrogen gas.
- Elute the cartridge with 5 mL methanol containing 4% ammonia, and collect the eluate into a glass test tube, and then dry the eluate at 50 Celsius under a gentle flow of nitrogen (ca. 1 mL/min).

Derivatization and detection

Heat the sample tube in an oven at 50 Celsius for a few minutes to remove water. Add 100 µL of toluene and 100 µL of N,O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) to the test tube. Vortex mix for 20 s. Seal the test tube and place it in oven at 80°C for 1 h. Cool and add 300 µL of toluene to the test tube. The solution is ready for GC-MS analysis (GC column: DA-5MS, 30 m×0.25 mm×0.25 µm, P/N: 1525-3002).

Results

Recovery
Samples of pig liver, spiked with 1, 2, 5, 10, and 100 µg/L, respectively, were extracted by liquid-liquid partitioning. Four batches of samples were tested. In each batch, 5 replications were used for each concentration. The results of the recovery test are listed in the following table.

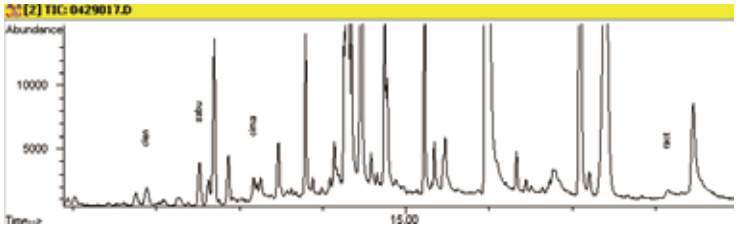
Results of analysis of pig liver samples:

Spiked concentration (μg/L)	Measured concentration (μg/L)	Mean measured concentration (μg/L)	Mean percent recovery (%)	RSD (%)
1	0.75	0.72	72.40	5.93
	0.67			
	0.72			
	0.70			
	0.78			
2	1.62	1.63	81.30	1.23
	1.66			
	1.60			
	1.61			
	1.64			
5	4.02	4.24	84.80	4.16
	4.10			
	4.27			
	4.38			
	4.43			
10	8.24	8.45	84.45	2.81
	8.35			
	8.77			
	8.62			
	8.25			
100	90.24	9.12	91.15	2.86
	87.15			
	91.77			
	92.62			
	93.95			

Repeatability Experiment
Results of analysis of pig liver samples:

Batch	Spiked concentration (μg/L)									
	1		2		5		10		100	
	Mean recovery %	RSD%	Mean recovery %	RSD%	Mean recovery %	RSD%	Mean recovery %	RSD%	Mean recovery %	RSD%
1	72.40	5.93	81.30	3.49	84.80	6.16	84.45	3.59	91.15	2.86
2	75.37	6.12	80.47	5.37	84.74	7.55	87.46	4.68	90.05	3.86
3	70.09	7.85	80.80	6.57	83.10	8.17	83.21	5.39	89.53	4.16
4	76.73	4.90	78.50	8.35	82.90	5.11	85.95	5.72	88.27	5.93
Average	73.65	6.20	80.25	5.95	83.88	6.75	85.27	4.84	89.75	4.20
RSD%	12.95		10.79		9.43		7.00		5.75	

Figure: Typical total ion chromatography (TIC) of spiked pig liver samples at six concentrations: 0.5 μg/L, 1 μg/L, 2 μg/L, 5 μg/L, 10 μg/L and 100 μg/L: liver + 1 ppb (pcx)



Detection of Five Sulfonamides in Pork (Cleanert® SUL-5, P/N: SUL-5)

EMF10002

Material

SPE cartridge: Cleanert® SUL-5 (2g / 12mL)
Five sulfonamides: sulphadimidine (SM2), Sulfamonomethoxine (SMM), Sulfamethoxazolum (SMZ), Sulfadimoxine (SDM), and Sulfaquinoxaline (SQ).

Experiment

Spike sample was prepared by diluting 50 or 100 µL of working solution in 3.00 mL acetonitrile/water (95:5, v/v). Use the follow steps for the experiment:

(1) Add 5 mL acetonitrile/water (95:5, v/v) to activate the cartridge
(2) Load the sample (3 mL) onto the cartridge
(3) Wash the cartridge with 5 mL acetonitrile/water (95:5, v/v), and discard the eluate.
(4) Elute the cartridge with 10 mL acetonitrile/water (7:3, v/v), and collect the eluate.
(5) Inject 20 µL of the collected eluate to HPLC for analysis.

Cartridge batch	Drugs	Recovery (%)			Mean recovery (%)	RSD (%)
Batch 1	SM_2	94.37	96.10	95.19	95.22	0.91
	SMM	96.97	95.38	96.10	96.15	0.83
	SMZ	89.95	90.96	98.50	93.14	5.01
	SDM	94.66	98.15	95.08	95.96	1.99
	SQ	93.48	91.85	90.55	91.96	1.59
Batch 2	SM_2	94.18	95.49	101.13	96.93	3.81
	SMM	97.55	88.94	96.90	94.46	5.07
	SMZ	91.73	87.87	94.46	91.35	3.62
	SDM	87.22	99.60	97.87	94.90	7.06
	SQ	92.18	94.80	93.17	93.38	1.43
Batch 3	SM_2	95.08	94.76	94.52	94.79	0.30
	SMM	99.63	95.11	95.96	96.90	2.48
	SMZ	96.40	98.16	87.26	93.94	6.23
	SDM	96.79	96.66	94.55	96.00	1.31
	SQ	96.32	91.38	92.49	91.93	0.85

Sample spiked recovery.
Add 5 g (accurate to 0.01 g) of tissue sample to a centrifuge tube containing 10 g of anhydrous sodium sulfate. Add 25 mL of acetonitrile. Homogenize at 10000 r/min for 1 min and centrifugate at 3000 r/min for 5 min. Dissolve the residue in 25 mL of acetonitrile and centrifugate at 3000 r/min for 5 min. Combine the supernatants of two centrifugations. Add 30 mL of hexane and vortex mix for 10 min. Centrifugate at 3000 r/min for 5 min and discard the supernatant layer. Add 10 mL of n-propanol and evaporate to dryness under reduced pressure at the temperature below 50°C. Dissolve the residue in 3 mL of 95% acetonitrile and load onto basic alumina cartridge. Wash the cartridge with 5 mL of 95% acetonitrile and then elute with 10 mL of 70% acetonitrile. Collect the eluate for HPLC analysis.

Results

Cartridge retention test

SUL-5 retention test was conducted at two concentrations, 100 and 200 μg/Kg.The results are summarized in Tables 1 and 2. The mean recovery ranges of SM2, SMM, SMZ, SDM, and SQ are 92.47~99.37%, 93.69~99.44%, 88.61~96.27%, 90.87~96.06% and 91.83~95.92%, respectively. The intra-batch RSD of Cleanert cartridge is 0.30~9.38%, showing good stability.

Sample recovery

Table 3 shows the results of recovery test of spiked samples with fortified concentration 100 and 200 μg/Kg, respectively. 3. The mean recovery and intra-batch RSD for spiked pork samples (100 and 200 μg/kg) are 80.62~94.49% and 3.98~7.79%, respectively, indicating that the Cleanert Series SPE cartridge has high recovery and good stability.

Table 1 Cartridge retention recovery of Cleanert® SUL-5 cartridge (corresponding to 100 μg/kg sulfonamides in tissues)

Cartridge batch	Drugs	Recovery (%)			Mean recovery (%)	RSD (%)
Batch 1	SM_2	94.37	96.10	95.19	95.22	0.91
	SMM	96.97	95.38	96.10	96.15	0.83
	SMZ	89.95	90.96	98.50	93.14	5.01
	SDM	94.66	98.15	95.08	95.96	1.99
	SQ	93.48	91.85	90.55	91.96	1.59
Batch 2	SM_2	94.18	95.49	101.13	96.93	3.81
	SMM	97.55	88.94	96.90	94.46	5.07
	SMZ	91.73	87.87	94.46	91.35	3.62
	SDM	87.22	99.60	97.87	94.90	7.06
	SQ	92.18	94.80	93.17	93.38	1.43
Batch 3	SM_2	95.08	94.76	94.52	94.79	0.30
	SMM	99.63	95.11	95.96	96.90	2.48
	SMZ	96.40	98.16	87.26	93.94	6.23
	SDM	96.79	96.66	94.55	96.00	1.31
	SQ	96.32	91.38	92.49	91.93	0.85

Table 2 Cartridge retention test recovery of Cleanert® SUL-5 cartridge (corresponding to 200 μg/kg sulfonamides in tissues)

Cartridge batch	Drugs	Recovery (%)			Mean recovery (%)	RSD (%)
Batch 3	SM_2	99.66	99.26	98.75	99.22	0.46
	SMM	97.46	100.25	96.37	98.03	2.04
	SMZ	96.35	94.42	97.06	95.94	1.42
	SDM	94.97	94.86	98.34	96.06	2.06
	SQ	98.22	91.96	97.59	95.92	3.59
Batch 4	SM_2	100.45	85.90	91.06	92.47	7.97
	SMM	94.67	88.58	97.83	93.69	5.02
	SMZ	95.96	81.25	88.63	88.61	8.30
	SDM	99.83	82.87	89.90	90.87	9.38
	SQ	96.21	87.89	91.40	91.83	4.54
Batch 5	SM_2	98.69	100.31	99.11	99.37	0.85
	SMM	101.51	100.78	96.04	99.44	2.99
	SMZ	91.35	98.78	98.68	96.27	4.43
	SDM	92.75	97.29	97.88	95.97	2.92
	SQ	84.27	98.61	98.20	93.69	8.71

Table 3 Results of recovery test of spiked samples

Spiked concentration (μg/kg)	Drugs	Recovery (%)					Mean Recovery (%)	RSD (%)
100	SM2	93.39	97.95	98.87	90.33	98.27	94.49	4.83
	SMM	97.46	90.66	94.33	81.83	93.64	92.40	6.17
	SMZ	83.78	88.45	94.40	80.24	82.04	84.87	6.56
	SDM	91.26	96.75	91.79	87.57	85.88	90.28	4.30
	SQ	88.44	85.14	92.61	81.03	79.57	85.06	5.69
200	SM2	80.85	77.37	88.39	90.53	73.80	82.02	7.79
	SMM	91.79	88.87	96.23	92.96	87.01	90.70	3.98
	SMZ	79.60	77.27	87.34	86.98	75.44	81.52	6.09
	SDM	84.31	72.35	84.52	81.92	82.53	80.62	5.79
	SQ	91.93	81.86	91.83	87.89	86.17	87.76	4.33

Detection of Terramycin, Tetracycline and Aureomycin in Aquatic Products and Meat (Cleanert® PS, P/N: PS2003)

EMF10003

Material

Cleanert® PS, 200mg / 3mL

Experimental

Procedure

- To a 100 mL centrifuge tube, add 5 g homogenized sample
- Add 2 x 20 mL citric acid/EDTA solution to extract target compounds. Vibrate the mixture in a mechanical shaker for 15 minutes.
- Centrifuge the mixture for 10 minute at 4000 rpm.
- Transfer the supernatant into a separating funnel, and add 20 mL hexane (to remove fat), and the shake the mixture for 5 minutes.
- Transfer the aqueous substratum into a sample flask for SPE cleanup.

Note: Citric acid/EDTA solution was prepared by dissolving 1.86 g EDTA-2Na into a mixture of 307 mL 0.1 M acid buffer solution and 193 mL 0.5 M disodium hydrogen phosphate solution.

Cleanert® PS Procedure

- Activate the PS cartridge by sequentially adding 10 mL methanol, 10 mL water and 5 mL EDTA-2Na solution.
- Load sample solution prepared from above procedure; and then wash the cartridge with 10 mL water, and discard the eluate.
- Dry the cartridge via positive pressure.
- Elute the cartridge with 10 mL methanol and collect the eluate.

Sample concentration and reconstitution

- Evaporate the sample eluate under rotovap to nearly dryness.
- Reconstitute the sample residue with 1 mL HPLC mobile phase.
- Filter sample through 0.45 um membrane before HPLC analysis.

Condition of HPLC
Column: Unisol C18, 5 μm, 4.6 × 250 mm
Mobile phase: A:B = 77:23

A: Iminazole buffer solution—dissolve 68.08 g of iminazole, 10.72 g of magnesium acetate and 0.37 g of EDTA2Na in 100 mL of water; modify pH to 7.2 with glacial acetic acid; bring the solution to 1000 mL with water.

B: Acetonitrile

Results

Compound	Recovery-1	Recovery-2
Terramycin	128%	137%
Tetracycline	91.8%	97.4%
Aureomycin	88.5%	87.1%
Doxycycline	97.3%	95.3%

Detection of Tetracycline in Honey (Cleanert® PEP, Cleanert® COOH, P/N: PE5006, CH5003)

EMF10004

Material

Cleanert® PEP, 500mg / 6mL
Cleanert® COOH, 500mg / 3mL

Experimental

Sample preparation

Dissolve 6.00 g honey sample in 30 mL extract solution; Vortex the mixture until the solution is clear; then the sample is ready for cleanup.
(Extract solution: Dissolve 10.5 citric acid and 8.88 g disodium hydrogen phosphate and 30.3 g EDTA-2Na in ca. 800 mL water; adjust pH to 4.0 by adding 2 M HCl and bring up total volume to 1000 mL with additional water if needed.)

Cleanert® PEP and COOH cleanup

(1) Cleanert® PEP (500mg / 6mL)
- Add 5 mL methanol and then 5 mL water
- Load sample solution and then wash the cartdrige with 5 mL methanol/water (5:95, v/v)
- Dry the cartridge under positive pressure

(2) Cleanert® COOH (500mg / 3mL)
Condition: 5 mL of ethyl acetate

(3) Cleanert® PEP (top) – Cleanert® COOH (down) in series
Washing: ethyl acetate (15 mL), remove Cleanert® PEP cartridge, dry Cleanert® COOH cartridge (5 min)
Elution: mobile phase (0.01 M oxalic acid: acetonitrile: methanol = 350:100:50) 4.5 mL
Dilute the eluate with 0.01 M oxalic acid in volumetric flask to 5 mL. Shake the volumetric flask to obtain homogeneous solution. Filter the solution before analyzed by UPLC.

Detection of Chloramphenicol Residue in Aquatic Products by Gas Chromatography (Cleanert® C18, P/N: S180006)

EMF10005

Material

Cleanert® C18, 1g / 6mL
DA-5, 30 m×0.53 mm×1.5 μm, P/N: 0153-3015.

Experimental

Sample preparation

For Fish: remove scales and skins, take the back muscles.
For Shrimp and Crab: remove the head, shell and limbs, take the eatable muscles.
Cut the muscle samples into small pieces (no bigger than 0.5 cm×0.5 cm×0.5 cm) and mix. Freeze in fridge for later use.

Extraction

Put 5.00 g fresh or fully defrozen sample into a glass centrifuge tube, and then add 20 mL ethyl acetate. Homogenize the mixture for 1 minute to extract target compound. Centrifuge the mixture at 4000 rpm for 3 minutes, and then tranfer ethyl acetate layer to a 100 mL pear-shaped flask. Add 10 mL ethyl acetate to the sample sediment to extract one more time following the previous procedure. Evaporate the combined extract to dryness under a rotovap at 40 Celsius.

Degreasing

- Add 1 mL methanol to reconstitute sample in the pear-shaped bottle from last step.
- Add 2 × 15 mL n-hexane and 2 × 25 mL 4% NaCl solution into the sample to remove lipids. For each replicate, first vortex the mixture for 1 minute followed by 2 minute centrifuging at 4000 rpm. Discard the hexane layer; and then combine the aqueous layer for next step operation.
- Add 2 × 15 mL ethyl acetate to the aqueous layer; then vortex the mixture for 2 minutes; and then centrifuge the mixture at 3000 rpm for 3 minutes.
- Collect the ethyl acetate layer; and pass the solution through a bed of sodium sulfate anhydrous to remove water content.
- Rotovap the solution to nearly dryness at 40 Celsius.
- Dissolve the sample residue in 2 mL ethyl acetate for SPE procedure.

Cleanert® C18 (1g / 6mL) Procedure

- Sequentially add 5 mL each of methanol, chloroform, methanol and water, respectively, to activate and to condition C18 cartridge.
- Load the sample on the C18 cartridge; and adjust the elution speed to below 1 mL/min.
- Wash the cartridge with 6 mL water and discard eluate.
- Dry the cartridge under positive pressure.
- Elute the cartridge with 5 mL acetonitrile and collect the eluate.
- Evaporate the sample solution under nitrogen to nearly dryness in a 50 Celsium sand bath.
- Rinse the sample vail with 1 mL ethylacetate; and then evaporate the solvent again under nitrogen to dryness.

Derivatization

Add 100 µL of derivatization reagent (N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) /trimethylchloro-silane (TMCS), 99/1, v/v) to the dried residue. Cap the tube and vortex and mix for 10 s. Allow to react in oven at 70 for 30 min. Remove the excess reagent under nitrogen stream in sand bath at 50~55°C until the tube is just dried. (Note: Too long time of evaporation may result in loss of analytes.) Add 0.5 mL of n-hexane, vortex and mix for 10 s for the analysis of GC (DA-5, 30 m×0.53 mm×1.5 µm, P/N: 0153-3015.)

Detection of Nitrofuran Residues in dairy and meat products (Cleanert® PEP, P/N: PE0603)

EMF10006

Material

Cleanert® SPE Cartridge: PEP (60mg / 3mL)

Experimental

Sample preparation

(1) Treatment of milk powder and milk

1) Sample treatment: add 15 mL of solution mixed by trichloroacetic acid and water (2 and 15 mol) in milk powder of 1 g (milk of 5 g); add the internal standard and mixed standard; hydrolyze in water bath at 37.5°C for 5 h; centifuge at 4000 rpm for 5 min and then get the supernatant for usage.

2) Initial cleanup and derivation of the sample: activate the Cleanert® PEP cartridge with 5 mL of methanol and 5 mL of water; pass the treated supernatant in SPE cartridge and wash the cartridge with 5 mL of trichloroacetic acid; collect the solution into another test tube; derive the derivating agent of 100 μL (dissolve 20 mg 2-nitrobenzaldehyde in dimethylsulfoxide of 1 mL) at 37.5°C for 16 h in the water bath (overnight).

(2) Treatment of the pork, beef, chicken, pork liver, aquatic product and honey sample.

1) Bulk processing of samples: add 15 mL of mixed solution of methanol and water in 2 g of pork, beef, chicken, pork liver and aquatic product sample respectively (5 g of honey) and vortex; centrifuge at 4000 rpm for 5 min and add the interior label and mixed label into the supernate.

2) Deriving add 1 mL of derivating agent (dissolve 20 mg of 2-nitrobenzaldehyde in 1 mL dimethylsulfoxide) into the supernate; derivate the solution in the water bath of 37.5°C for 16 h (overnight); add dipotassium phosphate of 5 mL (pH = 7.4) and centrifuge at 4000 rpm for 10 min. (add 5 mL of n-haxane to the supernate if there is too much fat in the sample; adsorb and remove n-haxane by vibration for 2 min and centrifuging at 4000 rpm for 10 min).

Cleanert® PEP Cleanup

(1) Activation: activate the cartridge with 5 mL of methanol and 5 mL of water.

(2) Sample loading: add the buffer solution of dipotassium phosphate 5 mL in the derivative solution; adjust the pH to 7.4 with 1 mol/L of sodium hydroxide solution; centrifuge at 4000 r/min for 10 min; keep the supernate (add 5 mL of n-haxane to the supernate if there is too much fat in the sample; absorb and remove n-haxane by vibration for 2 min and centrifuging at 4000 rpm for 10 min) going through the PEP cartridge with the flow rate less than 2 mL/min.

(3) Washing: elute SPE cartridge with 10 mL of water and remove all outflow; dry SPE cartridge of PEP for 15 min under the negative pressure of 65 kPa with the vaccum pump.

(4) Elution: elute the tested sample with 5 mL of ethylacetate into 25 mL-brown centrifuge tube.

(5) Concentration: dry the eluate with nitrogen at 40°C; dissolve the solution and fix the volume to 1.0 mL with the sample of constant volume solution; filter through the filter membrane of 0.2 μm by LC-MS after mixing.

Detection of 19 Quinolone Residues in Honey by LC-MS/MS (Cleanert® PAX, P/N: AX0603)

EMF10007

Material

(1) Quinolone standards: Enrofloxacin (ENR), Ciprofloxacin (CIP), Norfloxacin (NOR), Ofloxacin (OFL), Flumequine (FLU), Oxolinic acid (OXO), Difloxacin HCl (DIF), Sarafloxacin HCl (SAR), Sparfloxacin (SPA), Danofloxacin (DAN), Fleroxcain (FLE), Marbofloxacin (MAR), Enoxacin (ENO), Orbifloxacin (ORB), Pipemidic acid (PIP), Pefloxacin (PEF), Lomefloxacin (LOM), Cinoxacin (CIN), Nalidixic acid (NAL). The purities of all above standards are all ≥99%.
(2) SPE cartridge: Cleanert® PAX, 60mg / 3mL.
(3) Internal standard stock solution of deuterated Norfloxacin (NOR-D5): dissolve proper amount of NOR-D5 standard in methanol to obtain internal standard stock solution of 100 μg/mL. Dilute proper amount of the internal standard stock solution with methanol to obtain working solution at the concentration of 1 μg/mL and store at 4°C.

Experimental

Cleanert® PAX Procedure

Weigh 5 g (accurate to 0.01 g) of sample and add to a 50 mL of centrifuge tube with stopper. Add 50 μL of 1 μg/mL internal standard solution and 5 mL of 0.1 mol/L sodium hydroxide solution. Vortex mix to dissolve the honey thoroughly. Activate Cleanert® PAX SPE mini-cartridge with 5 mL of methanol followed by 3 mL of water. Load the sample solution onto the mini-column. Wash the cartridge sequentially with water and methanol. Elute with 3 mL of methanol containing 5% formic acid. Collect the eluate and evaporate to dryness by rotary evaporator in water bath at 40°C. Dilute to 1.0 mL with 20% methanol in water. Filter through 0.45 μm membrane to sample vial for LC-MS analysis.

Determination

(1) LC conditions:
a) Column: Unisol C18, 3 μm, 150 mm × 2.0 mm i.d or equivalent;
b) Mobile phase: methanol + water containing 0.1% formic acid;
c) Flow rate: 0.20 mL/min;
d) Gradient elution procedure: (omitted);
e) Temperature: room temperature;
f) Injection volume: 25 μL;
(2) MS methods: (omitted)
Please download the details at the website of Bonna-Agela: www.bonnaagela.com

Detection of Nitroimidazole Drugs and Metabolites Residues in Royal Jelly Honey with LC-MS/MS (Cleanert® PAX, P/N: AX0603)

EMF10008

Material

(1) Standards: Metronidazole (MNZ), Dimetridazole (DMZ) and related metabolite-2 - hydroxymethyl--1 - methyl-5–nitroimidazole (HMMNI), Ipronidazole (IPZ) and related metabolite-2-(2-hydroxy isopropyl)-1-methyl-5 –nitroimidazole (IPZOH), Ronidazole (RNZ). The purities of all above standards are all ≥99%.

(2) SPE cartridge: Cleanert® PAX, 60mg / 3mL

(3) Preparation of Internal standard solution of deuterated Norfloxacin (NOR-D5): dissolve proper amount of NOR-D5 standard in MeOH to obtain internal standard solution at 100 μg/mL. Dilute proper amount of the internal standard solution with MeOH to a concentration of 1 μg/mL and store at 4°C.

Experimental

Sample Extraction and Cleanert® PAX Cleanup

Add 5 g (accurate to 0.01 g) of sample to a 50 mL centrifuge tube with cap. Add 50 μL of mixture of three internal standards (1.3) and 10 mL of 0.5 mol/L sodium hydroxide solution. Mix for 15 s to dissolve the sample. Add 10 mL of ethyl acetate and mix for 30 s. Centrifugate at 2500 r/min for 3 min. Transfer the supernatant ethyl acetate layer to a 50 mL glass test tube. Add 10 mL of ethyl acetate again and repeat the extraction procedures. Combine the ethyl acetates and evaporate to dryness by rotary evaporator in water bath at 40°C. Dissolve the residue with 5 mL of acetonitrile containing 10% formic acid. Activate the SPE cartridge with 3 mL of methanol and 3 mL of water. Load the sample onto the catridge. Wash the cartridge with 3 mL of water and draw almost to dryness. Elute with 3 mL of methanol containing 5% ammonia. Collect the eluate and evaporate to dryness by rotary evaporator in water bath at 45°C. Dilute with 1.0 mL of water containing 20% methanol. Filter through membrane to sample vial for LC-MS/MS analysis.

Determination

(1) Reference conditions for LC analysis;

a) Column: C18 (end capped), 3 μm, 150 mm × 2.0 mm i.d or equivalent;

b) Mobile phase: methanol (A) + 5 mmol/L ammonium acetate(B);

c) Flow rate: 0.20 mL/min;

d) Gradient elution procedure: (omitted);

e) Temperature: room temperature;

f) Injection volume: 25 μL;

Please download the details from the website of Bonna Agela: www.bonnaagela.com

Detection of Glucocorticoids Drugs Residues in Animal-derived Foods(Cleanert® Silica, P/N: SI5006)

EMF10009

Material

Cleanert® Silica, 500mg / 6mL

Experimental

Extraction

(1) Muscle tissue sample

Weigh 5 g (±0.05 g) of tissue sample in a 50 mL centrifuge tube. Add 30 mL of ethyl acetate and 10 g of anhydrous sodium sulfate. Homogenize for 1 min and vibrate on shaker at 200 r/min for 20 min. Centrifugate at 4200 r/min for 10 min and take the ethyl acetate layer. Add 25 mL of ethyl acetate to the residue. Homogenize for 1 min and vibrate on shaker at 200 r/min for 20 min. Centrifugate at 4200 r/min for 10 min and take the ethyl acetate layer. Combine the ethyl acetate layers and evaporate by rotary evaporator in water bath at 40°C almost to dryness. Dissolve the residue in 1 mL of ethyl acetate and 5 mL of n-hexane for cleanup.

(2) Bovine milk, egg sample

Weigh 5 g (±0.05 g) of bovine milk or egg sample in a 50 mL centrifuge tube. Add 30 mL of ethyl acetate. Homogenize for 1 min and vibrate on shaker at 200 r/min for 20 min. Centrifugate at 4200 r/min for 10 min and take the ethyl acetate layer. Add 25 mL of ethyl acetate to the residue. Homogenize for 1 min and vibrate on shaker at 200 r/min for 20 min. Centrifugate at 4200 r/min for 10 min and take the ethyl acetate layer. Combine the ethyl acetate layers and evaporate by rotary evaporator in water bath at 40°C almost to dryness. Dissolve the residue in 1 mL of ethyl acetate and 5 mL of n-hexane for cleanup.

Cleanert® Silica Cleanup

Load the extract onto silica cartridge activated with 6 mL of n-hexane. Wash the cartridge with 6 mL of n-hexane and dry it. Elute with 6 mL of n-hexane-acetone (6/4 v/v). Evaporate the eluate under a stream of nitrogen at 50°C to dryness. Dissolve the residue in 0.5 mL of water containing 20% acetonitrile and transfer to a 1.5 mL centrifuge tube. Centrifugate at 4200 r/min for 20 min. Filter supernatant layer through 0.22 μm membrane for HPLC-MS/MS analysis.

Detection of Zearanol in Animal-derived Foods (Cleanert® NH_2, PN: NH5006; PAX, P/N: AX1506)

EMF10010

Material

Cleanert® NH_2: 500mg / 6mL, Cleanert® PAX: 150mg / 6mL

Experimental

Muscle tissue sample

(1) Extraction: Weigh 5 g (±0.05 g) of tissue sample in a 50 mL centrifuge tube. Add 15 mL of methanol and vortex mix for 1 min. Centrifugate at 4000 r/min for 10 min and transfer the supernatant layer to another centrifuge tube. Extract again and combine the extracts. Add 20 mL of n-hexane and shake 20 times by hand. Centrifugate at 3000 r/min for 5 min and discard the n-hexane layer. Add 20 mL of n-hexane again to repeat the degreasing process. Transfer the underlayer to a 100 mL pear-shaped bottle and evaporate by rotary evaporator in water bath at 50°C almost to dryness. Add 5 mL of ethyl acetate and vortex mix for 1 min. Stand for 10 s and transfer the supernatant to the same centrifuge tube. Wash the pear shape tube once again with 10 mL of n-hexane. Combine the solutions for later use.

(2) Cleanert® NH_2 cleanup: load 2 g of anhydrous sodium sulfate on the NH_2 cartridge and knock to uniformity with a glass stick. Activate the cartridge with 5 mL of ethyl acetate followed by 5 mL of n-hexane. Load the prepared sample onto the cartridge. Wash sequentially with 5 mL of n-hexane and 5 mL of n-hexane-ethyl acetate (60/40 v/v). Elute sequentially with 4 mL of n-hexane-ethyl acetate (20/80 v/v) and 4 mL of ethyl acetate. Combine the eluates and dry under a stream of nitrogen at 50°C. add 0.5 mL of acetonitrile to the residue and vortex mix for 1 min. Add 0.5 mL of water and mix. Filter the solution through 0.2 μm organic membrane for LC-MS/MS analysis.

Liver tissue sample

(1) Weigh 5 g (±0.05 g) of sample in a 50 mL centrifuge tube. Add 15 mL of methanol and vortex mix for 1 min. Centrifugate at 4000 r/min for 5 min and transfer the supernatant layer to another centrifuge tube. Extract again and combine the two extracts. Add 10 mL of n-hexane and shake by hand. Centrifugate at 3000 r/min for 5 min and discard the supernatant n-hexane layer. Dry the underlayer under a stream of nitrogen at 50°C. Add 5 mL of n-hexane and vortex mix for 1 min. Add 20 mL of n-hexane and vortex mix for 30 s. Centrifugate at 4000 r/min for 5 min and take the supernatant for later use.

(2) Cleanup: load 2 g of anhydrous sodium sulfate on the NH_2 cartridge and knock to uniformity with glass stick. Load the prepared sample onto the cartridge activated with 5 mL of ethyl acetate followed by 5 mL of n-hexane. Wash sequentially with 5 mL of n-hexane and 5 mL of n-hexane-ethyl acetate (45/55 v/v). Elute sequentially with 5 mL of n-hexane-ethyl acetate (20/80 v/v) and 5 mL of ethyl acetate containing 2% methanol. Combine the eluates and dry under a stream of nitrogen at 50°C.Add 0.5 mL of acetonitrile to the residue and vortex mix for 1 min. Add 0.5 mL of water and mix. Add 2 mL of n-hexane and vortex mix for 30 s. Centrifugate at 9000 r/min for 5 min. Filter the underlayer through 0.2 μm organic membrane for LC-MS/MS analysis.

Bovine milk sample

(1) Extraction: add 5.0 mL of sample to a 50 mL centrifuge tube. Add 0.1 mL of 18% H_2SO_4 solution and vortex mix to uniformity. Stand for 10 min. Add 10 mL of n-hexane and 20 mL of acetonitrile and vortex mix at 300 r/min for 10 min. Centrifugate at 4000 r/min for 10 min and discard the n-hexane layer. Transfer 12.5 mL of the extract to a centrifuge tube. Evaporate under a stream of nitrogen at 50°C to less than 0.1 mL. Add 10 mL of water and adjust with 5 mol/L sodium hydroxide to pH=11. Centrifugate at 9000 r/min for 5 min for later use.

(2) Cleanert® PAX cleanup: activate and equilibrate Cleanert PAX SPE cartridge sequentially with 2 mL of methanol and 2 mL of water. Load the sample onto the cartridge. Wash sequentially with 1 mL of methanol-ammonia-water (5/5/90 v/v/v) and 0.5 mL of methanol. Elute with 4 mL of 2% ethyl acetate. Collect the eluate and dry under a stream of nitrogen at 50°C.add 0.5 mL of acetonitrile to the residue and vortex mix for 1 min. Add 0.5 mL of water and mix. Filter the solution through 0.2 μm organic membrane for LC-MS/MS analysis.

Egg sample

(1) Extraction: add 5 g (±0.05 g) of sample to a 50 mL centrifuge tube. Add 10 mL of acetonitrile and vortex mix for 1 min. Centrifugate at 9000 r/min for 5 min. Transfer the supernatant to another centrifuge tube. Extract again and combine the two extracts. Transfer 12.5 mL of the extract to a centrifuge tube. Evaporate under a stream of nitrogen at 50°C to less than 0.1 mL. Add 10mL of water and adjust pH = 11.0 to with 5 mol/L sodium hydroxide. Centrifugate at 9000 r/min for 5 min for later use.

(2) Cleanup: activate and equilibrate Cleanert® PAX SPE cartridge sequentially with 2 mL of methanol and 2 mL of water. Load the sample onto the cartridge. Wash sequentially with 1 mL of methanol-ammonia-water (5/5/90 v/v/v) and 0.5 mL of methanol. Elute with 4 mL of 2% ethyl acetate. Collect the eluate and dry under a stream of nitrogen at 50°C. Add 0.5 mL of acetonitrile to the residue and vortex mix for 1 min. Add 0.5 mL of water and mix. Filter the solution through 0.2 μm organic membrane for LC-MS/MS analysis.

Detection of β-estradiol Residues in Muscles of Fish and Shellfish with Deuterium Isotope by GC-MS (Cleanert® C18, P/N: S185003)

EMF10011

Material

Cleanert® C18, 500mg / 3mL

Experimental

Extraction

Muscle tissues of fish or shellfish are minced by domestic blender and stored in refrigerator at -18°C for later use. Transfer 5.00 g of sample, accurately weighed, into a 50 mL centrifuge tube. Add 100 µL of internal standard working solution and 5 mL of sodium acetate buffer. Homogenize the mixture at 18000 r/min twice, by homogenizer, each time for 30 s. Add 10 mL of acetonitrile and vortex mix for 1 min. Ultrasonic extract at room temperature for 15 min. Centrifugate the mixture at 10000 r/min, 4°C, for 10 min. Transfer the supernatant to another centrifuge tube. Add 10 mL of acetonitrile to the residue and repeat the extraction steps. Combine the supernatants.

Cleanert® C18 cleanup

Add 10 mL of n-hexane to the supernatant. Cap the tube and shake tempestuously for 1~2 min. Centrifugate at 1000 r/min at 4°C for 5 min. Discard the n-hexane layer and wash the underlayer with n-hexane again. Transfer the remaining solution to a pear-shaped bottle. Add 0.5 mL of n-propanol and evaporate to dryness by rotary evaporator in water bath at 45°C. Add 1 mL of acetonitrile to the residue and wash the bottle for 1 min. Transfer the solution to a 5 mL syringe. Repeat the extraction with 1 mL of acetonitrile and combine the solution to the syringe. Filter the solution through organic membrane. Dilute the filtered solution with water to 10 mL. Load the sample solution onto the C18 SPE cartridge (activated sequentially with 6 mL of methanol, 3 mL of 0.1% acetic acid and 3 mL of water) at the flow rate of 1~2 mL/min. Wash the C18 cartridge with 3 mL of water then elute the cartridge with 9 mL of acetonitrile. Collect the eluate and dry under nitrogen stream.

Derivatization

For sample,accurately add 100 µL of MSTFA2DTE2TM IS derivatization reagent to the residue. Cap the tube and vortex mix for 1 min. Allow to react in oven at 60°C for 30 min and then cool to room temperature. Analyze the sample by GC-MS within 48 h. For standard solution, β-estradiol and internal standard working solution should be added to a tube in which the sample deactivation is conducted.
(Agilent, U.S.) then mix the solution with vortex and dry under nitrogen stream before derivatization as above.

GC/ MS conditions

DA-5MS capillary column (25 m × 0.32 mm × 0.52 µm);
column temperature: 120°C (2 min)---- 250°C (15°C/min),
300°C (5 min, 5°C/min);
Carrier gas: He (≥99. 999%);
Flow rate: 1. 0 mL /min;
Injector port temperature: 250°C;
Splitless injection volume: 1 µL;

EI source temperature: 230°C;
Quadrupole temperature: 150°C;
Interface temperature: 280°C;
Ionization voltage: 70 Ev;
Solvent detention time: 3 min;
Electron motiplier voltage: 1106 V;
Mass scan range: 40~500 amu.

The Detection of Sulfa Drug Residues in Pork (Cleanert® PCX 150mg / 6mL P/N: CX1506)

EMF10012

Abstract

In this study, solid phase extraction combined with liquid chromatography - tandem mass spectrometry (SPE - LC/MS/MS) method were used to established the detection method of 16 kinds of sulfa drugs under the multiple reaction monitoring (MRM) mode according to GB/T20759-2006 standard. The target object in the samples was extracted by ethyl acetate, purification by Cleanert® PCX solid-phase extraction column, separated by Venusil® ASB C18 HPLC Column (2.1×150 mm,3 μm, 150 Å) and quantitated by external standard method with water and acetonitrile as mobile phase in gradient elution. The results indicated that the sample amount of 10 ug/kg can meet the requirements for the detection with the recovery rate of each material between 70% and 110%.

Keywords

SPE-LC/MS/MS; Metronidazole;16 kinds of sulfa drugs; Cleanert® PCX SPE cartridge; Venusil® ASB C18 HPLC Column

Introduction

Sulfa drugs amino benzene sulfonamide (SAs) is the generic terms of the drugs with aminobenzene sulfonamide structure. They are commonly used broad-spectrum antibiotics with aromatic amino and sulfonamide and widely used in aquaculture. However, excessive use of these antibacterial agents can lead to drug residues in aquatic products, affecting human health. SAs can lead to the side effects such as micturition and hematopoietic disorders. SAs residues can lead to hemolytic anemia. And sulfadimidine even has the potential to cause cancer.

Experimen

Instruments, reagents and materials

(1) Main instruments and equipment
AB SCIEX API 4000+ HPLC-MS

(2) Reagents material
Methanol, acetonitrile, n-hexane, ethyl acetate are chromatography pure; ultrapure water; Sodium chloride, hydrochloric acid, ammonia are analytically pure; standard substance Sulfanilacetamide, Sulfachlorpyridazine, Sulfisoxazole, Sulfisoxazole, Sulfamethoxazole, Sulfamethizole, Benzenesulfonamide, Sulfapyridine, Sulfathiazole, Sulfamonomethoxine, Sulfadiazine, Sulfamerazine, Sulfamethazine, Sulfaquinoxaline, Sulfadoxine, Sulfamethoxypyridazine, Sulfameter, Sulfamonomethoxine and so on(Not less than 98% purity); 0.1mol/Lhydrochloric acid aqueous solution:add water to dilute 9 mL concentrated hydrochloric acid and capacity to 100 mL; Disposable sterilized syringe; Microporous membrane filter (0.22 μm, 13 mm in diameter); Cleanert® PCX SPE cartridge:150mg / 6mL

Sample preparation

(1) Sample extraction

Put (5±0.05)g samples to the centrifuge tube of 50 mL and then add 15 mL ethyl acetate vortex vibrate for 2 min. Centrifuge the samples for 10 min under 5000 rpm and separate the liquid supernatant in a 100 mL heart of bottles. Repeat extract the residue with the same method and combine the ethyl acetate layer.

Put 5 mL hydrochloric acid (0.1 mol/L) to the extracting solution above and steamed out of ethyl acetate under 45°C. Transfer rudimental hydrochloric acid layer to 10 mL centrifuge tube and wash heart of bottles with 2 mL hydrochloric acid (0.1 mol/L) twice. Transfer the cleaning mixture to the same centrifuge tube. Wash heart of bottles with 5 mL n-hexane and transfer the n-hexane to the centrifuge tube containing hydrochloric acid. Vibrations with the hand shake to make them mixed evenly and then centrifuge for 5min under 3500 rpm. Remove n-hexane and then washed with 3 mL n-hexane again. Take the subnatant as the target purifying liquid.

(2) Sample clean-up

Wash column of Cleanert® PCX (150mg / 6mL) with 3 mL methyl alcohol and 3 mL hydrochloric acid (0.1 mol/L). Transfer the target purifying liquid to the column and washed the column with 2 mL hydrochloric acid (0.1 mol/L) and 2 mL V(water):V(methyl alcohol):V(acetonitrile)=55:25:20. Finally elution with 2 mL V(water):V(methyl alcohol):V(acetonitrile)=:V(ammonia)=75:10:10:5, collect the eluent and dried with N2 under 45℃ . Capacity to 1 mL with water, filtered with 0.2 um organic membrane for future examination.

Experiment condition

(1) Liquid condition

HPLC Column: Venusil® ASB C18, 2.1×150 mm, 3 μm, 150 Å; Mobile phase: A - water, B - acetonitrile;
Column temperature: 35℃ ; Sample size: 5 μL;
Gradient elution: See table 1.

Table 1. HPLC gradient elution conditions

Time/min	Speed/(mL/min)	A/%	B/%
0.00	0.20	99.0	1.0
5.00	0.20	90.0	10.0
25.0	0.20	50.0	50.0
26.00	0.20	40.0	60.0
26.01	0.20	99.0	1.0
30.00	0.20	99.0	1.0

(2) Mass spectrometry conditions

Ion source: electrospray ion source
Scanning mode: positive ion scanning
Electrospray voltage: 5500 V
Atomization gas pressure: 65 psi
CUR: 12 psi
Aux Gas Pressure: 60 psi
Ion source temperature: 550℃
Acquisition methods: multiple reactions monitoring (MRM)
Q1 and Q3 are resolution ratio for the unit
Mass spectrum parameters (see table 2)

Compound	Q1	Q3	CE/V
Sulfanilacetamide	215.4	156.1	22
		108.2	30
Sulfachlorpyridazine	285.0	156.1	30
		108.2	44
Sulfisoxazole	254.2	156.1	30
		147.2	40
Sulfamethoxazole	268.0	156.1	22
		113.2	22
Sulfamethizole	271.6	156.1	24
		108.1	37
Benzenesulfonamide	315.1	156.1	22
		108.2	35
Sulfapyridine	249.9	156.1	30
		184.4	37
Sulfathiazole	255.8	156.1	25
		108.2	26
Sulfamonomethoxine	281.2	156.1	25
		215.4	23
Sulfadiazine	251.1	156.1	24
		108.2	20
Sulfamerazine	265.1	156.1	26
		172.2	20
Sulfamethazine	279.2	156.1	21
		186.1	35
Sulfadoxine	311.2	156.1	22
		108.2	33
Sulfamethoxypyridazine	281.2	156.1	25
		215.4	35
Sulfameter	281.4	156.1	25
		215.1	25
Sulfamonomethoxine	311.2	156.1	28
		108.2	28

Results and discussion

Experimental results

Table 3 illustrates that when using solid phase extraction combined with liquid chromatography - tandem mass spectrometry method to detection 16 kinds of sulfa drugs, the sample amount is 10 μg/kg and the recovery rate is 70%-110%, which can meet the requirements for the detection. Table 1 showed that processing with Cleanert PCX SPE cartridge and detection with Venusil® ASB C18 HPLC Column can make better separation for the 16 kinds of sulfa drugs. And each material peak shape is good, the retention time and the retention time is stable.

Table 3. The standard addition recovery experiment results of sulfonamides (n = 3)

Name of the substance	Add amount/(μg/kg)	Recovery rate /%	RSD//%	RT/min
Sulfanilacetamide	10	92.54%	3.87%	13.57
Sulfachlorpyridazine	10	73.08%	5.55%	21.46
Sulfisoxazole	10	81.48%	10.20%	23.52
Sulfamethoxazole	10	75.16%	14.18%	22.48
Sulfamethizole	10	92.56%	3.86%	19.15
Benzenesulfonamide	10	79.01%	11.72%	26.11
Sulfapyridine	10	87.80%	6.49%	16.45
Sulfathiazole	10	92.24%	4.04%	16.26
Sulfamonomethoxine	10	103.10%	1.53%	18.33
Sulfadiazine	10	96.21%	1.93%	15.22
Sulfamerazine	10	95.33%	2.39%	17.35
Sulfamethazine	10	106.93%	3.35%	18.94
Sulfadoxine	10	70.48%	7.31%	25.60
Sulfamethoxypyridazine	10	98.15%	0.93%	19.37
Sulfameter	10	99.94%	0.03%	19.37
Sulfamonomethoxine	10	88.43%	6.14%	20.84

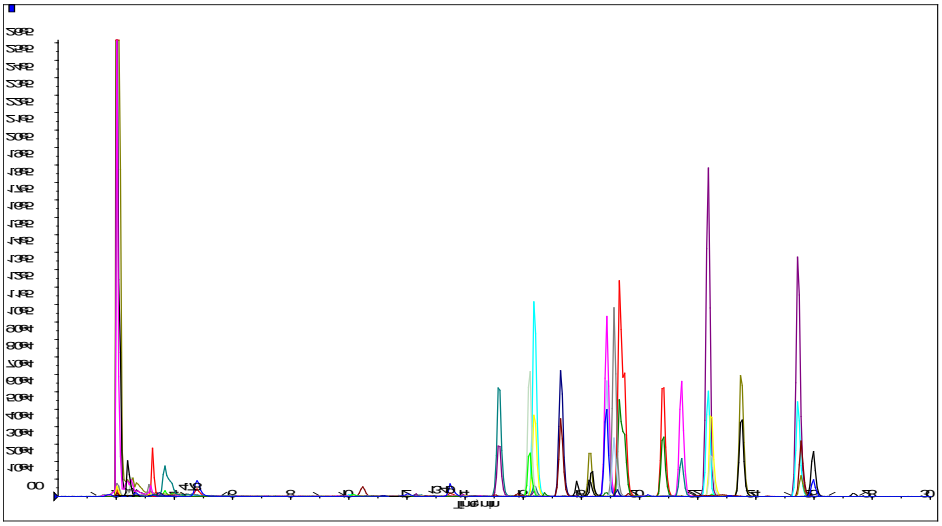


Fig 1. Liquid chromatography/tandem mass spectrometry mass spectrometry of sulfa samples with add amount of 10 μg/kg

Conclusion

We established the LC-MS/MS method for the sulfonamides residues detection. Combined with solid phase extraction techniques, the sulfa drugs residues in pork were detected. The recovery rate of the samples with the add scalar of 10 μg/kg is 70%-110%, which meet the requirement of national standard. Solid phase extraction method is stable and HPLC Column has good reproducibility, so this method can be used to detect sulfonamides residues in food of animal origin.

Ordering Information

Products	Specification	Cat.No.
Venusil ASB C18® column	3 μm, 150 Å; 2.1×150 mm	VS931502-0
Cleanert® PCX	150 mg/6 mL	CX1506
Guard cartridge holder	Suitable for 4.6×10 mm and 2.1×10 mm	SH-100
Direct-connection Guard Cartridge	5 μm, 100 Å; 4.6×10 mm	DN850105-0S
1.5 mL vial	Screw neck vial, graduated, 32×11.6 mm 9 mm center hole;	1109-0519
Cap for 1.5 mL vial	red silicone/ white PTFE septa, slitted 45` Shore A 1.0 mm	0915-1819
Micropore Membrane	Monofilm, 13 mm, 0.22 μm	AM021320
Syringe	2 mL Without Needle	LZSQ-2ML
Acetonitrile UV grade	4 L/bottle, chromatographic pure	015-4
Methanol	4 L/bottle, chromatographic pure	AH230-4

The Quantification of Fluoroquinolones Residues in Pork Using LC-MS/MS Method (Cleanert® PEP-2 60mg / 3mL P/N: PE0603-2)

EMF10013

Fluoroquinolones, also called the pyridine acid class, are a series of new synthetic sterilization antimicrobial drugs in recent years. Because of its broad antimicrobial spectrum, strong antibacterial activity, and no cross with other antibacterial drugs and small side effects, it is widely used in the prevention of various infectious diseases of animal and human. Besides the side effects of fluoroquinolone residues can cause harm to human directly, the more serious is that long-term consumption of animal source foods containing low concentration of FQs drugs may easily induce human disease to become bacterial resistant and may have the potential teratogenic and carcinogenic risk.China and EU stipulate the maximum residue levels for the series of quinolones is 10~90 μg/kg in animal tissues.

Sample extraction

Weigh 5.0 g (accurate to 0.01 g) homogenized sample, then put it into 50.0 mL centrifuge tube, and 20 mL 0.1 mol/L buffer solution was added. Mixed it for 1 min at 1000 r/min speed and ultrosonic extraction for 10 min then centrifuged for 10 min at 8000 r/min speed. Repeat the same process for three times and finally combined the supernatant for detection.

Purification

After activating the Cleanert® PEP-2 cartridge using 6.0 mL methanol and 6mL water in sequence, the purifying liquid was loaded on the column, and flowed through the column at the speed of 2 to 3 mL/min. Then put away the filtrate.The SPE columnwas washed using 3mL methanol and put away the waste before drying it in negative pressure. At last, the sample was eluted using 6 mL methanol into a pipe then further dried it at 45℃ in nitrogen. As the last step, the residues were dissolved by 1 mL 0.2 % formic acid solution and filtered (0.22 μm) in order to detection.

Analytical method

(1) Chromatographic conditions

Column: Unisol C18, 2.1×50 mm, 5 μm, 100 Å;
Mobile phase: A: 0.1 % formic acid and water, B: acetonitrile;
Column Temperature: 30℃ ;
Injection Volume: 10 μL;
Gradient elution is shown in table 1

Table 1 Gradient elution conditions of HPLC chromatography

Time (min)	Flow (mL/min)	A%	B%
0.00	0.3	85	15
6.00	0.3	80	20
7.00	0.3	10	90
7.10	0.3	85	15
12.00	0.3	85	15

Table 2 Mass spectrum parameters of fluoroquinolones

Compound	Q1	Q3	CE/V
Norfloxacin	320.3	302.3	25
		276.3	25
Ciprofloxacin	332.2	314.3	23
		288.3	23
Pefloxacin	334.3	290.3	29
		233.2	29
Lomefloxacin	352.3	265.2	27
		308.3	27
Enrofloxacin	360.3	316.4	27
		342.3	27
Ofloxacin	362.2	318.3	27
		261.2	27

Results

The standards ofdifferent drugs were added in samples at 2.0 μg/kg, 20 μg/kg and 100 μg/kg followed by detection using SPE-HPLC-MS/MS.As showed in table 3, the spiked recoveries were in the range of 70%-120%. As showed in fig.1to fig.3, the peak shape of six different drugs was satisfactory and its retention time was stable after purification and separation by Cleanert® PEP-2 SPE cartridge and Unisol C18 column.

Table 3 The spike recoveries and retention time for fluoroquinolones(n = 3)

Compound	Adding standard(mg/kg)	Recovery (%)	RSD (%)	RT/min
Norfloxacin	2.0	78.77	0.15	3.26
	20	84.95	0.12	
	100	100.34	0.03	
Ciprofloxacin	2.0	87.98	0.08	3.79
	20	71.63	0.11	
	100	79.66	0.01	
Pefloxacin	2.0	86.21	0.08	3.57
	20	83.65	0.15	
	100	92.67	0.18	
Lomefloxacin	2.0	101.39	0.07	4.34
	20	92.97	0.12	
	100	108.73	0.04	
Enrofloxacin	2.0	94.27	0.03	5.02
	20	88.12	0.16	
	100	93.05	0.05	
Ofloxacin	2.0	102.73	0.08	3.23
	20	103.91	0.15	
	100	114.35	0.12	

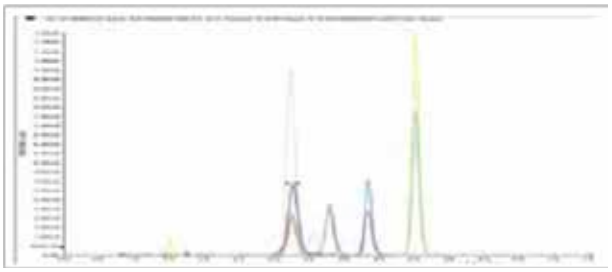


Fig.1 the HPLC-MS/MS chromatogram of fluoroquinolones(adding standards 2.0 μg/kg)

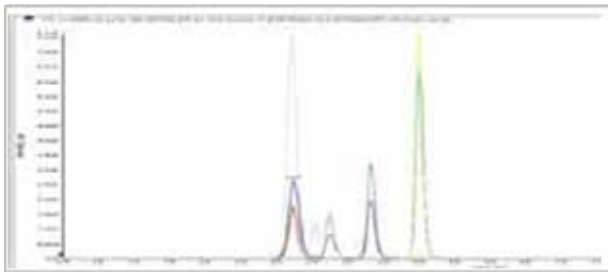


Fig.2the HPLC-MS/MS chromatogram of fluoroquinolones(adding standards 20 μg/kg)

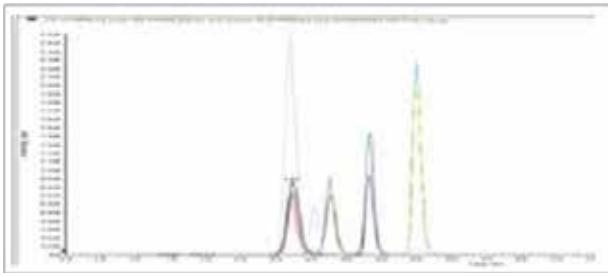


Fig.3the HPLC-MS/MS chromatogram of fluoroquinolones(adding standards 100 μg/kg)

Conclusion

This study developed a LC-MS/MS method for the detection offluoroquinolones. Combined with solid phase extraction, it also achieved quantification of fluoroquinolonesresidues in pork. With this method, 2.0 μg/kg, 20 μg/kg and 100 μzg/kgspiked samples could be directly analyzed, and the spiked recoveries were in the range of 70%-120%, which meet the standards of GB. Solid phase extraction method showed good stability and the columns showed excellent reproducibility, which pointed out that the method could be used for the quantification of fluoroquinolonesresidues in animal derived food.

Ordering Information

Products	Specification	Cat.No.
Unisol C18	2.1×50 mm, 5 μm, 100 Å	VA950502-0
Cleanert® PEP-2	200mg / 6mL,30/pk	PE2006-2
Qdaura® Automatic SPE Workstation	4 channel, 24 position	SPE-40
Guard Column Holder	For 4.6×10 mm and 2.1×10 mm	SH-100
Direct-connected Guard Column	5 μm,100 Å, 2.1×10 mm	VA950102-0S
1.5mL Vial	Short-thread and transparent, 32×11.6 mm, 100/pk	1109-0519
1.5mL Vial Cap	9mm center bore, blue cap, red rubber/beige PTFE pad, 45. Shore A 1.0 mm, 100/pk	0915-1819
Syringe Filter (Nylon)	0.22 μm, diameter 13 mm, 200/pk	AS021320
Disposable Syringe	2 mL, 100/pk	LZSQ-2ML
Acetonitrile (UV)	4 L/bottle, chromatographic grade	015-4
Methanol	4 L/bottle, chromatographic grade	AH230-4

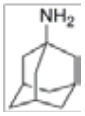
The Detection of 1-Aminoadamantane in Meat and Tissues Food with SPE-LC/MS/MS Method (Cleanert® PCX 60mg / 3mL P/N: CX0603)

EMF10014

The synthesis of amantadine begins with halogenation of adamantane and bromine, and then the intermediate products react with acetonitrile in sulfuric acid to get amide. After base catalyzed hydrolysis, amantadine can be got. Amantadine can be used for the prevention and early treatment of type A-influenza in Asian. Combined with antibiotics, it's useful to the treatment of septicemia and viral pneumonia, and it is effective to bring down a fever.It is also effective against quiver paralysis, thus it can be used for the treatment of parkinsonism. Based on its pharmacological action, amantadine is mainly used for the prevention and early treatment of chicken or swine flu, and the prevention of swine transmissible gastroenteritis in China.

In consideration of the residue, amantadine has been forbidden from prevention and treatment of viral disease caused by pathogenic microorganisms such as highly pathogenic avian influenza. There is no report before about the residual quantity of amantadine in animal derived food in China, and no national standard or industrial standard has been made before, even certain request to residues or detection method has not been ever raised internationally.

This study developed new method to quantify residues of amantadine in animal derived food using methanol-1%trichloroacetic acid extraction, Cleanert® PCX solid phase extraction, and LC-MS/MS technology.

Compound	CAS	Structure
1-Aminoadamantane	768-94-5	

Sample Extraction

Weigh accurately 2 g (accurate to 0.01 g) sample in 50mL centrifuge tube, and added 10 mL methanol 1% trichloroacetic acid (1:1,v/v) mixture in it, swirl 30 s, then ultrasound for 30 min, centrifuge for 10min at 8000 r/min speed. Got the supernate for further filtration, then transfered it using syringe into a sample tube of Qdaura® SPE Workstation, and Cleanert® PCX SPE cartridge was placed into the tube as well waiting for purify.

Purification

Set the program of Qdaura® fully automated SPE instrument as below: activate the column using 3mL methanol and 3mL water in sequence, after loading 5mL sample, wash it using 3mL 2% hydrochloric acid,and 3mL methanol in turn. Afterblow-drying the column in air, eluted the sample using 5mL solution mixed with ammonia, methanol, and isopropyl alcohol (5+80+15, v/v/v), and collected the eluent. All the programs above were operated at the flow rate 1mL/min.

Put the collection tube in a water baths at 50°C, and dried it in nitrogen. Followed by filtration(0.22 μm), it was dissolved in 1mL solution mixed with methanol, water, and formic acid (10+90+0.1, v/v/v), and then detected it using LC-MS/MS.

AnalyticalMethod

(1) Chromatographic conditions:

Column: Venusil® ASB C18, 2.1×150 mm, 3 μm, 150 Å;
Flow Rate: 200 μL/min;
Column Temperature: 30℃ ;
InjectionVolume: 5 μL;

Table 1 Mobile phase conditions of HPLC chromatography

Total time(min)	Flow Rate (μL/min)	0.1%formic acid–water	Acetonitrile(%)
0	200	70	30
10	200	70	30

(2) Mass spectrum conditions:

Ionization mode: ESI in positive mode;
Detection mode: multiple – reaction monitoring(MRM);
Ion source temperature: 550℃ , Curtian Gas: 10, Ion Source Gas 1: 70, Ion Source Gas 2:75;

Table 2 Mass spectrum parameters

compound	Retention time(min)	Q1	Q3	Decluster Potentia	Collision Energy
1-Aminoadamantane	6.88	152.2	134.9	77	24
			92.9	87	36
			78.9	78	40

"_" stands for ion detected for quantify.

Results

Table 3 Linearity and detection limit

Compound	Retention time(min)	Average recovery	RSD%
1-Aminoadamantane	6.8	79.8%	8.37

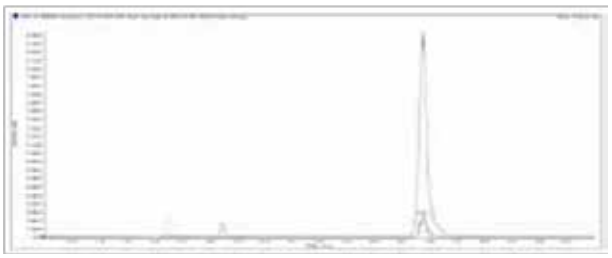


Fig.1 Standard mass spectrogram of 1-Aminoadamantane

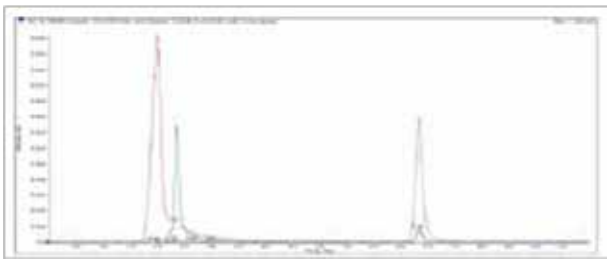


Fig.2 Mass spectrogram of chicken sample added 1-Aminoadamantane(concentration was 50 μg/mL)

Ordering Information

Products	Specification	Cat.No.
Qdaura®Automated SPE Workstation	4 channel, 24 position	SPE-40
Cleanert®PCX	60mg / 3mL, 50/pk	CX0603
Venusil®ASB C18	2.1×150 mm, 3 μm, 150 Å	VS931502-0
Syringe Filter(PTFE, prefilter, double membrane)	1μm, diameter 25 mm, 100/pk	AS082501-G
Syringe Filter (Nylon)	0.22μm, diameter 13mm, 200/pk	AS021320
Disposable Needle-Free injection systems	5 mL,100/pk	ZSQ-5ML
1.5mL vial	Short-thread and transparent,32×11.6 mm, 100/pk	1109-0519
1.5mL vial cap	9mm center bore, blue cap, red rubber/beige PTFE pad, 45.Shore A1.0 mm,100/pk	0915-1819
Isopropyl alcohol	4 x 4 L/bottle	AH232-4
Methanol	4 x 4 L/bottle	AH230-4

The Quantification of Lincomycin Residues in Pork Using LC-MS/MS Method (Cleanert® PEP-2 500 mg/6 mL P/N: PE5006-2)

EMF10015

Lincomycin, also called lincomycin , is mainly obtained by fermentation of streptomyces. It is often used in the 50S subunit of the bacterial ribosome, inhibiting bacterial protein synthesis by extending the inhibitory peptide. Then it removes the bacterial surface protein A and fluffy coat and makes it easily devoured and killed.Lincomycin can enhance the immune regulation of the immune system, improve nuclear leukocyte phagocytosis and bactericidal function and change the bacterial surface activity, inhibit of bacterial toxins. It can be used in the treatment of gram positive bacteria and mycoplasma infection and it has a strong effect in many treatments especially has strong effect on Staphylococcus aureus, Streptococcus and it also has inhibitory effect on anaerobic bacteria.Used as a feed additive, it can promote the growth of broilers and pigs. Nowadays, people are concerned about the animal derived food safety problems and therefore,China and EU stipulate the maximum residue levels of lincomycin is 1 μ g/kg in animal derived food.

Sample extraction

After weighing 5.0 g (accurate to 0.01 g) homogenized drug, the sample was put into a 50.0 mL centrifuge tube that had been added in 15.0 mL acetonitrile. Then homogenized it with homogenizer for 1 min, and oscillated it for 10min before centrifuging for 5 min at 4200 r/min speed. The supernate was transferred to another 50 mL centrifuge tube and added 10 mL acetonitrile to the residues that still in the first tube, then took the same extraction programs again. After that, two tubes of supernate werecombined in a 50 mL centrifuge tube before adding 2 g NaCl and 10.0 mL n-hexane in it.Then oscillated the sample for 10min and centrifuged it for 10 min at 4200 r/min speed again. Then 10 mL liquid was absorbed from the middle acetonitrile layer into a centrifuge tube for concentration until nearly dry in 55°C water bath and nitrogen. As the last step, the dried residues was dissolved in 7 mL phosphate buffer in twice in order to be purified.

Purification

The Cleanert® PEP-2 (500mg / 6mL) cartridge was acticated using 10.0 mL methanol, 10.0 mL water, 5.0 mL 0.2% NaCl solution and 5.0 mL phosphate buffer solution in sequence. After that,the liquid sample to purify was loaded on the column (the flow rate was controlled at 1 mL/min).The SPE columnwas washed using 10.0 mL water and 5.0 mL methanol: water (2:3. v/v) in turn, then put away the waste and dried it using vacuum pump. The sample was eluted using 10.0 mL methanol into a pipe then further dried it at 45°C in nitrogen. As the last step, the residues were dissolved by 1 mL 10mmolacetic ammonia solution (contained 15%formic acid) andFilter (0.22 mm) in order to detection.

Analytical method

(1) Chromatographic conditions

Column: Venusil® ASB C18, 2.1×100 mm, 5 μm, 150 Å;
Mobile phase: A: 0.3% formic acid and water mixture, B: 0.3% formic acid and acetonitrile mixture;
Column Temperature: 35°C;
Injection Volume: 10 μL;
Gradient elution is shown in table 1

Table 1 Gradient elution conditions of HPLC chromatography

Time (min)	Flow (mL/min)	A%	B%
0.00	0.25	95	5
2.00	0.25	95	5
5.00	0.25	5	95
6.00	0.25	5	95
6.01	0.25	95	5
10.00	0.25	95	5

(2) Mass spectrum conditions:

Ion source: ESI
Scan mode: positive
Electrospray voltage: 5500 V
Atomizer pressure: 45 psi
Curtain Gas pressure: 15 psi
Aux Gas Pressure: 50 psi
Ion source temperature: 550°C
Detection mode: MRM
Mass spectrum parameters were shown in table 2.

Table 2 Mass spectrum parameters of lincomycin

Compound	Q1	Q3	CE/V
Lincomycin	407.8	126.0	45
		359.2	45

Results

The standards of Lincomycin were added in samples at 1.0 μg/kg followed by detection using SPE-HPLC-MS/MS. As showed in table 3, the spiked recoveries were in the range of 84%-105%, and the RSDs under 10% showed good reproducibility. As showed in fig.1 and fig.2, the peak shape of Lincomycinwas satisfactory and its retention time was stable after purification and separation by Cleanert® PEP-2 SPE column and Venusil® ASB C18 column.

Table 3 The spike recoveries and retention time for Lincomycin (n = 3)

Compound	Concentration(μg/kg)	Recovery (%)	RSD (%)	RT (min)
Lincomycin	1.0	90.92	5.34	1.63

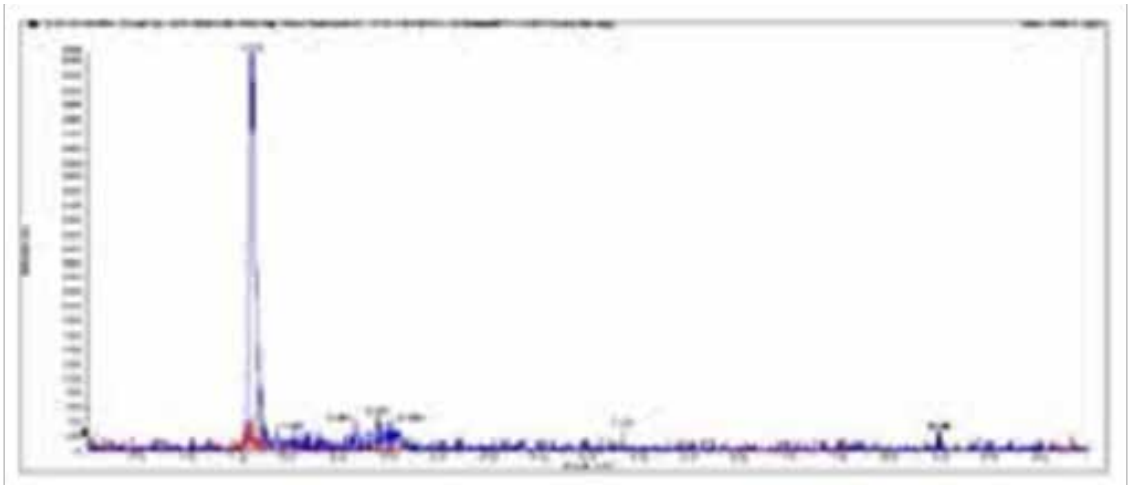


Fig.1 the HPLC-MS/MS chromatogram of Lincomycin(adding standards 1.0 μg/kg)

Conclusion

This study developed a LC-MS/MS method for the detection ofLincomycin. Combined with solid phase extraction, it also achieved quantification of Lincomycinresidues in pork. With this method, 1.0 μg/kg spiked samples could be directly analyzed, and the spiked recoveries were in the range of 84%-105%, which meet the standards of GB. Solid phase extraction method showed good stability and the columns showed excellent reproducibility, which pointed out that the method could be used for the quantification of Lincom ycin residues in animal derived food.

Ordering Information

Products	Specification	Cat.No.
Venusil® ASB C18	2.1×100 mm, 5 μm, 150 Å	VS951002-0
Cleanert® PEP-2	500mg / 6mL,30/pk	PE5006-2
Guard column sleeve	For 4.6×10 mm and 2.1×10 mm	SH-100
Direct-coupled protect column core	5 μm,150 Å,2.1×10 mm	VS950102-0S
1.5 mL vial	Short-thread and transparent, 32×11.6 mm, 100/pk	1109-0519
1.5 mL vial cap	9mm center bore, blue cap, red rubber/beige PTFE pad, 45. Shore A 1.0 mm, 100/pk	0915-1819
Syringe Filter (Nylon)	0.22 μm, diameter 13 mm, 200/pk	AS021320
Disposable Needle-Free injection systems	2 mL, 100/pk	LZSQ-2ML
Acetonitrile (UV)	4 L/bottle, chromatographic grade	015-4
n-hexane HPLC	4 L/bottle, chromatographic grade	AH216-4
Methanol	4 L/bottle, chromatographic grade	AH230-4

The Quantification of Tylosin Residues in Pork using LC-MS/MS Method (Cleanert® PEP-2 500mg / 6mL P/N: PE5006-2)

EMF10016

Tylosin, also known as Tai, tylosin, is a macrolide antibiotic which is obtained from Streptomyces fradiae culturemedium in USA in 1959. It has the specific effects on the mycoplasma and it not only has the very strong antibacterial effects on a variety of G+ bacteria, but also has an inhibitory effect on the part of the G- bacterium, campylobacter, spiral and coccidiosis. It exists in the form of tartaric acid salt, phosphate, chloride, sulfate and lactate and it is soluble in water. It is widely used in the prevention and control of animal husbandry and feed additive. In 2006 the EU Member States fully ban the use of antibiotics as growth promoters. The EU provide the standard that the highest residue of tylosin is 100 μg/kg in the meat. In 2002 China's Ministry of agriculture announced "the highest on veterinary drug residues in animal food notice", it said that the maximum residue levels of tylosin is 200 μg/kg in muscle, fat, liver, kidney of chicken, pigs, cattle.

Sample Extraction

After weighing 5.0 g (accurate to 0.01g) homogenized pork, the sample was put into a 50.0 mL centrifuge tube and then added in 20.0 mL acetonitrile. Then homogenized it for 1 min, and oscillated it for 10min before centrifuging for 5 min at 4200 r/min speed. The supernate was transferred to another 50 mL centrifuge tube and added 20 mL acetonitrile to the residues that still in the first tube, then took the same extraction programs again. After that, two tubes of supernate were combined in a 50 mL centrifuge tube and diluted with acetonitrile to 50 mL. Then 10 mL liquid was transferred out and added in 30 mL n-hexane. Put away the n-hexane after oscillating for 2 min and centrifugation for 5 min at 4200 r/min speed. The under layer was concentrated until nearly dry in 50°C water bath and nitrogen. As the last step, the dried residues was dissolved in 10 mL phosphate buffer in twice in order to be purified.

Purification

The Cleanert® PEP-2 (500mg / 6mL) column was acticated and equilibrated using 5.0 mL methanol, 5.0 mL water and 5.0 mL phosphate buffer solution in sequence. After that, the liquid sample to purify was loaded on the column (the flow rate was controlled at 1 mL/min). The SPE column was washed using 5.0 mL water and 5.0 mL methanol : water (2:8. v/v) in turn, then put away the waste and dried it using vacuum pump for 30 min. The sample was eluted using 10.0 mL methanol into a pipe then further dried it at 45°C in nitrogen. As the last step, the residues were dissolved by 1 mL water contained 10% methanol and filtered (0.22 μm) in order to detection.

Analytical method

(1) Chromatographic conditions

Column: Venusil® MP C18, 2.1×50 mm, 5 μm, 100Å;
Mobile phase: A: water contained 0.1% formic acid, B: acetonitrile contained 0.1% formic acid;
Column Temperature: 30°C;
Injection Volume: 10 μL;
Gradient elution is shown in table 1

Table 1 Gradient elution conditions of HPLC chromatography

Time (min)	Flow (mL/min)	A%	B%
0.00	0.30	80	20
1.00	0.30	80	20
2.50	0.30	5	95
4.00	0.30	5	95
6.00	0.30	80	20
15.00	0.30	80	20

(2) Mass spectrum conditions:

IIon source: ESI
Scan mode: positive
Electrospray voltage: 5500 V
Atomizer pressure: 50 psi
Curtain Gas pressure: 15 psi
Aux Gas Pressure: 45 psi
Ion source temperature: 550°C
Detection mode: multiple reaction monitoring (MRM)
Mass spectrum parameters were shown in table 2.

Table 2 Mass spectrum parameters of Tylosin

Compound	Q1	Q3	CE/V
Tylosin	916.5	174.2	61
		145.1	61

Results

The standards of Tylosin were added in samples at 4.0 μg/kg and 20.0 μg/kg followed by detection using SPE-HPLC-MS/MS. As showed in table 3, the spiked recoveries were in the range of 80%-100%, and the RSDs under 10% showed good reproducibility. As showed in fig.1 and fig.2, the peak shape of Tylosin was satisfactory and its retention time was stable after purification and separation by Cleanert® PEP-2 SPE column and Venusil® MP C18 column.

Table 3 The spike recoveries and retention time for Lincomycin (n = 3)

Compound	Concentration(μg/kg)	Recovery (%)	RSD (%)	RT (min)
Tylosin	4.0	91.56	4.35	9.59
	20.0	88.74	1.31	

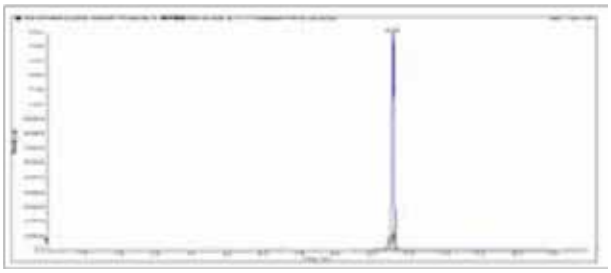


Fig.1 the HPLC-MS/MS chromatogram of Tylosin (adding standards 4.0 μg/kg)

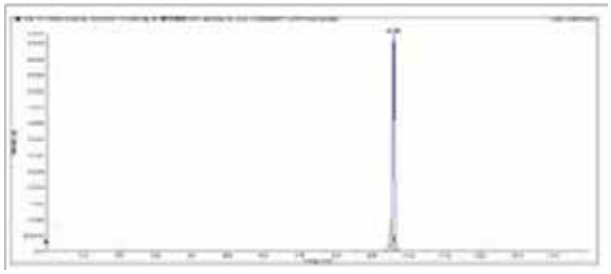


Fig.2 the HPLC-MS/MS chromatogram of Tylosin (adding standards 20 μg/kg)

Conclusion

This study developed a LC-MS/MS method for the detection of Tylosin. Combined with solid phase extraction, it also achieved quantification of Tylosin residues in pork. With this method, 4.0 μg/kg and 20.0 μg/kg spiked samples could be directly analyzed, and the spiked recoveries were in the range of 80%-100%, which meet the standards of GB. Solid phase extraction method showed good stability and the columns showed excellent reproducibility, which pointed out that the method could be used for the quantification of Tylosin residues in animal derived food.

Ordering Information

Products	Specification	Cat.No.
Venusil® MP C18	2.1×50 mm, 5 μm, 100 Å	VA950502-0
Cleanert® PEP-2	500mg / 6mL, 30/pk	PE5006-2
Guard column sleeve	For 4.6×10 mm and 2.1×10 mm	SH-100
Direct-coupled protect column core	5 μm,100 Å,2.1×10 mm	VS950102-0S
1.5mL vial	Short-thread and transparent, 32×11.6 mm, 100/pk	1109-0519
1.5mL vial cap	9mm center bore, blue cap, red rubber/beige PTFE pad, 45. Shore A 1.0 mm, 100/pk	0915-1819
Syringe Filter (Nylon)	0.22 μm, diameter 13 mm, 200/pk	AS021320
Disposable Needle-Free injection systems	2 mL, 100/pk	LZSQ-2ML
Acetonitrile (UV)	4 L/bottle, chromatographic grade	015-4
n-hexane HPLC	4 L/bottle, chromatographic grade	AH216-4
Methanol	4 L/bottle, chromatographic grade	AH230-4

The Quantification of Tilmicosin Residues in Livestock Meat Using LC-MS/MS Method (Cleanert® PEP-2 500mg / 6mL P/N: PE5006-2)

EMF10017

Tilmicosin is a relatively new special macrolide antibiotic for livestock with tylosin for semi synthetic precursor. The British Elanco animal health care products company was the first one to develop the medicine successfully in 1980s. It is mainly used for infectious diseases of cattle, dairy cattle, goats, sheep, pigs, chicken and other animal caused by sensitive bacteria, especially for respiratory disease of livestock such as porcine Actinobacilluspleuropneumonia, cattle Hemolytic and Pasteurella multocida disease and avian mycoplasmosis. Besides, it has a good antibacterial effects on dairy cow mastitis mainly strains. GB/T20762-2006provides Tilmicosin residues detection limit is 1 μg/kg in the livestock and poultry meat.

Sample extraction

After weighing 5.00 g (accurate to 0.01 g) homogenized drug, the sample was put into a 50.0 mL centrifuge tubethat had been added in 15.0 mL acetonitrile. Thenhomogenized it with homogenizer for 1 min,and oscillated it for 10min beforecentrifuging for 5 min at 4200 r/min speed. After that 2 g NaCl and 10.0 mL n-hexane were added into the separated supernate, then oscillated it for 10 min and centrifuged it for 5 min at 4200 r/min speed again. As the last step,transferred the middle acetonitrile layer 12.0 mL on another centrifuge tube carefully, and driedit in the nitrogen.

Purification

The Cleanert® PEP-2 (500mg / 6mL)column was acticated using 10.0 mL methanol, 10.0 mL water, 5.0 mL 2% NaCl solution and 5.0 mL ammonium dihydrogen phosphate buffer solution in sequence. After that, dissolved the residual liquid sample using 7.0 mL ammonium dihydrogen phosphate buffer in twice (the flow rate in the range of 2-3 mL/min) and put away the filtrate. The SPE columnwas washed using 10.0 mL water and 5.0 mL methanol: water (2:3. v/v) in turn, then put away the waste and dried it in negative pressure. The sample was eluted using 10.0 mL methanol into a pipe then further dried it at 45oC in nitrogen. As the last step, the residues were dissolved by 1 mL 0.1% acetic ammonia solution andFilter (0.22 μm) in order to detection.

Analytical method

(1) Chromatographic conditions:

Column: Venusil® ASB C18, 2.1×100 mm, 5 μm, 150 Å;
Mobile phase: A: 10 mM ammonium acetate solution, B: acetonitrile;
Column Temperature: 35 °C;
Injection Volume: 5 μL;
Gradient elution is shown in table 1.

Table 1 Gradient elution conditions of HPLC chromatography

Time (min)	Flow (mL/min)	A%	B%
0.00	0.35	95	5
1.50	0.35	95	5
2.00	0.35	60	40
4.00	0.35	60	40
4.01	0.35	10	90
5.00	0.35	10	90
5.01	0.35	95	5
7.00	0.35	95	5

(2) Mass spectrum conditions:

Ion source: ESI
Scan mode: positive
Electrospray voltage: 5500 V
Atomizer pressure: 60 psi
Curtain Gas pressure: 13 psi
Aux Gas Pressure: 65 psi
Ion source temperature: 600°C
Detection mode: MRM
Mass spectrum parameters were shown in table 2.

Table 2 Mass spectrum parameters of Tilmicosin

Compound	Q1	Q3	CE/V
Tilmicosin	869.8	696.7	58
		174.5	60

Results

The standards of Tilmicosinwere spiked in samples at 1.0 μg/kg and 20.0 μg/kg respectively, followed by detection using SPE-HPLC-MS/MS.As showed in table 3, the spiked recoveries were in the range of 90%-100%, and the RSDs under 10% showed good reproducibility.As showed in fig.1 and fig.2, the peak shape of Tilmicosin was satisfactory and its retention time was stable after purification and separation by Cleanert®PEP-2 SPE column and Venusil®ASB C18 column.

Table 3 The spike recoveries and retention time for Tilmicosin (n = 3)

Compound	Concentration(μg/kg)	Recovery (%)	RSD (%)	RT (min)
Tilmicosin	1.0	92.52	3.88	2.50
	20.0	91.91	4.21	

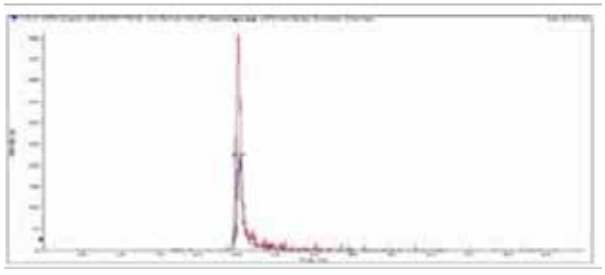


Fig.1 the HPLC-MS/MS chromatogram of Tilmicosin (1.0 μg/kg)

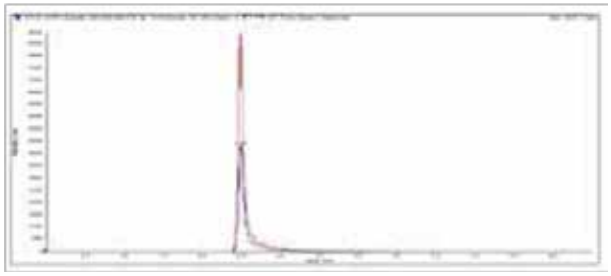


Fig.2 the HPLC-MS/MS chromatogram of Tilmicosin (20.0 μg/kg)

Conclusion

This study developed a LC-MS/MS method for the detection of Tilmicosin.Combined with solid phase extraction, it also achieved quantification of Tilmicosin residues in livestock meat.With this method,1.0 μg/kg and 20.0 μg/kg spiked samples could be directly analyzed, and the spiked recoveries were in the range of 90%-100%, which meet the standards of GB. Solid phase extraction method showed good stability and the columns showed excellent reproducibility, which pointed out that this method could be used for the quantification of Tilmicosin residues in livestock meat.

Ordering Information

Products	Specification	Cat.No.
Venusil® ASB C18	2.1×100 mm, 5 μm, 150 Å	VS951002-0
Cleanert® PEP-2	500mg / 6mL,30/pk	PE5006-2
Guard Column Holder	For 4.6×10 mm and 2.1×10 mm	SH-100
Direct-connected Guard Column	5 μm,150 Å,2.1×10 mm	VS950102-0S
1.5 mL vial	Short-thread and transparent, 32×11.6 mm, 100 /pk	1109-0519
1.5 mL vial cap	9mm center bore, blue cap, red rubber/beige PTFE pad, 45. Shore A 1.0mm, 100/pk	0915-1819
Syringe Filter (Nylon)	0.22 μm, diameter 13 mm, 200/pk	AS021320
Disposable Needle-Free injection systems	2 mL, 100/pk	LZSQ-2ML
Acetonitrile (UV)	4 L/bottle, chromatographic grade	015-4
n-hexane HPLC	4 L/bottle, chromatographic grade	AH216-4
Methanol	4 L/bottle, chromatographic grade	AH230-4

The Determination of Stilbestrol Residues in Pork Using LC-MS/MS Method (Cleanert® PEP-2 500mg / 6mL P/N: PE5006-2)

EMF10018

Stilbestrol is a synthetic non-steroidal estrogen substance, which can produce all pharmacological and therapeutic effects as the same natural estradiol does. It is mainly used for functional bleeding and amenorrhoea caused by estrogen low disease and hormone imbalance. It can also be used for stillbirth before induction of labor, in order to improve the muscular layer of uterine sensitivity to oxytocin.Pregnant women taking the drug, the female offspring in adolescence of cervical and vaginal adenosis and cancer incidence will rise and the male offspring reproductive tract abnormalities and abnormal sperm rate also will increase.Diethylstilbestrol can cause nausea, vomiting, loss of appetite, headache and other symptoms and long term using can make endometrial hyperplasia that may result in uterine bleeding and hypertrophy. The international standard provides stilbestrol residues detection limit is 1 μ g/kg in liver and muscle tissue in cattle and pigs.

Sample extraction

Weighed 5.00 g (accurate to 0.01 g) homogenized sample, then put it into 50.0 mL centrifuge tube, and 15.0 mLt-butyl methyl etherwas added, vortex for 1 min and oscillation for another 10 min. Centrifuged the solution for 5 min at 6000 r/min speed at -4 °C and transfered the supernate to the condensed bottle. Extracted the residue with another15.00 mL t-butyl methyl ether then combined the twice extracts. The solution was evaporated in the water bath at 45 °C. Then added 80 % acetonitrile solution 2 mL to the concentrated bottle, making the residue dissolved, and transferred it to the 10 mL centrifuge tube. The concentrated bottle was washed with 5 mL n-hexane and the n-hexane was transferred to the 10mL centrifuge tube. Then centrifuged the mixed solution for 10 min at 6000 r/min speed at 15 °C and the supernatewas removed.The lower layer would be used finally.

Purification

Activate the Cleanert® PEP-2 (500mg / 6mL) column using 3.0 mL t-butyl methyl ether, 3.0 mL methanol, 3.0 mL water in sequence. The lower layer solution flowed through the column at the speed of every 3~4 seconds per drop.Using 3.0 mL solution (methanol:water=3:7,V:V) to wash the column and added another 3.0 mL water to balance the system. Next with methanol: 2 % ammonia solution (1:9, v/v) 3 mL flushing, discarding the lotion, and drained. Elute the sample using 1 % formic acid / methanol: methyl t-butyl ether (1:9, v/v) 7 mL into a pipe, then dried it in nitrogen. Then the residues were dissolved by 1 mL 80 % acetonitrile water solution. Filter the samples (0.22 μm) for detection.

Analytical method

(1) Chromatographic conditions:

Column: Unisol C18, 2.1×50 mm, 2.5 μm, 100 Å;
Mobile phase: A: water, B: acetonitrile;
Column Temperature: 35 °C;
Injection Volume: 5 μL;
Gradient elution is shown in table 1.

Table 1 Gradient elution conditions of HPLC chromatography

Time (min)	Flow (mL/min)	A%	B%
0.00	0.35	85	15
1.50	0.35	85	15
4.00	0.35	10	90
5.00	0.35	10	90
5.01	0.35	85	15
7.00	0.35	85	15

(2) Mass spectrum conditions:

Ion source: ESI
Scan mode: negative
Electrospray voltage: -5500 V
Atomizer pressure: 55 psi
Curtain Gas pressure: 12 psi
Aux Gas Pressure: 50 psi
Ion source temperature: 550 °C
Detection mode: MRM
Mass spectrum parameters were shown in table 2.

Table 2 Mass spectrum parameters ofDiethylstilbestrol

Compound	Q1	Q3	CE/V
Diethylstilbestrol	267.1	251.2	-36.5
		221.9	-47

Results

The standards of Diethylstilbestrolwere added in samples at 2.5 μg/kg and 25.0 μg/kg followed by detection using SPE-HPLC-MS/MS.As showed in table 3, the spiked recoveries were in the range of 80 %-96 %, and the RSDs in the range of 2.26 % - 8.95 % showed good reproducibility. As showed in fig.1 and fig.2, the peak shape of different drugs was satisfactory and its retention time was stable after purification and separation by Cleanert® PEP-2 SPE column and Unisol C18 column.

Table 3 The spike recoveries and retention time for Diethylstilbestrol(n = 3)

Compound	Adding standard (μg/kg)	Recovery (%)	RSD (%)	RT (min)
Diethylstilbestrol	2.5	95.58	2.26	3.30
	25	83.56	8.95	

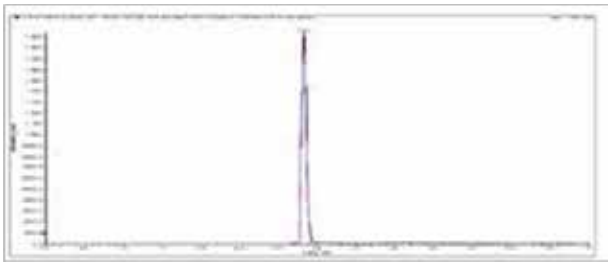


Fig.1 the HPLC-MS/MS chromatogram of Diethylstilbestrol(adding standards 2.5 μg/kg)

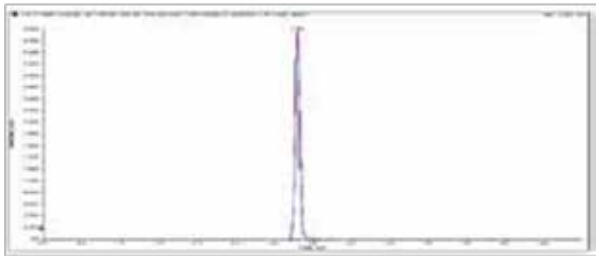


Fig.2the HPLC-MS/MS chromatogram of Diethylstilbestrol(adding standards 25 μg/kg)

Conclusion

This study developed a LC-MS/MS method for the detection ofDiethylstilbestrol. Combined with solid phase extraction, it also achieved quantification of Diethylstilbestrolresidues in pork. With this method, 2.5μg/kg and 25 μg/kg spiked samples could be directly analyzed, and the spiked recoveries were in the range of 80%-90%, which meet the standards of GB. Solid phase extraction method showed good stability and the columns showed excellent reproducibility, which pointed out that this method could be used for the quantification of Diethylstilbestrolresidues in animal derived food.

Ordering Information

Products	Specification	Cat.No.
Unisol C18	2.1×50 mm, 2.5 μm, 100 Å	UO920502-0
Cleanert® PEP-2	500mg / 6mL,30 /pk	PE5006-2
Guard Column Holder	For 4.6×10 mm and 2.1×10 mm	SH-100
Direct-connected Guard Column	2.5 μm,100 Å,2.1×10 mm	VA920102-0S
1.5 mL Vial	Short-thread and transparent, 32×11.6 mm, 100/pk	1109-0519
1.5 mL Vial Cap	9mm center bore, blue cap, red rubber/beige PTFE pad, 45. Shore A 1.0 mm, 100/ pk	0915-1819
Syringe Filter (Nylon)	0.22 μm, diameter 13 mm, 200 /pk	AS021320
Disposable Needle-Free Injection Systems	2 mL, 100/pk	LZSQ-2ML
Acetonitrile (UV)	4 L/bottle, chromatographic grade	015-4
Methanol	4 L/bottle, chromatographic grade	AH230-4
n-hexane HPLC	4 L/bottle, chromatographic grade	AH216-4
Diethylstilbestrol	Standard, 100mg	GBW(E)060984

The Determination of Tetracyclines Drug Residues in Pork and Chicken (Cleanert® PEP-2 500 mg/6 mL P/N:PE5006-2)

EMF10019

Abstract

In this study, solid phase extraction combined with liquid chromatography - tandem mass spectrometry (SPE-LC/MS/MS) method were used to established the detection method of tetracycline drugs such as tetracycline, oxytetracycline and chlortetracycline in port and chicken under the multiple reaction monitoring (MRM) mode according to GB/T 21317-2007 standard. Tetracycline drugs in the samples were extracted by Na2-EDTA-McIlvaine buffered solution, cleaned by Cleanert PEP-2 SPE cartridge, separated by Durashell C18 HPLC Column (2.1*150 mm, 2.5 μm,100 Å) and quantitated by external standard method with 0.3 % formic acid and 0.3 % formic acid acetonitrile acetonitrile as mobile phase in gradient elution. The result indicated that when the sample amount of tetracycline drugs is 10 μg/kg, 50 μg/kg, 200 μg/kg, the recovery rate is 70%-110%, which meet the National standard.

Keywords

SPE-LC/MS/MS; Tetracycline; Terramycin. Aureomycin; Cleanert® PEP-2 SPE cartridges; Durashell C18 HPLC Column.

Introduction

Tetracycline class (Tetracyclines, TCs) is a kind of broad spectrum antibiotics produced by Streptomyces with the basic frame structure including their tetracene. It mainly includes Tetracycline (TC), Oxytetracycline (OTC), Chlortetracyline (CTC) and so on. It has been widely used in the treatment of edible animal disease and medicated premix for the prevention and treatment of animal diseases.

Tetracycline class antibiotics are instable both in acidic and alkaline conditions. They contain many hydroxyl groups, enolic hydroxyl and carbonyl group, which can form insoluble chelation with a variety of metal ions under neutral condition. Such as form insoluble calcium or magnesium salts with calcium or magnesium ions, form red complex with iron ion and form yellow complex compound with aluminum ions.

European Union, Japan and the United States set the maximum residue limits of animal tissues and milk in order to effectively prevent the abuse of tetracycline drugs, which stipulated the maximum residue limits in animal tissues is 0.1 mg/mL.

Experiment

(1) Instruments, reagents and materials

1) Main instruments and equipment

AB SCIEX API 4500 HPLC-MS

2) Reagents material

Methanol, acetonitrile, formic acid are chromatography pure; Citric acid, disodium hydrogen phosphate, sodium hydroxide and ethylenediamine tetraacetic acid disodium are analytically pure;

Citric acid solution: 0.1 mol/L, dissolve 21.01 g citric acid in water and constant volume to 1L;

Disodium hydrogen phosphate solution: 0.2 mol/L, dissolve 28.41g disodium hydrogen phosphate ($Na_2HPO4•H_2O$) with water and constant volume to 1 L;

McIlvaine buffer solution:Mixture 1 L citric acid (0.1 mol/L) with 625 mL disodium hydrogen phosphate (0.2 mol/L), adjust the pH value to 4.0±0.05 with sodium hydroxide;

Na2-EDTA-McIlvaine buffer solution:0.1 mol/L, dissolve 60.5 gethylenediamine tetraacetic acid disodium into 1625 mL McIlvaine buffer solution shaking for future use;

5% methanol aqueous solution;

Methanol-acetic acid ethyl ester (1:9, v/v);

Acycline hydrochloride are no less than 97%;

Cleanert® PEP-2 cartridge: 60mg / 3mL

(2) Sample preparation

1) Sample extracts

Put 5.0 g (accurate to 0.01 g) sample to the centrifuge tube of 50 mL and then 20 mL,20 mL,10 mL 0.1 mol/L EDTA-McIlvaine buffer solution, respectively. Vortex mixed the sample for 1 min under 1000 rpm, supersonic extraction for 10 min and then centrifuged for 5 min under 3000 rpm. Merge the supernatant (control the volum of the supernatant no more than 50 mL) and constant volume to 50 mL. Mixed the sample and centrifuged for 10 min under 5000 rpm. Filter the sample with rapid filters for future purification.

2) Sample purification

Condition the PEP-2 cartridge with 5 mL methyl alcohol and 5 mL activated water. Take 10 mL extracting solution to pass the SPE cartridge with the speed of 1drop/s. Wash the cartridge with 5 mL water and 5 mL 5% methanol aqueous solution after all the extracting solution outflow and discard all the effluent. Reduced pressure and swab off the cartridge for 5 min and finally elution with 10 mL methyl alcohol+ ethyl acetate (1:9,v/v). Dry the eluent with N2 under 40℃ and then dissolve solution residue with 1 mL methyl alcohol solution (3:7, v/v). Filtered with 0.45 μm filter membrane for future examination.

(3) Experiment condition

1) Liquid condition

HPLC Column: Durashell C18, 2.5 μm;100 Å; 2.1x50 mm

Mobile phase:A-0.3% formic acid aqueous solution, B-0.3% formic acid acetonitrile

Column temperature: 30℃

Sample size: 5 μL

Gradient elution: See table 1

Table 1. HPLC gradient elution conditions

Time (min)	Flow (mL/min)	A%	B%
0.00	0.2	90.0	10.0
1.00	0.2	10.0	90.0
8.00	0.2	60.0	40.0
8.10	0.2	10.0	90.0
9.00	0.2	90.0	10.0
15.00	0.2	90.0	10.0

2) Mass spectrometry conditions
Ion source: electrospray ion source
Scanning mode: positive ion scanning
Electrospray voltage: 5500 V
Atomization gas pressure: 65 psi
CUR:12 psi
Aux Gas Pressure: 60 psi
Ion source temperature: 550˚C
Acquisition methods: multiple reactions monitoring (MRM)
Q1 and Q3 are resolution ratio for the unit
Qualitative ion pair, quantitative ion pair, cluster voltage and collision voltage (see table 2)

Table 2. Spectrometry conditions of tetracycline drugs

Substances	Q1	Q3	CE/V
(Tetracycline hydrochloride)	445	410	37
		428	37
(Oxytetracycline)	461	426	27
		443	27
(Chlorotetracycline hydrochloride)	479	444	29
		462	29

Results and discussion

(1) Experimental results

Table 3 illustrates that when using solid phase extraction combined with liquid chromatography - tandem mass spectrometry method to detection three kinds of tetracycline drugs, the sample amount is 10 μg/kg, 50 μg/kg, 200 μg/kg, the recovery rate is 90%-105%, which can meet the requirements for the detection. Fig.1-3 showed the clean-up with Cleanert® PEP-2 SPE cartridge and detection with Durashell C18 HPLC column can make better separation to the 3 kinds of tetracyclines drugs. And each material peak shape is good, the retention time and the retention time is stable.

Table 3. Tetracycline drugs standard addition recovery experiment results (n = 3)

Substances	Add amount/(μg/kg)	Recovery rate /%	RSD/%	RT/min
(Tetracycline hydrochloride)	10	97.05	3.63	4.91
	50	100.46	2.08	
	200	96.71	0.12	
(Oxytetracycline)	10	96.66	2.22	3.84
	50	99.82	0.87	
	200	97.49	3.77	
(Chlorotetracycline hydrochloride)	10	91.87	7.62	7.06
	50	92.52	0.21	
	200	94.94	1.94	

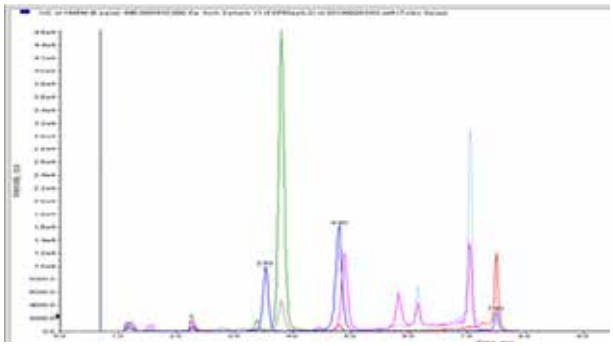


Fig 1. Liquid chromatography/tandem mass spectrometry mass spectrometry of tetracycline class sample with add amount of 10 μg/kg

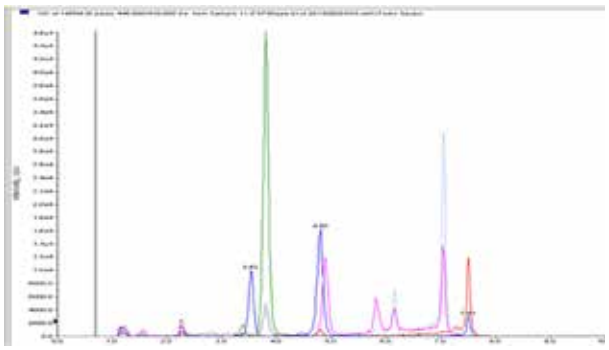


Fig 2. Liquid chromatography/tandem mass spectrometry mass spectrometry of tetracycline class sample with add amount of 50 μg/kg

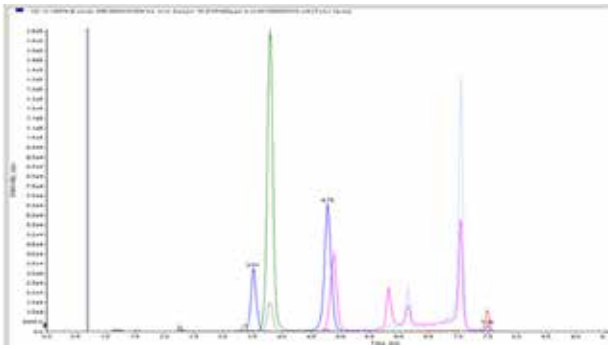


Fig 3. Liquid chromatography/tandem mass spectrometry mass spectrometry of tetracycline class sample with add amount of 200 μg/kg

Conclusion

We established the LC-MS/MS method for the detection of 3 kinds of tetracycline drugs. The samples with the add amount of 10 μg/kg, 50 μg/kg and 200 μg/kg were established and the results indicated that the recovery rate is 90%-105%, which meet the requirement of national standard. Solid phase extraction method is stable and HPLC Column has good reproducibility, so this method can be used to detect tetracycline drugs residues in food of animal origin.

Ordering Information

Products	Specification	Cat.No.
Durashell C18	2.5 μm, 100 Å; 2.1×50 mm	DC930502-0
Cleanert® PEP-2	60 mg/3 mL	PE0603-2
Qdaura® Automated SPE System	6 channel	SPE-40
Guard cartridge holder	Suitable for 4.6×10 mm and 2.1×10 mm	SH-100
Direct-connection Guard Cartridge	5 μm,100Å;4.6×10 mm	DN850105-0S
1.5 mL vial	Screw neck vial, graduated, 32×11.6 mm	1109-0519
Cap for 1.5 mL vial	9 mm center hole; blue cap; Red silicone/ white PTFE septa. 45. Shore A 1.0 mm	0915-1819
Micropore Membrane	Monofilm, 13 mm, 0.45 μm	AS021345
Syringe	2 mL Without Needle	LZSQ-2ML
Acetonitrile UV grade	4 L/bottle, chromatographic pure	015-4
Methanol	4 L/bottle, chromatographic pure	AH230-4

Simultaneous QuEChERs-HPLC Analysis of Clopidol, Diclazuril and Sulfonamides in Animal Tissues (Cleanert® PSA, C18, Alumina-N, P/N: PA0010,180010, AL0010-N)

EMF10020

Material

PSA absorbent: Cleanert® PSA
C18 absorbent: Cleanert® C18
Neutral alumina absorbent: for chromatography (roast at 600°C in muffle furnace before use)

Experimental

Sample preparation with QuEChers method

Weigh 5 g (accurate to 0.01 g) of minced and mixed sample in a 50 mL centrifuge tube. Add 14 mL of acetonitrile/chloroform (10/1) solution, 1.0 mL of 10% sodium sulfate solution and homogenize. Centrifugate at 5000 r/min for 5 min. Take 10 mL of the extract precisely and concentrate or evaporate almost to dryness under nitrogen stream. Dilute with acetonitrile/chloroform (10/1) to 2 mL. Choose and add proper amount of C18, PSA or alumina absorbents. Vortex mix for 2 min to purify sample by dispersed solid phase extraction. Centrifugate at 5000 r/min for 3 min. Transfer 1.0 mL of the supernatant precisely into another 15 mL centrifuge tube. Dry under nitrogen stream and vortex dissolve the residue in 1.0 mL of acetonitrile/water (12/88). Filter through 0.45 μm membrane for analysis.

HPLC Conditions

Column: Venusil® ASB C18 (5 μm, 3.9 × 150 mm)
Mobile phase: A: acetonitrile; B: acetic acid/water (3/1000)
Gradient elution: see table 1
Flow rate: 1.0 mL/min; Injection volume: 20 μL;
Column temperature: 40°C; UV: 270 nm.

Table 1 Program of gradient elution

Time/min	A %	B %	Gradient Curve
0.00	12	88	linear
8.50	12	88	linear
8.51	35	65	linear
15.00	35	65	linear
15.01	60	40	linear
20.00	60	40	linear
20.01	12	88	linear
25.00	12	88	linear

Results

(1) Linear range and limit of detection (LOD)
The limits of detection of different samples could not be exactly the same due to the difference of recovery even at the same spiked concentration. LOD of 7 compounds could reach: 0.05 mg/kg for clopidol, 0.05 mg/kg for sulfonamides, 0.10 mg/kg for diclazuril.
(2) Recovery and precision
Recovery and precision experiments are carried out with spiked chicken meat and liver samples at three concentration levels, respectively. For each level, experiments are repeated six times. At the concentrations between 0.10 mg/kg~1.0 mg/kg, the recovery and RSD are 65%~100% and 1%~10%, respectively. Chromatograms of spiked chicken meat and liver samples are shown as figure 1 and 2, respectively.

Discussion

The original QuEChERS method for pesticide residues in vegetables needs 15 g of sample and uses shaking for extraction. The extract is taken directly for next cleanup. The improved method for veterinary drug residues in animal tissues uses 5 g of sample and extraction by homogenization has better extraction efficiency. Take 10 mL of the extract and evaporate almost to dryness by rotary evaporator. Dissolve the residue and dilute with extractive solvent to 2 mL for next cleanup. The improved method enriches more analytes than the original one. Therefore the sensitivity could be improved by 5 times theoretically.

PSA is used in the original QuEChERS method to remove fatty acids and pigments. As animal tissues have large amounts of proteins and fats, C18 and neutral alumina absorbent also need to be considered during the cleanup process. Sample cleanup is carried out by mixed dispersed solid phase extraction. The amount of different adsorbents, which usually is between 100 and 250 mg, is adjustable with different sample matrices. As for recovery, C18 and neutral alumina have strong adsorption towards diclazuril, thus reducing its recovery. But these adsorbents can hardly adsorb other 6 veterinary drugs. PSA could adsorb 7 compounds to different extents. In practice, it is best to choose appropriate absorbent and its amount according to the properties of matrix and the target compounds.

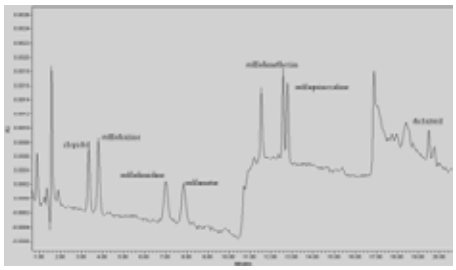


Figure 1. Chromatogram of spiked chicken meat sample

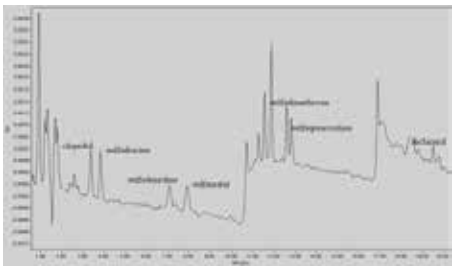


Figure 2. Chromatogram of spiked chicken liver sample

Pesticide Residues

Detection of Pesticide Residues in Food Products of Mult-pesticide Residues in Tea Leaves by GC-MS and LC-MS/MS(Cleanert® TPT, P/N: TPT200010)

EMF10021

Material

Cleanert® TPT, 2g / 12mL

Experimental

Extraction

Weigh 5 g (accurate to 0.01 g) of tea leaves sample in a 80 mL centrifuge tube, add 15 mL of acetonitrile. The solution is homogenized at 15000 r/min for 1 min, then centrifugated at 4200 r/min for 5 min. Transfer the supernatant into a 100 mL pear-shaped bottle. Extract the residue with 15 mL of acetonitrile and centrifugate. Combine the two supernatants and evaporate to 1 mL by rotary evaporator in water bath at 40°C for further cleanup.

Cleanert® TPT cleanup

Method for GC/MS: load 2 cm high of anhydrous sodium sulfate onto Cleanert® TPT cartridge. Wash the cartridge with 10 mL of acetonitrile/toluene (3:1, v/v), and place it on a fixed rack mounted with a pear-shaped bottle under it. Load the concentrated sample onto the Cleanert® TPT cartridge. Wash the sample bottle with 2 mL of acetonitrile/toluene (3:1, v/v) 3 times and combine the solutions onto the cartridge. Add a 50 mL liquid reservoir on the cartridge and wash the cartridge with 25 mL of acetonitrile/toluene (3:1, v/v). Collect all the eluate in a pear-shaped bottle and evaporate to 0.5 mL by rotary evaporator in water bath at 40°C. Exchange the solvent with 5 mL of n-hexane twice and obtain 1 mL of solution. Add 40 µL of internal standard solution and mix for GC-MS analysis.

Method for LC-MS/MS: Except that the sample amount is 2 g, the sample extraction and cleanup method is the same as above. Evaporate the collected eluate to 0.5 mL by rotary evaporator in water bath at 40°C. Dry under a stream of nitrogen at 35°C. Redissolve the residue in 1 mL of acetonitrile/water (3:2, v/v). Filter the solution through 0.2 µm membrane for LC-MS/MS analysis.

Detection of Mult-pesticide Residues in Ramulus Mori, Honeysuckle and the Fruit of Chinese Wolfberry Using GS-MS and LC-MS/MS Respectively (Cleanert® TPH, P/N: TPH200010)

EMF10022

Material

Cleanert® TPH, 2g / 12mL

Experimental

Extraction

Weigh 5 g (accurate to 0.01 g) of honeysuckle, medlar samples, or 2.5 g (accurate to 0.01 g) of lotus leaves, ramuli mori samples in 50 mL centrifuge tubes. Add 15 mL of acetonitrile. (For medlar sample, another 5 mL of water is needed). Homogenize at 15000 r/min for 1 min. Add 2 g of sodium chloride and homogenize again for 1 min. Centrifugate at 4200 r/min for 5 min and transfer the supernatant into a 150 mL pear-shaped bottle. Add 15 mL of acetonitrile to the centrifuge tube again and homogenize for 1 min. Centrifugate at 4200 r/min for 5 min and combine the supernatant to the pear-shaped bottle. Evaporate to 1~2 mL by rotary evaporator in water bath at 40°C for further cleanup.

Sample Preparation

(1) Extraction

Weigh 2 g (accurate to 0.01 g) of honeysuckle, medlar, (lotus leaf) and ramuli mori samples in 50 mL centrifuge tubes. Add 15 mL of acetonitrile. (For medlar sample, another 5 mL of water is needed). Homogenize at 15000 r/min for 1 min. Add 2 g of sodium chloride and homogenize again for 1 min. Centrifugate at 4200 r/min for 5 min and transfer the supernatant into a 150 mL pear-shaped bottle. Add 15 mL of acetonitrile to the centrifuge tube again and homogenize for 1 min. Centrifugate at 4200 r/min for 5 min and combine the supernatant to the pear-shaped bottle. Evaporate to 1~2 mL by rotary evaporator in water bath at 40°C for further cleanup.

(2) Cleanup

Fill a 2 cm bed height of sodium sulfate anhydrous onto the head of TPH cartridge. Place it on a fixed rack. Wash the cartridge with 10 mL of acetonitrile/toluene (3:1, v/v). When the washing solution reaches the upper surface of the sodium sulfate, load the concentrated sample (3.1) onto the cartridge. Collect the eluate in a pear-shaped bottle. Wash the pear-shaped bottle (3.1) with 2 mL of acetonitrile/toluene (3:1, v/v) 3 times. Combine the washing solutions onto the cartridge. Add a 25 mL liquid reservoir on the cartridge. Wash the cartridge with 25 mL of acetonitrile/toluene (3:1, v/v) to elute the pesticides and related compounds. Collect the eluate and evaporate to 1~2 mL by rotary evaporator in water bath at 40°C. Dry under a stream of nitrogen. Redissolve the residue in 1 mL of acetonitrile/toluene (3:1, v/v). Filter the solution through 0.22 μm membrane for LC-MS/MS analysis.

QuEChERS Application in Fast Analysis of Multiple Pesticide Residues (Cleanert® PSA, C18, PestiCarb, NH_2, P/N: PA0010, 180010, PC0010, NH0010)

EMF10023

Material

PSA absorbent: Cleanert® PSA
C18 absorbent : Cleanert® ODS C18
Graphite carbon black absorbent: Cleanert® PestiCarb
NH_2 absorbent : Cleanert® NH_2

Experimental

Sample preparation

Take edible parts of samples, mince and mix. Weigh 15 g (accurate to 0.01 g) of sample and place it into a 100 mL plastic centrifuge tube. Add 15 mL of 0.1% acetic acid / acetonitrile solution, 6.0 g of anhydrous magnesium sulfate, 1.5 g of sodium acetate and homogenize.
Centrifugate at 5000 r/min for 5 min. Take 10 mL of organic phase accurately into a 15 mL plastic centrifuge tube. Dry the solution under nitrogen stream. Vortex mix to dissolve the residue in 2.0 mL of 0.1% acetic acid / acetonitrile solution. According to the interference of sample matrix, select and weigh proper amount of absorbents like C18, PSA, graphite carbon black or NH_2, and place into another 15 mL plastic centrifuge tube. Transfer 2 mL of the above dissolved solution to the centrifuge tube. Vortex mix for 2 min and centrifuge at 5000 r/min for 3 min. Take the supernatant with a disposable syringe and filter through 0.45 μm membrane for analysis.

GC analysis

The treated sample can be analyzed with GC-FPD for organophosphorus pesticide determination, GC-ECD for organochlorine pesticides determination or GC-MS for multi-residue organophosphorus, organochlorine, carbamate et al. Here GC-MS is applied for simultaneous multiple residues. The conditions of instrument are as follows:
Column: Bonna Agela DA-35MS capillary column, (30m × 0.25mm × 0.25μm, P/N: 3525-3002);
Temperature programming in column box: 50°C (for 2 min), 10°C/min to 180°C (hold on for 1 min), 3°C/min to 250°C (hold on for 1 min),
2°C/min to 270°C (hold on for 15 min);
Inlet temperature: 250°C;
Carrier gas: He (>99.999%), constant flow, flow rate 1.0 mL/min;
Injection volume: 1 μL;
Injection type: Splitless injection. After 0.8 min switch the splitting valve on.
Electron impact ionization source: 70 eV;
Temperature of ionization soure: 250°C
Temperature of GC/MS interface: 250°C
Selected ion monitoring: For each compound, select one ion for quantitative analysis and 2~3 ions for qualitative analysis. All the ions to be determined in each group are monitored respectively at different time, according to their retention times.

Results

Recovery and precision experiments are carried out with spiked spinach, cabbage and yellow peach samples at three concentration levels. For each level, experiments are repeated six times. At the concentrations between 0.05 mg/kg~1.0 mg/kg, the recovery and RSD are 65%~120% and 1%~13.5%, respectively.

Discussion

(1) Improvement of sample extraction

In a typical QuEChERS cleanup for analysis of multiple pesticide residues, 'shaking' the sample mixture is employed to extract target compounds, and 2 mL aliquot of the organic solution is taken for analysis. In this study, however, it is found that homogenization for sample extraction improves QuEChERS procedure with higher efficiency. This is useful for samples that contain high content of fibers. On the other hand, this study also shows that a concentration step is necessary to enhance the detectability of target compounds. 10 mL Aliquot was used in the procedure, which results in a 5 magnitude concentration comparing to 2 mL. The drying effect of anhydrous magnesium and sodium sulfates was compared. Before sample introduction to GC analysis, magnesium sulfate anhydrous is more effective to remove water content in the sample solution of reconstitution This is helpful to reduce interfering background when using electron capture detector (ECD) in the GC analysis.

(2) Improvement of sample cleanup

It is shown that Cleanert® PSA is more effective to remove fatty acids in sample matrices while Cleanert® C18 and graphite carbon black (PestiCarb) have good cleanup performance on pigments, sterols and vitamins. Cleanert® NH_2 is more adsorptive to interference than PSA. Therefore, besides the evaluation of PSA in this paper, Cleanert® C18, NH_2 and PestiCarb were also tested. Even though the amount of sorbents used cannot be specified explicitly for given sample matrices, it always falls into the range from 100 to 350 mg. The extract of spinach was clear and colorless, for example, with our improved QuEChERS procedure while the solution was greenish with the 'standard' QuEChERS. In the recovery experiment, we found that C18 has no impact upon recovery. But PSA, NH_2 and PestiCarb could reduce recovery of some pesticides if the amount of sorbent used was too much since the analytes can be strong adsorbed. In practice, it is recommended that the type and the amount of sorbents should be screened and optimized based on the properties of target compounds and nature of matrices.

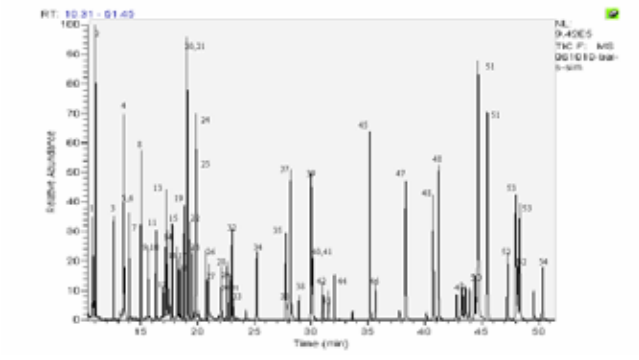


Figure 1. GC-EI-MS SIM spectrum of spiked cabbage sample

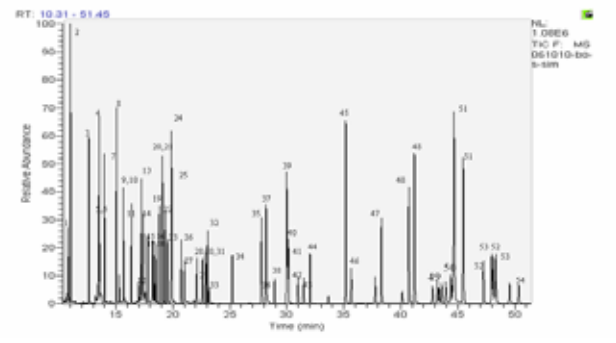


Figure 2. GC-EI-MS SIM spectrum of spiked spinach sample

The spiked concentration is the level 3 in table 4.

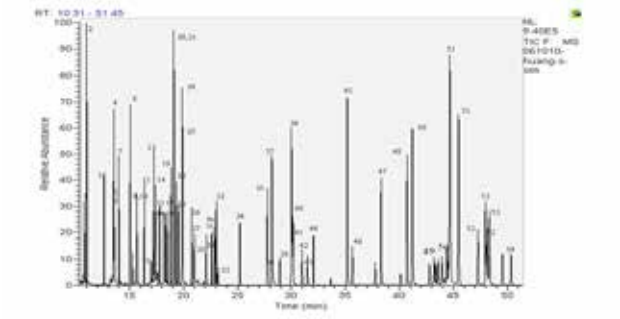


Figure 3. GC-EI-MS SIM spectrum of spiked yellow peach sample

Full length of this paper is available at www.bonnaagela.com: Imidacloprid, tebufenozide, avennectins and hexythiazox in vegetables by QuEChERS-GC.

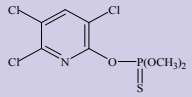
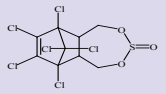
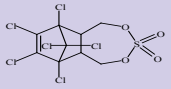
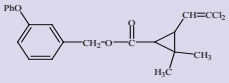
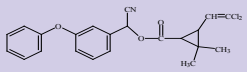
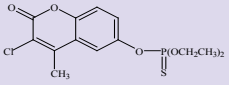
Detection of Pesticide Residues in Honey by Modified QuEChERS Extraction

EMF10024

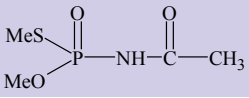
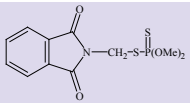
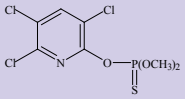
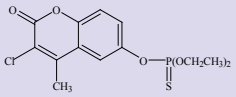
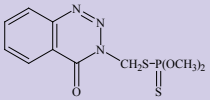
Dispersive sample preparation, referred as “QuEChERS”, is a sample and straightforward sample extraction technique. It can be used for multi-residue pesticide analysis in a wide variety of food and agricultural products.
Bonna-Agela’s MAS-Q is a well proven, high throughput method. After study of more than 60 of the fruit, vegetable samples (contain 27 of different sample matrix) and honey samples, the recoveries are very well proved for both Bonna-Agela’s high quality SPE sorbents and MAS-Q method.

Material

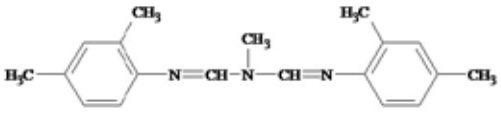
honey
Analytes: 10 pesticides

Organochlorines	Molecular Structure	Type
Chlorpyrifos Methyl		Insecticides
Endosulfans		Insecticides
Endosulfan sulfate		Insecticides
Permethrins		Insecticides (Pyrethroid)
Cypermethrins		Insecticides (Pyrethroid)
Coumaphos		Insecticides

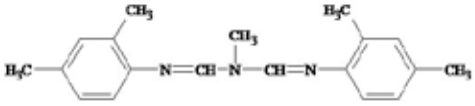
Organophosphates:

Acephate		Insecticides
Phosmet		Insecticides
Chlorpyrifos Methyl		Insecticides
Coumaphos		Insecticides
Azinphos Methyl		Insecticides

Organonitrogen Pesticide: Amitraz



The New Challenge of LOD: Fluvalinate

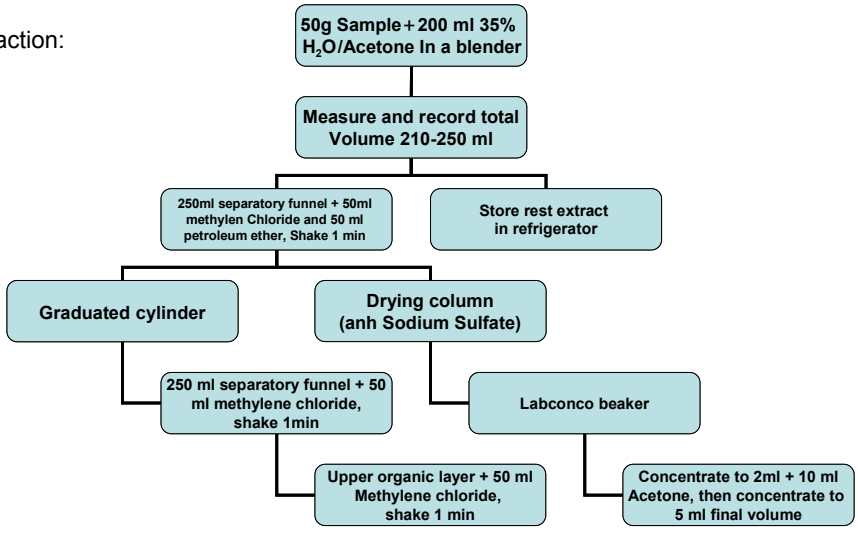


Tolerance Level in Honey:

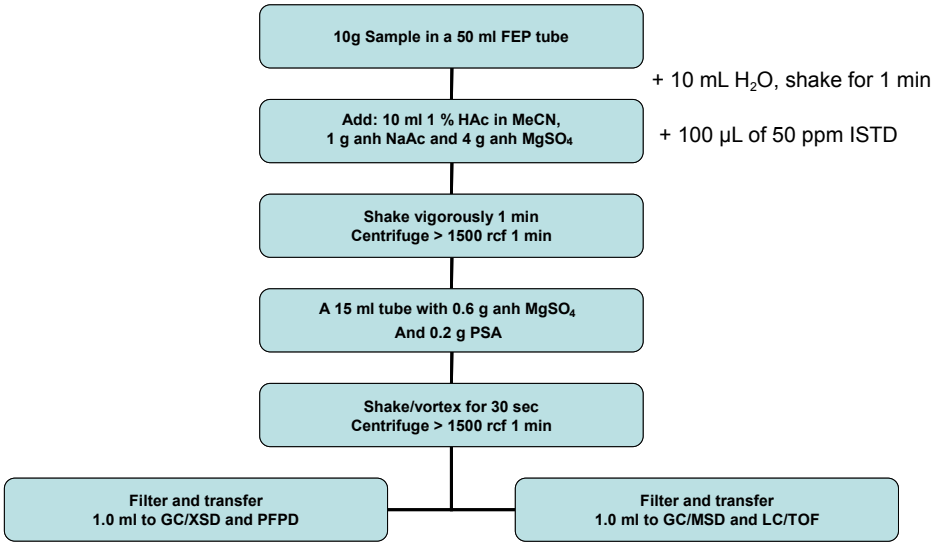
Fluvalinate	0.05 ppm
Coumaphos	0.1 ppm
Amitraz	1.0 ppm

Experimental

The Old Luke method for Honey Extraction:

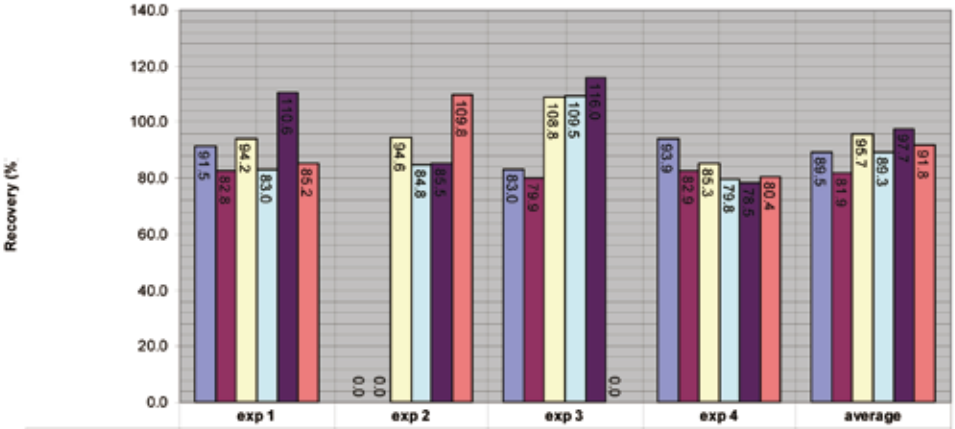


The new QuEChERS method for honey extraction



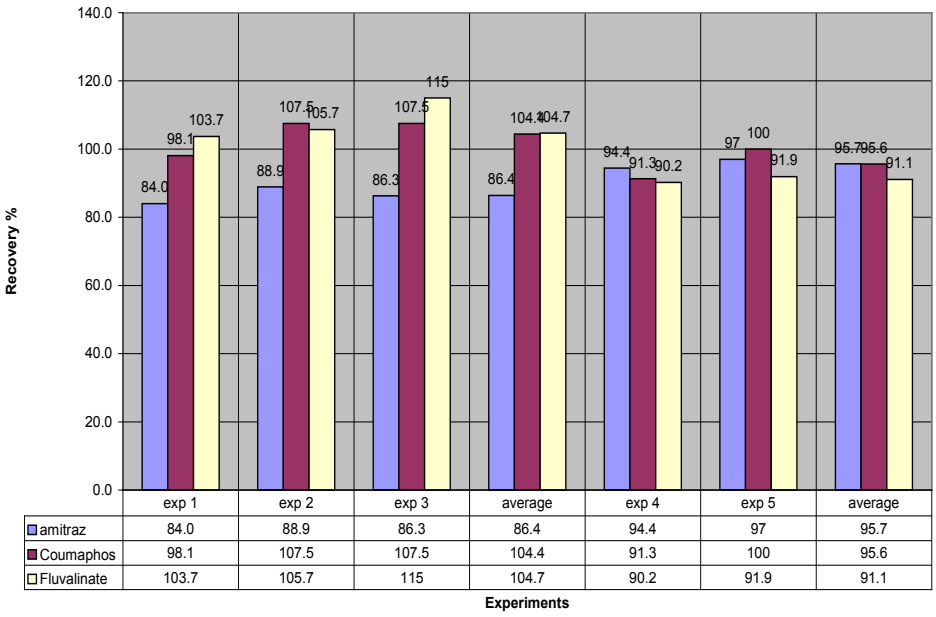
Results

Pesticide Recoveries by PFPD
Amitraz Recovery by MSD



	exp 1	exp 2	exp 3	exp 4	average
m-CPRF	91.5	0.0	83.0	93.9	89.5
endosulfans	82.8	0.0	79.9	82.9	81.9
endosulfan sulfate	94.2	94.6	108.8	85.3	95.7
permethrins	83.0	84.8	109.5	79.8	89.3
cypermethrins	110.6	85.5	116.0	78.5	97.7
coumaphos	85.2	109.8	0.0	80.4	91.8

LC/MS/TOF recovery



	exp 1	exp 2	exp 3	average	exp 4	exp 5	average
amitraz	84.0	88.9	86.3	86.4	94.4	97	95.7
Coumaphos	98.1	107.5	107.5	104.4	91.3	100	95.6
Fluvalinate	103.7	105.7	115	104.7	90.2	91.9	91.1

Experiments

Conclusion

Method	Sample (gram)	Solvent (mL)	Experiment time	Equipment	Toxicity	Others
Old Luke	50	400	4 hrs	250 ml Glass separate funnels, Drying columns	Methylene Chloride	A lot of Dish washings And Waste Most tubes
QuEChERS	10	10	1 hr	Tubes	MeCN	disposable, and less waste Environment
Advantages	Save 80% sample	Save 97% solvent	Save 75% time	Cost Cut	Less toxic	Friendly

Detection of Cyromazine Residues in Vegetables with HPLC (Cleanert® SCX, P/N: SC5006)

EMF10025

Material

Cleanert® SCX, 500mg / 6mL

Experimental

Sample Preparation

Mince the edible part of vegetable sample. Collect the minced sample by quartation into a PE container and store it at 16 to 20 Celsius. Defreeze the sample at room temperature before use.

Sample Extraction

Weigh 20.00 g sample into a beaker, and then add 50 mL mixture of 0.05 M ammonium acetate solution and acetonitrile (1:4, v/v). Homogenize the sample mixture at 14000 rpm for 2 minutes. Filter the sample slurry through a Buchner funnel into a 100 mL volumetric flask. First rinse of the sample beaker with 35 mL ammonium/acetonitrile solution, and then homogenize the remaining sample at 14000 rpm for 30 seconds, and transfer the mixture into the funnel. Add additional ammonium/acetonitrile solution into the collecting flask to bring up volume to 100 mL and mix well the sample solution. Pipette 10 mL sample solution into a round-bottom flask, and then rotovap the sample at 40 Celsius to remove the organic solvent. Adjust the pH of the sample concentrate to 2 with 0.1 M HCl solution, and the concentrate is ready for SPE cleanup.

SCX Procedure

- Precondition SCX cartridge by adding 5 mL methanol first followed by 5 mL de-ionized water.
- Add sample mixture from the last step to the cartridge, and discard the eluate.
- Wash the cartridge first with 5 mL methanol and then with 5 mL de-ionized water, and discard the eluate and dry the cartridge.
- Elute the cartridge three times subsequently with 5 mL 5% ammonina/methanol a time, and collect the eluate into a round-bottom flask.
- Evaporate the collected eluate to nearly dryness with a rotovap at 40 Celsius, then blow the sample to dryness under nitrogen.
- Reconstitute the sample residue with 1 mL acetonitrile/water (93:7, v/v), and filter the solution through 0.45 µm membrane into a HPLC vial.

Detection

Column: Venusil® XBP NH_2 column, 4.6 × 250 mm, 5 µm (VN852505-0)
Mobile phase: acetonitrile/water = 97:3 (v/v)
UV: 215 nm
Flow rate: 1.0 mL/min
Injection volume: 10 µL
Column temperature: 35°C

Detection of Organophosphorus, Organic Chloride and Carbamates Residues in Vegetables (Cleanert® Florisil, P/N: FS0006)

EMF10026

Material

Cleanert® Florisil, 1g / 6mL

Experimental

Sample Preparation

Cole plants or tomato samples are minced into small pieces followed by blending using a food processor. Store the samples in subpackages at 16°C~ -20°C.

Extraction

Weigh 25 g of sample accurately and place in homogenizer. Add 50 mL of acetonitrile and homogenize for 2 min. Filter the solution through filter paper into a 100 mL graduated cylinder with cap containing 5~7 g sodium chloride in it. Collect 40~50 mL of filtrate. Cap the graduated cylinder and shake for 1 min. Stand at room temperature for 30 min to allow acetonitrile and water phases separated.

Florisil Cleanup

Transfer 10 mL of acetonitrile solution into a 150 mL beaker. Heat the beaker in water bath at 80°C and evaporate the solution under nitrogen or air stream almost to dryness. Add 2 mL of n-hexane and cover the beaker with aluminium foil for further cleanup. Pre-washand condition Florisil cartridge sequentially with 5 mL of acetone/hexane (10:90, v/v) and 5 mL of hexane. When the solvent reaches the adsorption layer of the cartridge, load the sample in beaker onto the cartridge. Collect the eluate in a 15 mL centrifuge tube. Wash the beaker with 5 mL of acetone/hexane(10:90, v/v) twice. Combine the solutions onto the cartridge and collect the eluates into the centrifuge tube. Evaporate under nitrogen stream in water bath at 50°C to less than 5 mL. Dilute to 5 mL with hexane again. Vortex mix and transfer the solution to two 2 mL sample vials for GC analysis. (column: DA-50+, 30 m × 0.53 mm × 1.0 μm, P/N: 5053-3010, DA-1, 30 m × 0.53 mm × 1.5 μm, P/N: 0153-3015).

Results

Recoveries of tomato and cole samples, with low and high levels of pigments respectively, are studied (spiked concentrations: 0.1 mg/kg and 0.2 mg/kg). For each concentration level, experiments are repeated three times. The results are summarized in Table 1.

Table 1 Recoveries of tomato and cole samples

Pesticides	Spiked concentration 0.1 mg/kg		Spiked concentration 0.2 mg/kg		Average
	Tomato	Cole	Tomato	Cole	
Chlorothalonil	72.6	68.8	70.0	70.9	70.6
Ketotriazole	88.6	84.7	87.8	82.7	88.4
Cyfluthrin	91.0	97.6	97.1	98.0	93.4
Cypermethrin	81.8	77.7	82.0	83.0	81.1
Fenvalerate	77.0	72.0	78.0	79.8	76.7
Fenpropathrin	77.7	77.1	81.2	79.0	78.5

Detection of 466 Pesticide Residues in Vegetables and Fruits (Cleanert® PestiCarb/NH_2, Cleanert® C18, P/N: PN0006, S18200012)

EMF10027

Material

Cleanert® C18, 2g / 12mL; Cleanert® PestiCarb/NH_2, 500mg / 500mg / 6mL

Experimental

Extraction

Weigh 20 g (accurate to 0.01 g) of sample in a 80 mL centrifuge tube. Add 40 mL of acetonitrile and homogenize at 15000 r/min for 1 min. Add 5 g of sodium chloride and homogenize for 1 min again. Centrifugate at 3000 r/min for 5 min and take 20 mL (corresponding to 10 g of sample) of the supernatant for further cleanup.

Cleanert® C18 and PestiCarb/NH_2 cleanup

(1) Activate Cleanert® C18 cartridge with 10 mL of acetonitrile. Load the sample (2) onto the cartridge and elute with 15 mL of acetonitrile. Collect the solutions in a pear-shaped bottle and evaporate to 1 mL by rotary evaporator in water bath at 40°C for later cleanup.

(2) First fill PestiCarb/NH_2 cartridge with sodium sulfate anhydrous to a 2 cm bed height; and use a pear-shaped bottle under the cartridge for collection. Wash the cartridge with 4 mL of acetonitrile/toluene (3:1, v/v). When the washing solution reaches the upper surface of the sodium sulfate, load the concentrated sample (3.1) onto the cartridge. Wash the pear-shaped bottle (3.1) with 2 mL of acetonitrile/toluene (3:1, v/v) 3 times. Combine the washing solutions onto the cartridge. Wash the cartridge with 25 mL (divided into several times) of acetonitrile/toluene (3:1, v/v). Collect the eluate in pear-shaped bottle and evaporate to 0.5 mL by rotary evaporator in water bath at 40°C. Add 5 mL of n-hexane to exchange the solvent and evaporate by rotary evaporator in water bath at 40°C. Repeat the solvent exchange process and obtain 1 mL of sample. Add 40 µL of internal standard solution and mix for GC-MS analysis. (Column: DA1701, 30 m × 0.25 mm × 0.25 µm, P/N: 6125-3002)

Detection of Food Additives

Detection Phthalates in Different Food Matrices

EMF10028

Introduction

Phthalic acid esters or phthalates are commonly used as plasticizers. Although phthalates are being phased out in many developed countries over health concern, occurrence of such substances recently in food products in other parts of the world has drawn considerable publicity over the same health issue. SPE products from Bonna-Agela provide effective tools to rapidly process food samples for detection and analysis of such substances. Detail procedure and methodology are illustrated here for such applications with plenty of real-life samples.

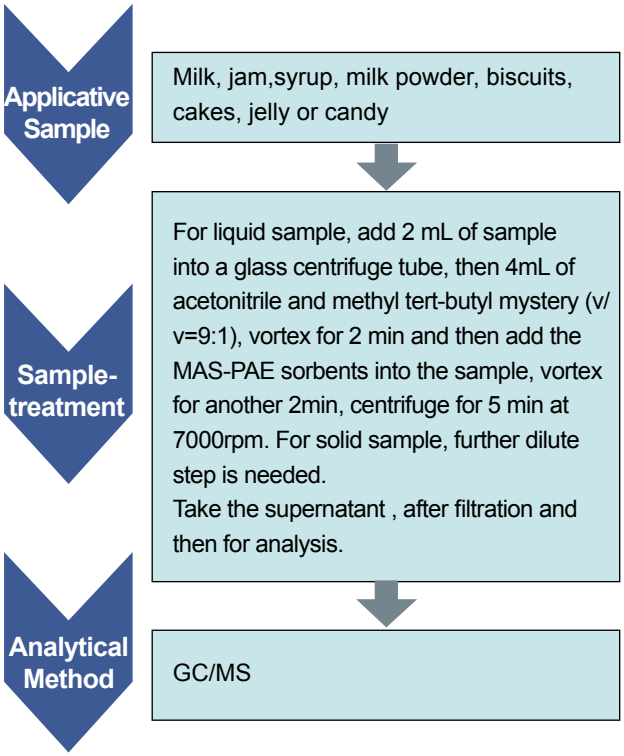
Experimental

Suggested Processing Method

Applicative Sample	Bottled Drinking water and Soft drink
SPE Process	activation: 5 mL of methanol, 5 mL of water Sample loading: 10 mL liquid sample Washing: 5 mL water contained 5% methanol, vacuum for 20min Elute: 5 mL of methanol. Concentrated the elution to dryness with N_2, and reconstitute with 1mL of methanol.
Analytical Method	HPLC

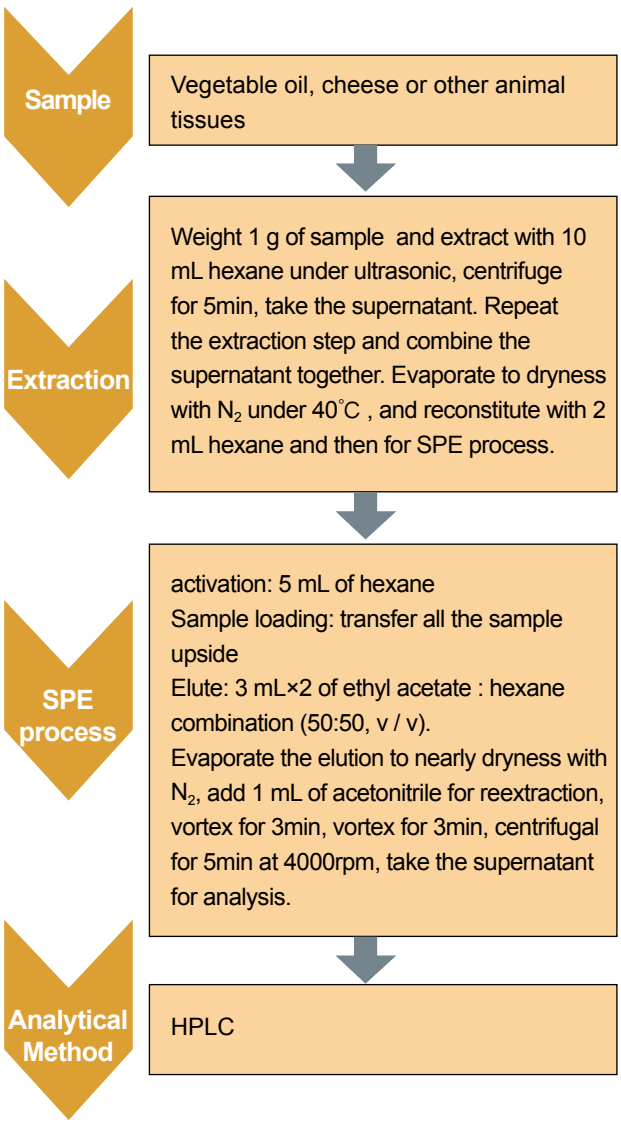
Ordering Information

SPE Cartridge	Cleanert® DEHP; 500 mg / 6 mL; Cat.No: DEHP5006-G, 30/pk
HPLC columnn	Venusil® XBP C18-L; 4.6×250 mm, 5 μm,150 Å; Cat.No: VX952505-L



Ordering Information

SPE Cartridge	Cleanert® MAS-PAE; Cat. No: MS-PAE40, 15/pk Cleanert® MAS-PAE (sample with colorant); Cat. No: MS-PAE40-C, 15/pk
HPLC columnn	PAE Syringe filters; Cat.No: PAE041345-T; 100/pk
GC Column	DA-5MS; 30m×0.25mm×0.25μm; Cat. No: 1525-3002, 1/pk



Ordering Information

SPE Cartridge	Cleanert® PAE, Cat. No:PAE30006-G, 30/pk Cleanert® PAE (Sample with colorant); Cat.No:PAE30006-C; 30/pk
GC Column	DA-5MS, 30 m×0.25 mm×0.25 μm, Cat. No: 1525-3002, 1/pk

Method 1 The detection of 15 pthalate esters with HPLC

Column	Venusil® XBP C18 L, 4.6 × 250 mm, 5 μm, 150 Å (Cat. No: VX952505-L)
Mobile Phase	A: water; B: methanol: acetonitrile = 50:50
Flow Rate	1.0 mL/min
Wavelength	242 nm
Loading	50 μL
Sample	15 pthalate esters
Concentration	10 ppm
Solvent	40% mobile phase A
ColumnTemperature	30 ℃

Elution gradient:

Time / min	A / %	B / %
0	60	40
2	50	50
10	40	60
12	30	70
20	30	70
31	0	100
40	0	100
40.01	60	40

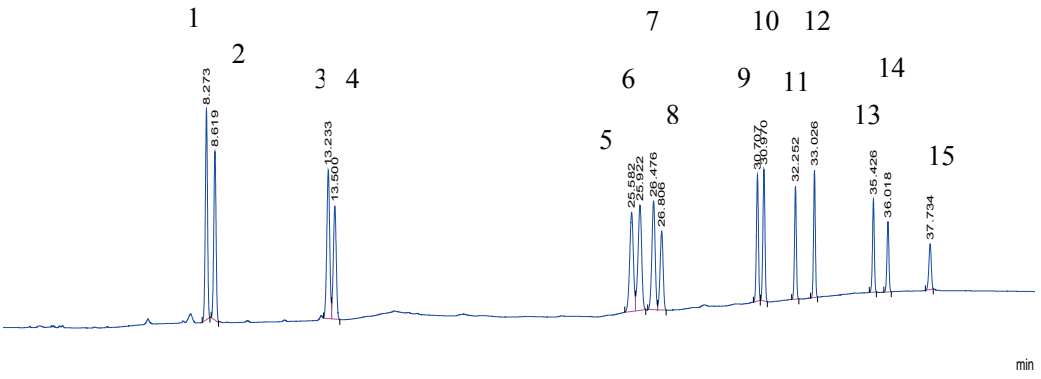


Fig 1. The chromatogram for pthalate esters standard (sample concentration: 10 ppm)
(1-DMP, 2-DEP, 3-DBP, 4-DEHP, 5-BBP, 6-DEHP, 7-DMEP, 8-DBEP, 9-DPP, 10-BMPP, 11-DEEP, 12-DCHP, 13-DIBP, 14-DNP, 15-DINP)

15 Pthalates were separated as shown in Figure 1 with good resolution.
Detection limit: 0.5 ppm
Linear relationship: Y = 26.8X-26.6, R2 = 0.941

Method 2 The detection of 15 pthalate esters with GC/MS

Instrument	Agilent 7890/5975 GC/MS
GC Column	DA-5MS 30 m × 0.25 mm × 0.25 μm (1525-3002)
Injection	250 °C, splitless injection
Temperature Program	50 °C (1 min) 20 °C/min 220 °C (1 min) 5 °C/min 280 °C (4 min)
Loading Volume	1 μL
Flow Rate	1 mL/min
Inlet Temperature	280
Ionization Mode	EI
Ionization Energy	70eV
Solvent Delay	7 min
Monitoring Methods	SIM mode, ion monitored as below

No	Retention Time (min)	Name	SIM
1	8.265	DMP	163, 77
2	9.135	DEP	149, 177
3	10.888	DIBP	149, 223
4	11.637	DBP	149, 223
5	11.979	DMEP	59, 149, 193
6	12.72	BMPP	149, 251
7	13.044	DEEP	45, 72
8	13.41	DPP	149, 237
9	15.552	DHXP	104, 149, 76
10	15.694	BBP	149, 91
11	17.153	DBEP	149, 223
12	17.81	DCHP	149, 167
13	18.056	DEHP	149, 167
14	20.444	DNOP	149, 279
15	22.98	DNP	57, 149, 71

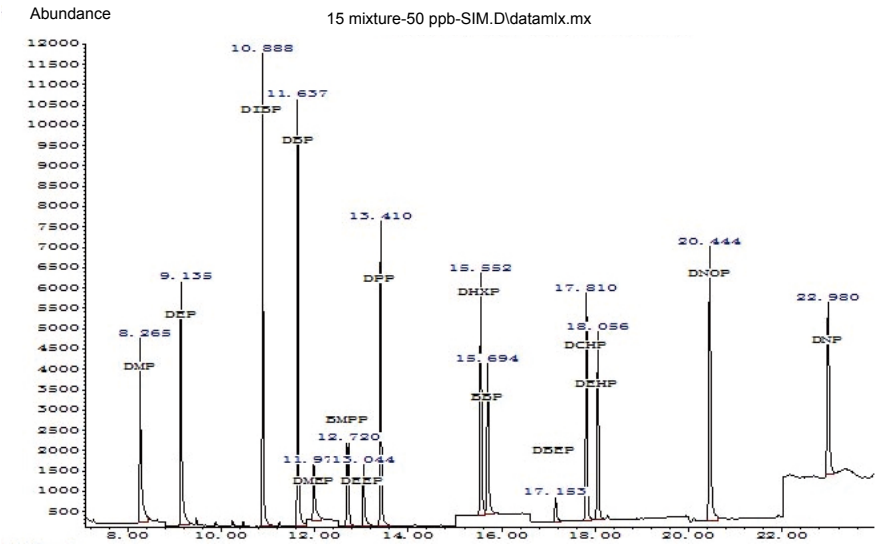


Figure 2. Detection of 15 pthalates using GC-MS method

Figure 2 shows the separation profile of 15 pthalates using GC-MS. Each component is well resolved, and the detection limit reaches ppb level.

The recoveries of 15 phthalate esters in vegetable oil

Validation of SPE procedures for 15 phthalates in vegetable oil shown with method recovery data when samples were spiked at 500 μg/L.

No	Retention time/min	Sample	Recovery
1	8.308	DMP	149.97%
2	9.185	DEP	93.49%
3	10.96	DIBP	125.70%
4	11.716	DBP	136.89%
5	12.064	DMEP	90.84%
6	12.778	BMPP	82.29%
7	13.144	DEEP	106.38%
8	13.518	DPP	88.14%
9	15.686	DHXP	75.32%
10	15.844	BBP	89.56%
11	17.295	DBEP	105.05%
12	17.967	DCHP	72.94%
13	18.206	DEHP	124.27%
14	20.625	DNOP	78.19%
15	23.297	DNP	75.27%

Detection of Acrylamide in Chips and Fried Bread Stick (Cleanert® ACA, P/N: ACA2006)

EMF10029

Introduction

Acylamide(CAS RN 79-06-1) is a colourless, odourless transparent platy crystals; it is soluble in water, alcohol, acetone, ether and chloroform, slightly soluble in toluene, insoluble in benzene and heptane. The relative molecular weight is 71.08, and the structural formula is shown in Figure 1. Acrylamide is a recognized neurotoxin and quasi carcinogens, both animal experiment and in vitro cell experiment prove that acrylamide also can lead to changes in the genetic material, and it has been listed as 2A class carcinogen by International Agency for Research on Cancer early in 2005. April 2002, the Swedish National Food Administration found that many foods containing starch will produce high levels of acrylamide after frying, baking, frying and other high-temperature cooking. Many studies suggest that potato products such as chips and crisps have highest content of acrylamide, and are 500 times of the maximum drinking water allowed limit.

Therefore, it is important to monitor the acrylamide in food to ensure the safety of food consumption by effective experimental technological method. This experiment reference to the first method of national standard method, using Cleanert ® ACA solid phase extraction cartridge to purify samples and established a quickly and effectively acrylamide in food by LC-MS / MS detection method.

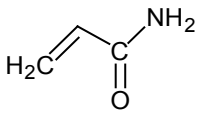


Figure 1. Structural formula of acrylamide

Experimental

Instruments, Reagents and Materials

(1) Main instruments and equipment
AB SCIEX API 4000+Liquid Chromatograph Mass Spectrometer;
Qdaura® automatic solid-phase extraction apparatus;
(2) Reagents material
Original taste chips and fried bread stick of a relevant brand;
Formic acid, methanol, and hexane are all chromatographically pure; Watsons water;
Acrylamide standard substance (purity > 99%), dissolved in water;
D3-acrylamide standard solution (CD2=CDCONH2) (concentration of 500 mg/L, acetonitrile as solvent);
The substrate standard working solution;
0.1% formic acid solution: take 100 μL formic acid and add 100 mL water, shake well;
The disposable sterile syringes; Nylon pin type filter(0.22 μm, diameter of 13 mm);
Cleanert® ACA solid phase extraction cartridge:200 mg/6 mL

Sample preparation

Sample extract

Accurately weigh 1 g crushed sample into 50 mL centrifuge tube, add 200 μL D3 - acrylamide internal standard (1 mg/L), then add 10 mL water, after ultrasonic oscillation 30 min, centrifuge at 8000 r/min for 5 min , transfer the supernatant to 15 mL centrifuge tube, add 5 mL n-hexane, vortex extract 1 min, centrifuge at 6000 r/min for 5 min, remove the upper organic phase, repeat extract again with 5 mL n-hexane, and then quickly take 5 mL water phase as the sample load solution.

Sample purification

Manual operation

Before using, activate Cleanert® ACA solid phase extraction cartridge(200 mg/6 mL) with 5 mL metanol and 5 mL water, load sample into Cleanert® ACA phase extraction cartridge, wash with 5 mL water, vacuum dry column, eluted with 5 ml methanol, collect all the eluate and nitrogen blowing concentrated to nearly dry at 40˚C , constant volume to 1 mL with water for LC-MS/MS detection.

Qdaura® automatic solid phase extraction apparatus operation procedure

First install Cleanert® ACA solid phase extraction cartridge on the instrument, and inject samples into the sample loading tube, automatic operation according to the procedure in the following figure:

HPLC Method

HPLC colum: Venusil® AQ C18, 5 μm100 Å2.1 × 150 mm;
Mobile phase: 0.1% formic acid aqueous solution;
Column temperature: 26˚C ;
Flow rate: 0.2 mL/min;
Injection volume: 2 μL;

Mass spectrometry conditions

Ion source:ESI+;
Electrospray voltage: 5500 V;
Atomization gas pressure: 45 psi;
Air curtain pressure: 10 psi;
Aux Gas Pressure: 45 psi;
Ion source temperature: 330˚C ;
Acquisition methods: multiple reactions monitoring (MRM).

Figur 2. Automatic solid phase extraction operation procedure

Table 1 Mass spectrum parameters of acrylamide

Substance	Q1	Q3	CE/V
acylamide	72	55	15
		44	15
D3- acylamide	75	58	16
		44	25

Results

Linear relation

Prepare acrylamide standard solution with the concentration of 0.01 mg/L, 0.05 mg/ L, 0.1 mg/L, 0.5 mg/L, 1 mg/L and 3 mg/L respectively (internal standard: 0.1 mg/L) for LCMS/MS detection. Take the injection concentration of each acrylamide standard solution (mg/L) as the abscissa, take the rate of acrylamide peak area and internal standard D3 - acrylamide peak area as the ordinate, draw the standard curve, as shown in figure 3, the fitting parameters are shown in table 2.

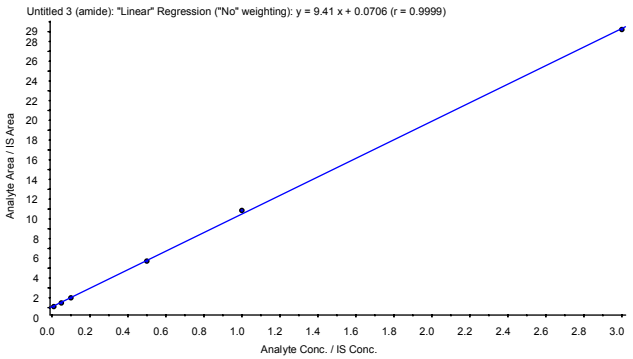


Figure 3. Standard solution curve of acrylamide

Table 2 Standard curve and the detection limit of acrylamide

Compound	Standard curve equation	Correlation index	Detection limit(mg/L,S/N = 3)
acylamide	Y = 9.41 X + 0.0706	0.9999	0.005

Experimental results

Table 3 indicate that when using Cleanert® ACA solid phase extraction cartridgecombined with LCMS/MS method to detect acrylamide, the recovery rate of 0.1 mg/kg and 2 mg/kg acrylamide spiked amount is 100% ~ 110%, which meet the detection requirement.

Table 3 Spiked recovery experiment results of acrylamide (n=3)

Substance	Operation mode	Spiked level/ (mg/kg)	Retention time/min	Average recovery rate/%		RSD/%	
				chips	fried bread stick	chips	fried bread stick
acylamide	automatic	0.1	3.52	101.7	101.2	4.5	1.6
	manual	2.0		100.4	102.7	1.4	2.0

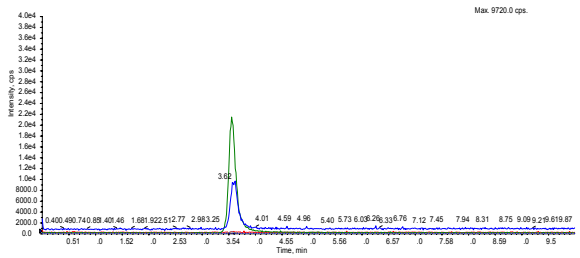


Figure 4. 0.05 mg/L LC-MS/MS spectra of acrylamide standard solution

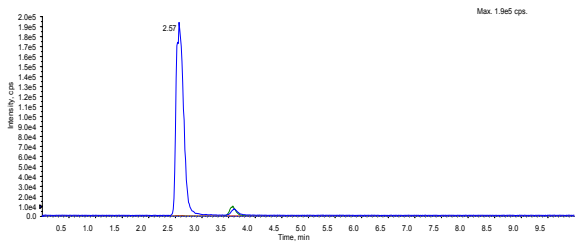


Figure 5. Matrix blank LC - MS/MS spectra of chips sample

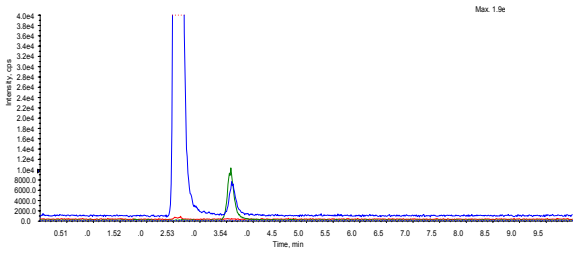


Figure 6. Enlarged spectra of chips sample

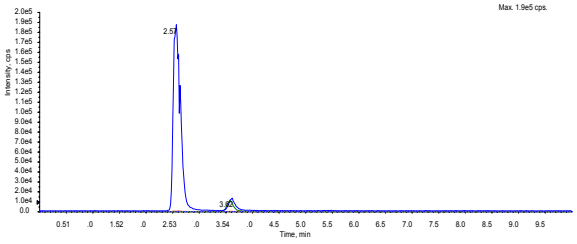
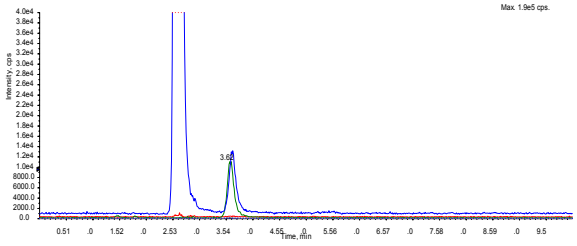


Figure 7. LC-MS/MS spectra of matrix spiked 0.1 mg/kg chips sample



Figur 8. 0.1 mg/kg Enlarged spectra of matrix spiked 0.1 mg/kg chips sample

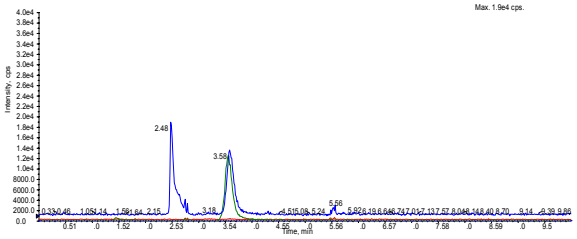
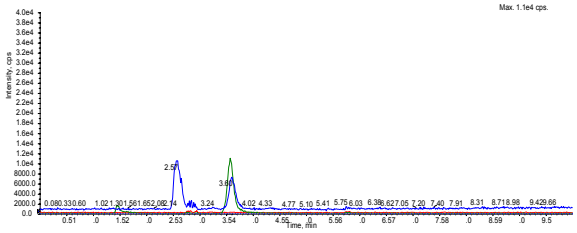


Figure 9. LC-MS/MS spectra of fried bread stick sample matrix standard work solution



Figur 10. LC-MS/MS spectra of blank fried bread stick sample matrix

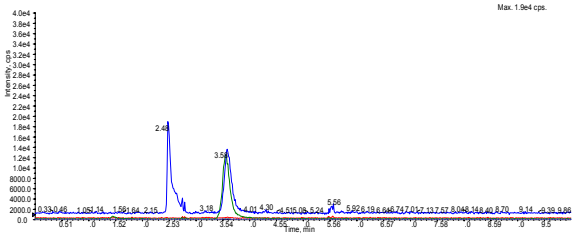


Figure 11. LC-MS/MS spectra of spiked 0.1 mg/k fried bread stick sample matrix

Ordering Information

Products	Specification	Cat.No.
Venusil® AQ C18	5 μm,100 Å, 2.1 × 150 mm	VA951502-0
Cleanert® ACA	200 mg/6mL	ACA2006
Qdaura® automatic solid-phase extraction apparatus	Maximal 24 sample, four channels	SPE-40
Nitrogen blowing instrument	Maximal 15 sample	NV15-G
1.5 mL sample bottle	short thread transparent belt with writing place 32 × 11.6 mm	1109-0519
1.5 mL sample bottle cap	9 mm center hole with blue cover, red rubber/beige PTFE insulation pad, 45°Shore A; 1.0 mm	0915-1819
Syringe Filter(Nylon)	monofilm, 13 mm, 0.22 μm	AS021320
disposable syringe	2 mL needle-free	LZSQ-2ML

Detection of Chloropropanol Content in Soy Sauce by SLE and GCMS Method (Cleanert® MCPD P/N: LBC400030)

EMF10030

Introduction

Chloropropanol is a kind of toxic carcinogenesis generated in chemical production of soy sauce. It also has inhibitory effect on the production of male hormones which decrease the reproductive ability. It is greatly harmful to human body. Daily common three kinds are as following: 1- chloro -2- propanol (ClCH2CHOHCH3); 3- chloro -1,2- propanediol (3-MCPD) and 1,3- dichloro-2- propanol (1,3-DCP).

Material

SPEcartridge

Cleanert® MCPD (chloropropanol special column),4 g/30 mL(P/N:LBC400030)

Standard solution preparation

Accurately weigh 0.1 g chloropropanols standard substance in to100 mL volumetric flask, constant volume to scale by ethyl acetate and get 1 mg/mL stock solution. Gradually dilute the stock solution with acetone to get 1 μg/mL standard working solution.

Saturated sodium chloride solution

Weigh 290 g sodium chloride, add water to dissolve and dilute to 1000 mL, and then ultrasonic for 20 min

Experimental

GC-MS operating conditions

Chromatographic column: DA-5MS 30 m × 0.25 mm × 0.25 μm
Injection port: 230℃ , splitless injection
Temperature programming: 50℃ (1 min) 2℃ /min 82℃
Injection volume: 1 μL
Flow velocity: 1 mL/min
Interface temperature: 250℃
Ionization mode: EI
Ionization energy: 70 eV
Solvent delay: 7 min
Ion source: 230℃
Quadrupole: 150℃
Detection mode: selective ion detection;
SIM ion: 253/275/289/291/453

Sample preparation

Weighs 2.5 g soy sauce directly loads to the Cleanert MCPD solid phase extraction column, static balance for 10min, wash the column with 15 mL ethyl acetate and collect the eluent. Nitrogen blow the eluent to nearly dry (not dry completely) at 35℃ . Add 2 mL n-hexane, shake, quickly add 50 μ L seven fluorine butyl acyl imidazole, tighten the sample bottle, vortex for 20 seconds, put the sample bottle into 70℃ thermotank to react for 30 min, take out and cool to room temperature, add 2 mL Saturated sodium chloride solution into sample bottle, vortex 1 min, stewing for 2 min, taking the upper organic phase to another clean sample bottle, repeat 1 times washing operation to remove impurities. After removing water with a small amount of anhydrous Na_2SO_4, transfer the organic phase to injection sample bottle for GC-MS detection.

Result

Chromatogram of standard solution

Under GC-MS operation condition, chromatogram of standard solution is shown in Figure 1.

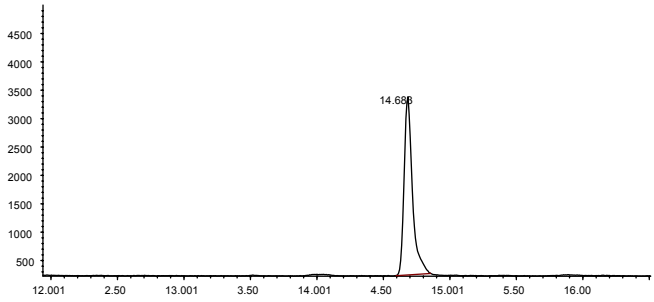


Figure1 Chromatogram of standard solution (concentration of 50 ng/mL)

Chromatogram of samples

Accurately weigh 6 pieces of the soy sauce, add 1 μg/mL standard solution into five pieces respectively. Purify derivative 6 samples according to the processing method above and get chromatogram of spiked soy sauce sample and chromatogram of soy sauce sample as shown in Figure 2 and Figure 3.

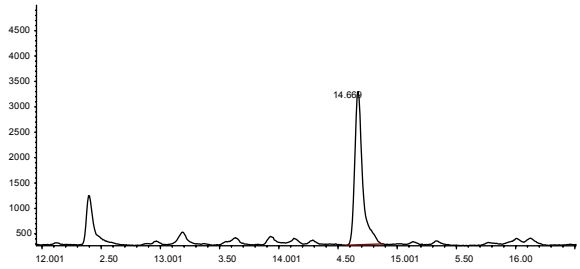


Figure 2 Chromatogram of spiked soy sauce sample (concentration of 50 ng/mL)

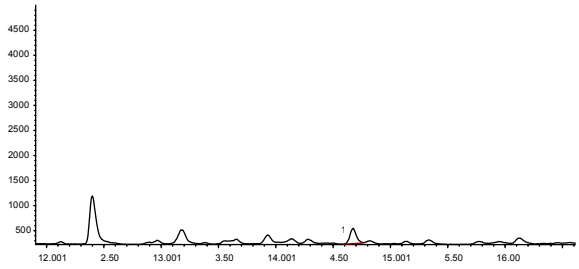


Figure 3 Chromatogram of soy sauce sample

Table 1 Recovery rate and precision

	1#	2#	3#	4#	5#	Average recovery rate/%	RSD/% n=5
Recovery Rate(%)	88.0	83.9	90.5	83.6	92.1	87.60	3.84

Ordering Information

Products	Specification	Cat.No.
Vacuum manifold	12 position	VM12
Cleanert® MCPD column	4 g/30 mL, 15/pk	LBC400030
Cleanert® MAS-MCPD	2 g, 50/pk	MS-MCPD01
DA-5MS GC columns	30 m × 0.25 mm × 0.25 μm	1525-3002
1.5 mL sample bottle	short thread transparent belt with writing place 32 × 11.6 mm, 100/pk	1109-0519
1.5 mL sample bottle cap	9 mm center hole with blue cover, red rubber/beige PTFE insulation pad, 45. Shore A1.0 mm, 100/pk	0915-1819

Detection of Benzo (a) pyrene in Grease by HPLC Method (Cleanert® BAP P/N: BAP2260-0)

EMF10031

Material

Cleanert® BAP, 22g / 60mL, P/N: BAP2260-0

Experimental

Sample Preparation

Weigh about 0.300 g grease sample, dissolved in 5 mL n-hexane and fully mixing on the vortex mixer.

SPE Method

Conditioning 30 mL n-hexane. (Attention: continuous adding n-hexane into the column and keep n-hexane above the upper sieve plate of column to avoid air entering the column.)
Loading sample: add the dissolved grease sample onto the Cleanert BAPcartridge, attention that the upper sieve plate cannot dry.
Elution: add 80 mL n-hexane and receive with 150 mL spin steaming bottle until 80 mL of n-hexane is completely dropped natural. In order to ensure the recovery rate, the elution volume of n-hexane can be increased, and the maximum volume up tp 120 mL.
Rotary evaporation: rotate evaporation the eluent in 45℃ water bath to dry, if there are still oil droplets can't dry out, it indicate that the grease content in the sample is higher, and purification is incomplete, it need to add 80 mL n-hexane into of oil droplets to made new sample, and using a new column to repeat the purification process;
Concentration: leaching spin steaming bottle three times with a total amount of 10 mL n-hexane, merge eluent to nitrogen blowpipe and blow dry with nitrogen. Add 300 μL n-hexane to nitrogen blowpipe, thoroughly mixing above the vortex mixer. Avoid n-hexane evaporation during vortex process. Transfer 300 μL n-hexane above into insert-pipe of 1.5 mL sample bottles and load sample for analysis.

Experimental

HPLC column: Venusil ASB C18 250 mm × 4.6 mm, 5.0 μm (P/N: VS952505-0)
Flow rate: 1.0 mL/min
Mobile phase: acetonitrile:water=88:12
Emission wavelength: 406 nm ;excitation wavelength: 384 nm

Results

This method using Cleanert® BAP SPE cartridge for the purification treatment of benzo (a) pyrene in a vegetable oil and the recovery rate of 5 μg/kg addition level is 99.49%. The chromatogram results of using Venusil® ASB C18 are shown in figure 1-3.

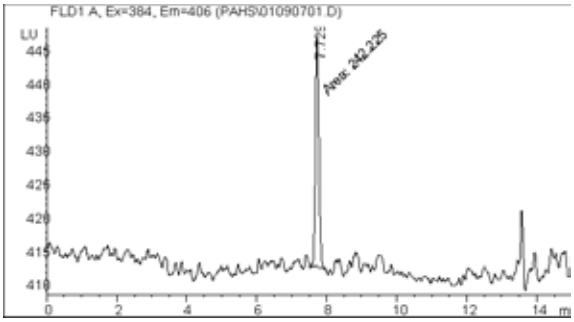


Figure1 Chromatogram of 5 μg/L benzo (a) pyrene standard sample

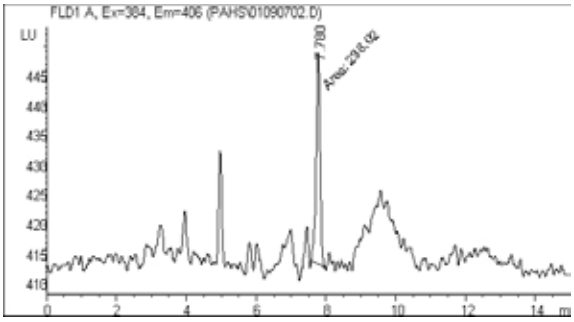


Figure 2 Actual sample chromatogram of a vegetable oil

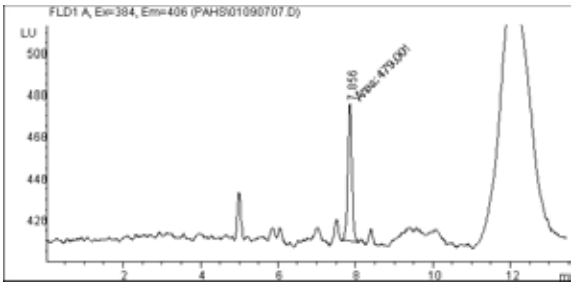


Figure 3 Chromatogram of a vegetable oil with the spiked matter amount of 5 μg/kg

Table1 Recovery rate of benzo (a) pyrene

	5 μg/L standard substance	actual grease sample	spiked grease sample	recovery rate
Response value of benzo (a) pyrene	242.225	238.02	479.001	99.49%

Detection of Benzo(a)pyrene in Vegetable Oil By Solid Phase Extraction(Cleanert® BAP-3 P/N: BAP5006)

EMF10032

Overview

The determination of benzo(a)pyrene in oil generally used prepacked alumina column, and big volume of elution is needed and take lots of labor and time.
Bonna-Agela develop a new SPE product with much more convenient method.The main challenge is to extract hydrophobic Benzo(a)pyrene from oil matrix. Hexane was chosen as the best solvent to dissolve vegetable oil after investigation.
In the new method, 0.5 g vegetable oil was dissolved in 3 mL hexane and loaded onto the Cleanert® BAP-3 cartridge. 10mL hexane was used to wash the cartridge and 5mL methylene chloride to elute the Benzo(a)pyrene out. After drying the eluant with nitrogen blowing, 1mL acetonitrile was used to reconstitute the sample. The detection was carried out on HPLC with fluorometric detection. In addition, sesame oil contained more impurities which was need to be treated with Cleanert® SI (500 mg/6 mL) firstly.This new method show better performance, and could be used for many kinds of vegetable oil.

Reagents and apparatus

n-Hexane (HPLC grade), Dichloromethane (HPLC grade), Acetonitrile (HPLC grad);
Benzo(a) pyrene (100 mg/mL); PTFE syringe filter(0.22 μm)
SPE cartridge: Cleanert® BAP-3 (P/N: Bap5006)
Bonna-Agela 12 Position Vacuum manifold (P/N: VM12), NV-8G Evaporator(P/N: NV-8G)

Experimental

Sample Preparation

Take 0.5g of oil sample in 12ml vial. Then added 3mL hexane and shocked it with ultrasound instrument to get the extraction solution. Spiked with Benzo(a)pyrene standard solution into 0.5g oil sample if recovery investigation of Benzo(a) pyrene was needed.

SPE Process:
(1) Condition: 5ml Dichloromethane and 5ml Hexane;
(2) Load all the 0.5g sample onto the cartridge;
(3) Washing: 10ml Hexane;
(4) Elution: 5ml Dichloromethane.
Collected the eluant in 12 mL vial and dried it by Nitrogen blowing at 40°C. Then 1mL Acetonitrile was added to reconstitute it. After 10s treatment by ultrasound and filtration by 0.22 μm PTFE syringe filter, then for further analysis. This method is adapt to peanut oil, soybean oil, corn oil, sunflower oil, rice oil, sesame oil, cooking oil, canola oil and olive oil.For sesame oil, and anothher Cleanert® Si (500mg/6mL, P/N:Si5006) cartridge was needed to place onto the Cleanert® BAP-3 cartridge, use the same process as describe upside and remove the Silica cartridge before elution.

HPLC Condition

HPLC Column: Venusil® ASB C18, 4.6 mm×250 mm×5.0 μm (P/N: VS952505-0);
Mobile phase: Acetonitrile: Water (88/12,V/V);
Flow rate: 1.0 mL/min;
Injection sample: 20 μL;
Fluorescence Detector: excitation spectrum 384, emission spectrum 406;
Column temperature: 30℃

Results

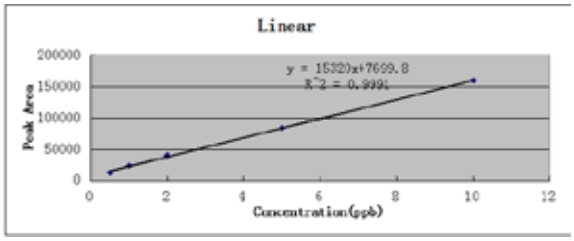


Figure 1 Benzo(a)pyrene calibration curve

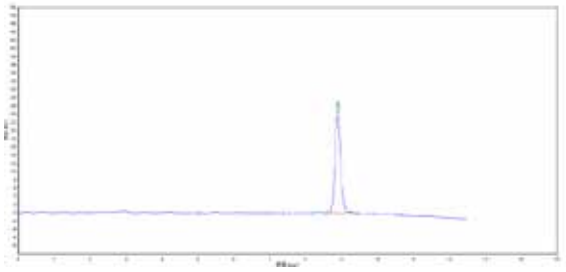


Figure 2 Benzo(a)pyrene standard solution (10μg/L)

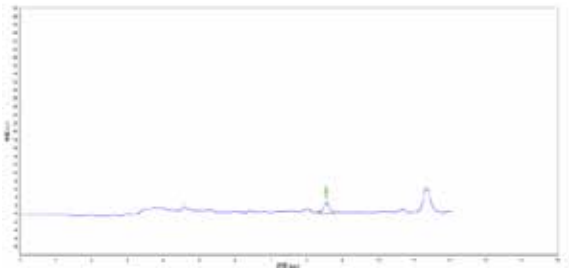


Figure 3 HPLC chromatogram of blank oil sample

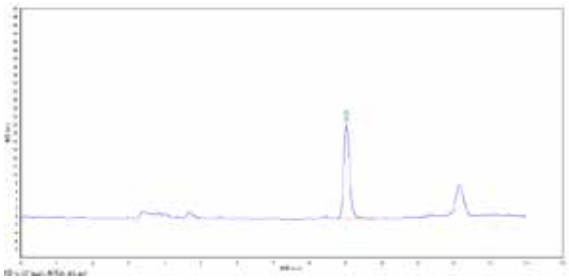


Figure 4 HPLC chromatogram of sample spiked with Benzo(a)pyrene(10μg/L)

Table1. Recovery of Benzo(a)pyrene

Spiked concentration	Recoveries	RSD %
10 (μg/L)	91.5%	2.3
5 (μg/L)	86.5%	4.5
1 (μg/L)	87.5%	6.8

A new detection method of Benzo(a)pyrene in Vegetable oil was developed with Cleanert® BAP-3 cartridge which was simple, quick and easily to be handled. The recovery rates of the method were between 84.1%~92.2% for 1 μg/L, 5 μg/L and 10 μg/L concentrations.

Detection of Melamine in Eggs (Cleanert® PCX, P/N: CX0603)

EMF10033

Material

(1) Instruments and reagents

Chromatographic column (Venusil® ASB C8, 4.6 × 250 mm, 5 μm), SPE(mixed cation exchange) (Cleanert® PCX, 60mg / 3mL), SPE manifolds of 12 port configuration, HPLC, high speed centrifuge, ultrasonic oscillators, vortex mixer, analytical balance(one out of ten thousand) , solvent filtrator with organic and aqueous filtering membrane of 0.45 μm and vaccum pump, acetonitrile (HPLC level), standard substance of melamine (≥99.0%), space citric acid (analytical reagent), heptane sulfonic acid sodium salt (HPLC grade), water (over second distilled water).

(2) HPLC condition

Column: Venusil® ASB C8, 4.6 × 250 mm, 5 μm (VS852505-0).

Mobile phase: acetonitrile: 10 mM/L citric acid + 10 mM/L heptane sulfonic acid sodium salt buffer solution = 7:93 (pH = 3.0).

Detection: 240 nm; Flow rate: 1 mL/min; Injection: 20 μL.

Experimental

Sample preparation

Prepare freshly whipped egg sample by mechanical blending for 3 minutes. Weigh 1.0 g of the sample each time into a centrifuge tube for experiment. Spike samples with 10, 20 and 100 μL of 100 mg/L melamine stock solution, respectively, to obtain three fortified samples at concentrations of 1, 2, and 10 mg/Kg. To each sample, add 10 mL 1% trichloroacetic acid and 2 mL 5% lead acetate solutions, respectively. Mix the sample thoroughly and sonicate it for 20 minutes, and then centrifuge the sample at 8000 rpm for 10 minutes.

Blank sample is prepared with the same procedure as illustrated above without fortification.

Cleanert® PCX (60mg / 3mL) cleanup

- Activate the SPE cartridge by adding 3 mL methanol followed by 3 mL water, then discard the eluate.
- Transfer supernate of the sample into the cartridge.
- Wash the loaded cartridge first with 3 mL water, and then with 3 mL methanol; dry the cartridge and discard the eluate.
- Add 5 mL 5% ammonium methanol (v/v) to elute the cartridge and collect the eluate.
- Evaporate the collected eluate under nitrogen at 50°C.
- Reconstitute the sample with 1 mL mobile phase and filter through 0.45 μm membrane for HPLC analysis.

Results

Figures 1 and 2 show the HPLC profiles of negative blank and spiked samples, respectively. The PCX cleanup procedure removes interference effectively and allows clear detection of melamine. The HPLC method was confirmed by 6 replicate injections of standard melamine solution at 1 and 5 mg/L, respectively. The results are summarized in Table 1, showing the consistence of the method. Calibration curve was established as shown in Table 2 and Figure 3. Recovery data of the spiked samples are shown in Table 3.

Table1 stable data of retention time and peak area ratio

Density (mg/mL)	Indicator	1#	2#	3#	4#	5#	6#	Mean value	RSD%
1.0	Retention time (min)	18.830	18.829	18.829	18.838	18.840	18.834	18.833	0.026
	Peak area ratio	89	81	84	88	84	80	84	4.286
5.0	Retention time (min)	18.949	18.952	18.947	18.949	18.950	18.946	18.949	0.011
	Peak area ratio	423	440	438	439	437	438	436	1.461

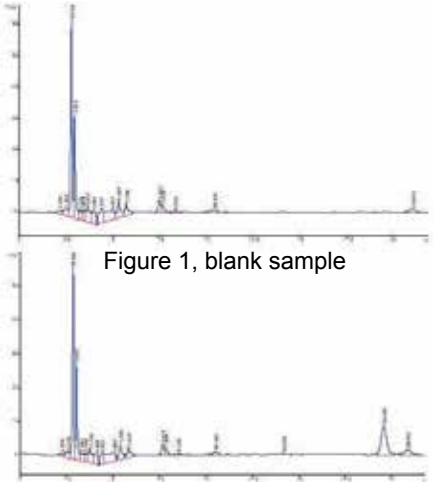


Figure 1, blank sample

Figure 2 Profile of sample spiked with 10 ppm melamine.

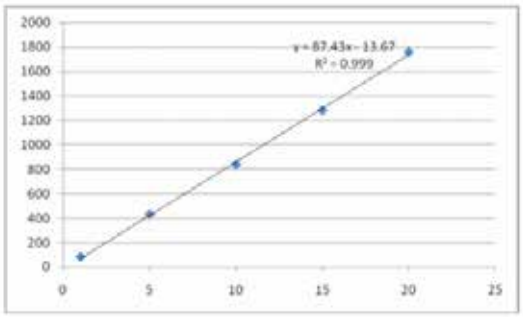


Figure 3 Regression curve of density and peak area ratio

Table 2 Experimental data of standard adjusted curve

Levels (mg/Kg)	Replicate 1	Replicate 2	Mean
1.0	89	79	84
5.0	423	440	431
10.0	832	844	838
15.0	1265	1299	1282
20.0	1689	1823	1756

Table 3 shows that the recovery ratio of melamine in the eggs is relatively good with the method

Concertration (mg/kg)	Peak area	Content	Recovery (%)
1.0	19.9	1.158892	115.89
1.0	21.0	1.214532	121.45
2.0	41.7	2.261587	113.08
2.0	40.8	2.216062	110.80
10.0	188.8	9.702247	97.02
10.0	219.6	11.26018	112.60

Detection of Sudan Red in Foods with HPLC (Cleanert® Alumina-N, P/N: AL5006-N)

EMF10034

Material

Cleanert® Alumina N, 500mg / 6mL

Experimental

Red chili and other powder samples

Put 1.00 to 5.00 g sample in a flask and then add 15 mL n-hexane. Extract the sample under sonication for 5 minutes followed by filtration. Use additional 10 mL hexane by batch to rinse the sample residue several times to colorless. Rotovap the combined supernatant to obtain sample concentrate in less than 5 mL.
Cleanert® Alumina N Cleanup: Slowly transfer the concentrate to Alumina N cartridge to reduce broadening of the pigment band. Wash the loaded cartridge with 10-30 mL hexane until the eluate is colorless, and discard the eluate. Elute the cartridge with 60 mL n-hexane/acetone (95:5, v/v) and collect the eluate. Evaporate the collected eluate in a rotovap. Transfer the final concentrate to a vial and bring up to 5 mL using acetone. Filter the solution through 0.45 μm membrane before analysis.

Red chili oil, hot pot ingredients, cream and other oil samples

Directly dissolve 0.50 to 2.00 g oil sample with 10 mL n-hexane in a beaker. Follow the Cleanert® Alumina N Cleanup procedure illustrated above to prepare sample.

Chili sauce, tomato sauce and other sample with high content of water

Weigh 10.00 to 20.00 g sample into a centrifuge tube and add 10 to 20 mL water and mix well the mixture, and then add 30 mL n-hexane/acetone (3:1, v/v). Homogenize the mixture for 5 minutes followed by centrifuging at 3000 rpm for 10 minutes. Transfer the n-hexane layer. Extract the sample paste again using 2 × 20 mL n-hexane. Dry the combined extract over 5 g sodium sulfate in a funnel and collect the solution. Evaporate the solution to dryness under rotovap. Reconstitute the residue in 5 mL hexane. Follow the procedure Cleanert® Alumina N Cleanup illustrated above to cleanup the sample for analysis.

Sausage and other meat products

Weigh 10~20 g (accurate to 0.01 g) of smashed sample in a triangular flask. Add 60 mL of n-hexane and homogenize for 5 minutes. Filter and obtain clear filtrate. Add 20 mL of n-hexane to repeat the extraction twice. Combine the n-hexane solutions of 3 times and dehydrate with 5 g of anhydrous sodium sulfate. Filter the solution and evaporate to less than 5 mL by rotary evaporator. Follow the procedure Cleanert® Alumina-N Cleanup illustrated above to further process the sample.

Detection of Malachite Green and Crystal Violet Residues in Aquatic Products (Cleanert® Alumina-N,P/N: AL0006-N; Cleanert® PCX, P/N: CX0603)

EMF10035

Material

Alumina Neutral cartridge: Cleanert® Alumina N, 1g / 6mL, activate with 5 mL of acetonitrile.
Cation exchange cartridge: Cleanert® PCX, 60mg / 3mL, activate sequentially with 3 mL of acetonitrile and 3 mL of formic acid solution.

Experimental

Fresh aquatic product

(1) Extraction

Weigh 5.00 g of smashed sample in a 50 mL centrifuge tube. Add 200 μL of mixed internal standard solution and 11 mL of acetonitrile.

Sonicate for 2 min; homogenate at 8000 r/min for 30 s; centrifugate at 4000 r/min for 5 min. Transfer the supernatant into a 25 mL colorimetric tube. Wash the blade of homogenizer with 11 mL of acetonitrile in another 50 mL centrifuge tube for 10 s and transfer the solution to the former centrifuge tube. Mash the sediments in the centrifuge tube with a glass rod and vortex mix for 30 s. Sonicate for 5 min and centrifugate at 4000 r/min for 5 min. Combine the supernatant into the 25 mL colorimetric tube, dilute with acetonitrile to 25 mL. Shake up for later use.

(2) Alumina-N Cleanup

Load 5 mL of sample onto the activated Alumina Neutral cartridge, and wash the cartridge with 4 mL of acetonitrile. Collect the eluate in a KD–concentrator. Evaporate the solution to approximately 1 mL by rotary evaporator in water bath at 45°C, dilute with acetonitrile to 1 mL. Ultrasonicate the solution for 5 min and then add 1 mL of 5 mmol/L ammonium acetate, ultrasonicate for 1 min.

Filter the sample solution through 0.2 μm membrane for HPLC-MS analysis.

Processed aquatic products

(1) Extraction

Weigh 5 g of mashed sample in a 100 mL centrifuge tube. Add 200 μL of mixed internal standard solution, 1 mL of 0.25 g/mL hydroxylamine hydrochloride, 2 mL of 1 mol/L p-toluenesulfonic acid, 2 mL of 0.1 mol/L ammonium acetate and 40 mL of acetonitrile, sequentially. Homogenize at 10000 r/min for 2 min and centrifugate at 3000 r/min for 3 min. Transfer the supernatant into a 250 mL separatory funnel. Extract the residue with 20 mL of acetonitrile again and combine the supernatant. Add 30 mL of dichloromethane and 35 mL of water to the separatory funnel and shake for 2 min. Stand to let two layers separated. Transfer the underlayer to a 150 mL pear-shaped flask. Extract with 20 mL of dichloromethane again and combine dichloromethane layers. Evaporate by rotary evaporator in water bath at 45°C almost to dryness.

(2) Coupled Cleaner® Alumin N and PCX procedure

Connect Cleanert® Alumina N cartridge (top) with Cleanert® PCX (down). Vortex mix to dissolve the residue (c) in 6 mL of acetonitrile (divided into three times) and load the solutions sequentially onto the connected cartridges. Keep the flow rate in Cleanert® PCX cartridge below 0.6 mL/min. Wash Cleanert® Alumina-N cartridge with 2 mL of acetonitrile and discard the eluate. Wash the Cleanert® PCX cartridge with 3 mL of 2% (V/V) formic acid, 3 mL of acetonitrile sequentially and discard the effluent. Elute with 4 mL of 5% ammonium acetate in methanol at the flow rate of 1 mL/min. Collect the eluate in a 10 mL scaled test tube and dilute with water to 10 mL. Filter the sample solution through 0.2 μm filter membrane for HPLC-MS analysis.

Melamine in Fish, Milk and Eggs by MAS-HPLC (MAS Purified Tube for Melamine, P/N: MS-SPM5001)

EMF10036

Material

Instruments: L6-1 series HPLC (Beijing purkinje general instrument Co., Ltd.); sample preparation method for melamine determination, including HCl 0.1 mol/L, 6% sulfosalicylic acid, mixed anion exchange packing material Cleanert® PAX; Venusil® SCX-M, 5 μm, 4.6 × 250 mm strong cation exchange cartridge (the achievement of Chinese `11 th Five-Year Plan` supported science and technology project, Bonna Agela Technologies) and its guard cartridge; needle type filters (Bonna Agela Clarinert™, 0.22 / 0.45 μm, nylon); melamine standard (>99%); homogenizer (T25 Basic, IKA).
Reagents: acetonitrile (chromatographic grade), potassium dihydrogen phosphate (analytical grade), ultrapure water.

Experimental

HPLC conditions
Column: Venusil® SCX-M, 4.6 × 250 mm, 5 μm, 300 Å;
Mobile phase: potassium dihydrogen phosphate (0.050 mol/L):acetonitrile = 70:30;
Flow rate: 1.5 mL/min; Column temperature: ambient temperature; UV wavelength: 240 nm;
All injection volume is 20 μL if not noted otherwise.

Preparation of melamine working standard solutions
(1) Stock standard solution of melamine: 1.00×10^3 mg/L.
Weigh 100 mg (accurate to 0.1 mg) of melamine standard and dissolve completely in water. Dilute with water to 100 mL and mix.
(2) Working standard solutions
1) Standard solution A: 2.00×10^2 mg/L.
Take 20.0 mL of standard stock solution of melamine (1.3.1) accurately into a 100 mL volumetric flask. Dilute with water to volume and mix for use.
2) Standard solution B: 0.50 mg/L.
Take 0.25 mL of standard solution A (1.3.2.1) into a 100 mL volumetric flask. Dilute with water to volume and mix for use.
3) Working standard solutions
Take different volumes of standard solution A (1.3.2.1) into volumetric flasks according to Table 1. Dilute with water to volume and mix. Filter the solutions through 0.45 μm membrane for determination. Take different volumes of standard solution B (1.3.2.2) into volumetric flasks according to Table 2. Dilute with water to volume and mix. Filter the solutions through 0.45 μm membrane for determination.

Table 1 Preparation of working standard solutions (high concentration)

Volume of standard solution A (mL)	0.1	0.25	1.00	1.25	5.00	12.5
Volumetric flask volume (mL)	100	100	100	50	50	50
Final concentration (mg/L)	0.20	50	2.00	5.00	20.0	50.0

Table 2 Preparation of working standard solutions (low concentration)

Volume of standard solution B (mL)	1.00	2.00	4.00	20.0	40.0
Volumetric flask volume (mL)	100	100	100	100	100
Final concentration (mg/L)	0.005	0.01	0.02	0.10	0.20

Sample preparation using MAS cartridge

(1) Milk sample

Take 15.0 g of milk into a 25 mL volumetric flask. Add 7 mL of 0.1 mol/L HCl, 3.0 mL of 60 g/L sulfosalicylic acid. Add the sample into the melamine MAS Tube (S/N:MS-SPM5001) After vortex mixing for 2 min and centrifugation at 1000 r/min, collect the supernatant and filter it through 0.45 μm membrane for LC analysis.

(2) Egg sample

Take 0.5 g of well mixed egg sample and add 10 mL of 0.1 mol/L HCl for 10 min ultrasound. Add 3 mL of 60 g/L sulfosalicylic acid and 2 mL of 0.1 mol/L HCl . Add the sample into the melamine MAS tube (S/N:MS-SPC5001). After vortex mixing and centrifugation at 1000 r/min for 10 min, collect the supernatant and filter it through 2 μm membrane for LC analysis.

(3) Fish sample

Take 1.0 g of chopped meat of fish back and 10 mL of 0.1 mol/L HCl. After ultrasound for 10 min , add 3.0 mL of 60 g/L sulfosalicylic acid and 2 mL of 0.1 mol/L HCl. Transfer the sample into MAS tube (S/N:MS-SPC5001). After vortex mixing and centrifugation at 1000 r/min for 10 min, collect the supernatant and filter it through 2 μm membrane for LC analysis.

Results

(1) Linear range

The curve of low concentration is made by 5 standard samples in different concentrations 0.005 mg/L, 0.01 mg/L, 0.02 mg/L, 0.1 mg/L, 0.2 mg/L. The standard working curve is shown as below.

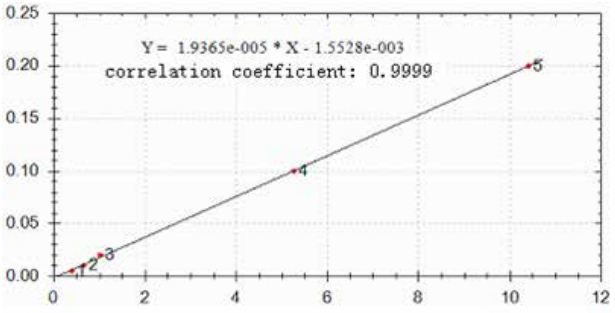


Figure 1. Linearity of low concentration samples

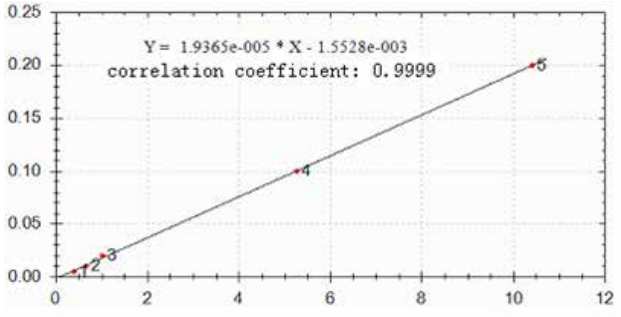


Figure 2. Linearity of high concentration samples

The curve of high concentration is made by 6 standard samples of different concentrations 0.2 mg/L, 0.5 mg/L, 2 mg/L, 5 mg/L, 20 mg/L, 50 mg/L. The standard working curve is shown as below.

Results show good linearity of melamine at concentrations between 0.005 mg/L~ 50.0 mg/L.

(2) Reproducibility

Analyze 20 μL of 5 mg/L standard solution 6 times. Calculate the RSD of retention time and peak area. The results are listed in the table 3.

Table 3 and figure 3 show good reproducibility of this method.

Table 3 Repeatability (precision) of retention time and peak area

Item / Mumber	1#	2#	3#	4#	5#	6#	Average	RSD%
Rentention time/min	6.817	6.833	6.817	6.817	6.858	6.808	6.825	0.26
Peak area/μAu·s	571360	574492	569813	572187	574525	575593	572995	0.39

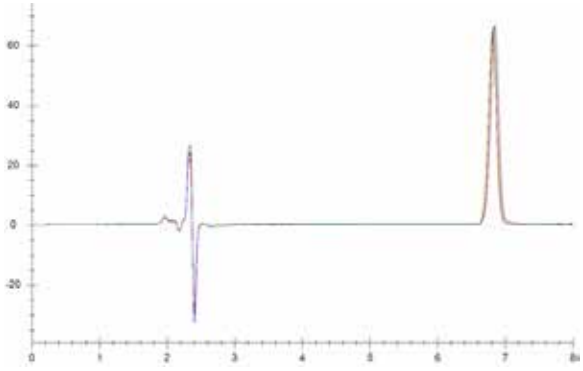


Figure 3. Chromatogram showing repeatability

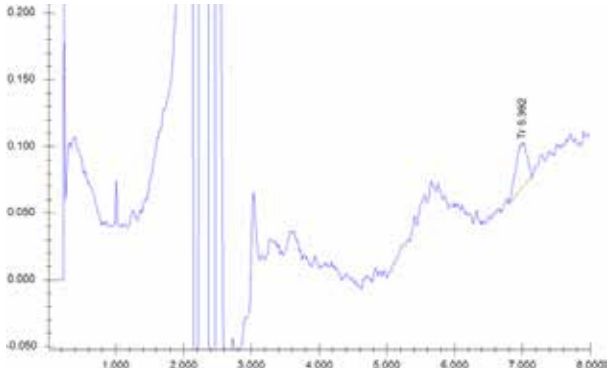


Figure 4. Chromatogram of 0.005 mg/L sample (indicating limit of detection)

(3) Limit of detection

Limit of detection (LOD) is estimated to be 0.0032 mg/L based on the peak height of response from 0.005 mg/L sample while considering signal to noise (S/N) ratio is 3.

(4) Real samples

1) Milk sample

A) Figure 5 shows the chromatogram of blank milk sample.

B) Recovery of spiked milk sample.

Figure 6 shows the chromatograms of spiked milk samples at concentrations of 1.0 mg/kg, 5.0 mg/kg and 10.0 mg/kg.

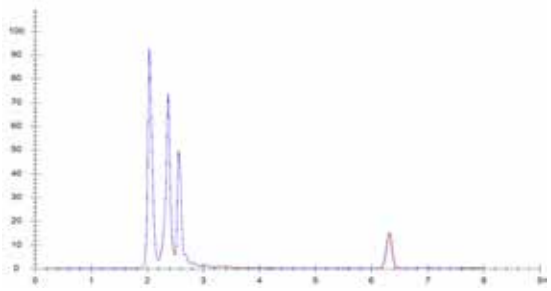


Figure 5. Chromatogram of blank milk sample

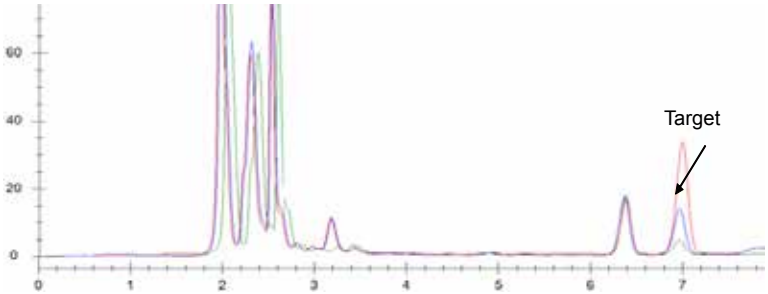


Figure 6. Chromatograms of spiked milk samples at different concentration levels

The recoveries of melamine obtained from above chromatograms are listed in table 4.

Table 4 Results of recovery of melamine spiked in milk

Sample	Amount of spiked melamine (mg/kg)	Amount of measured melamine (mg/kg)	Recovery(%)
Blank milk	0.00	-	-
1#	1.00	1.05	105.0%
2#	5.00	5.18	103.6%
3#	10.00	9.04	90.4%

The results show that the MAS cleanup method of melamine in milk has a good recovery and impurities are effectively removed. The melamine can be well separated from impurities.

2) Fish sample

A) Figure 7 shows the chromatogram of blank fish sample.

B) Recovery of spiked fish sample.

Figure 8 and 9 show the chromatograms of spiked fish samples at concentrations of 1.0 mg/kg, 5.0 mg/kg and 10.0 mg/kg. From the chromatograms, the recoveries of melamine spiked in fish samples are listed in table 5.

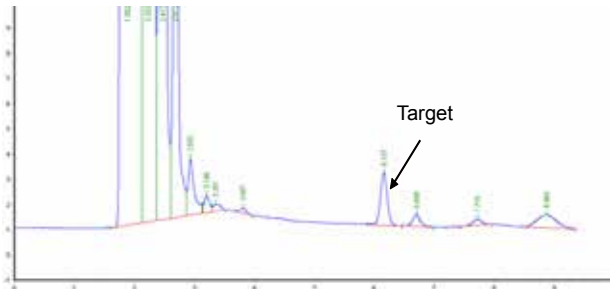


Figure 7. Chromatogram of blank fish sample

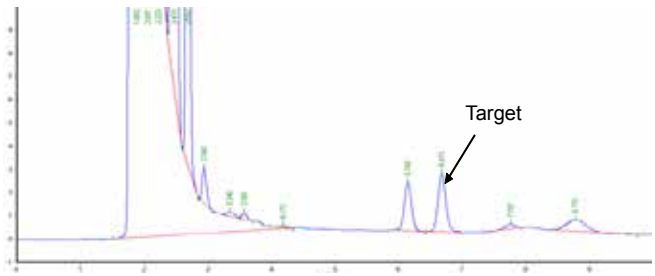


Figure 8. Chromatogram of spiked fish sample (1.0 mg/kg)

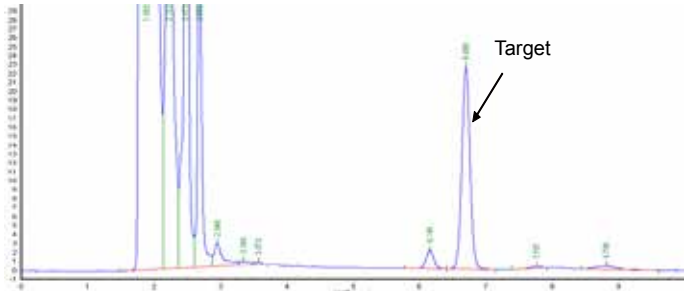


Figure 9. Chromatogram of spiked fish sample (10.0 mg/kg)

Table 5 Results of recovery of melamine spiked in fish

Sample	Amount of spiked melamine (mg/kg)	Amount of measured melamine (mg/ Kg)	Recovery(%)
Blank	0.00	-	-
1#	1	0.98	98.0%
3#	5	5.81	116.2%
5#	10	9.67	96.7%

The results show that the MAS pretreatment method of melamine in fish has a good recovery and impurities are effectively removed. The melamine can be well separated from impurities.

3) Egg sample

A) Figure 10 shows the chromatogram of blank egg sample.

B) Recovery of spiked egg sample.

Figure 11 shows the chromatograms of spiked egg samples at concentrations of 1.0 mg/kg, 5.0 mg/kg and 10.0 mg/kg. The recoveries of melamine spiked in egg samples are listed in table 6.

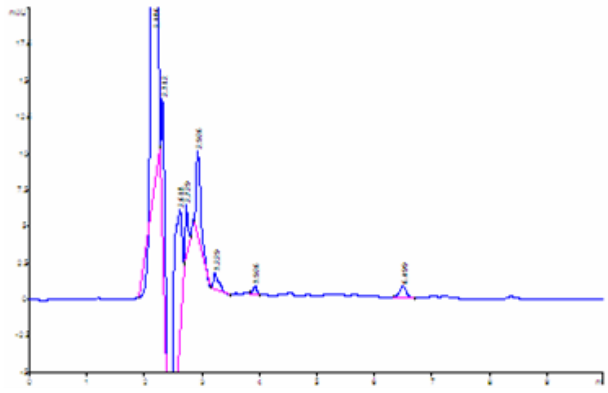


Figure 10. Chromatogram of blank egg sample

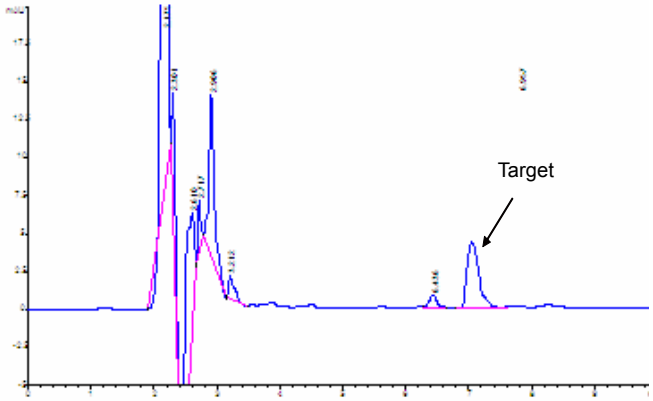


Figure 11. Chromatogram of spiked egg sample (5.0 mg/Kg)

Table 6 Recovery of melamine spiked in egg samples

Sample	spiked melamine (mg/kg)	measured melamine (mg/ Kg)	Recovery (%)
Blank	0.00	-	-
1#	1	0.89	89.0%
3#	5	5.55	111.0%
5#	10	10.48	104.8%

The results show that the MAS cleanup method of melamine in egg sample has a good recovery and impurities are effectively removed. The melamine can be well separated from impurities.

Conclusions

The above experiment results show that the MAS sample pretreatment method, which utilizes mixed anion exchange packing Cleanert PAX as extraction material, provides a fast and accurate approach for treatment of milk, fish and egg samples. Cation exchange column is used for HPLC analysis. The whole analysis method can be used for melamine in different samples, with low limit of detection, high repeatability, wide linear range and good recovery, which totally meets the demands of fast melamine analysis.

Environmental Applications

Detection of Extractable Petroleum Hydrocarbons (Cleanert® EPH, P/N: SI500025-30) by SPE-10 Automated Processing Station

EME10001

Introduction

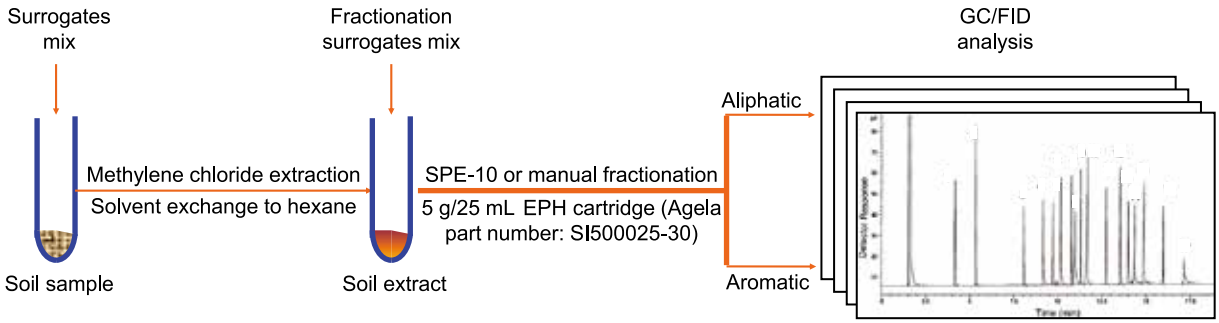
Extractable Petroleum Hydrocarbons (EPH) methodology was recently released by the New Jersey
Department of Environmental Protection (NJDEP) to replace the Total Petroleum Hydrocarbons (TPH) method based on Freon 113 extraction and IR. This method allows aliphatic and aromatic C9-C44 hydrocarbons to be measured separately. It can be used for quantitative analysis of environmental samples (water, soil, sediment and sludge) for residues of crude oils, diesel, sludge and many other types.

Silica gel cleanup and fractionation of aliphaticsand aromatics is a critical and sensitive step in this method. Slight changes, such as the volumes of eluting solvents used, fractionation equipments (including the preparation of the silica gel columns) and fractionation techniques, will have tremendous impact on the proportion of the hydrocarbons separated into their respective aliphatic and aromatic fractions.

Here we present a method based on an automated sample processing station and commercial silica cartridges for rapid, reproducible and efficient fractionation procedure.

Experimental

A. EPH methodSummary



10 Gram soil sample was mixed with pelletized diatomaceous earth, a drying agent, and was loaded into an extraction cell. After adding 100 µL of 1000 ppm surrogate mix (1-chlorooctadecane and o-terphenyl), sample was heated and pressurized with methylenechloride. Extract was collected, dried, concentrated to 1 mLand solvent exchanged to hexane.

With the addition of 1 mL 100 ppm fractionation surrogates mix in hexane (2-bromonaphthlene and 2-fluorobiphenyl), 1 mL extract was fractionated through a 5 g/25 mL silica cartridge (Bonna-Agela Technologies, P/N SI500025-30). Recovered fractions of the aliphatics and aromatics were then concentrated to 1 mL and analyzed separately by Gas Chromatography/Flame Ionization Detector (GC/FID).

A method blank (MB) was done in the same way except that 10 g diatomaceous earth was used to monitor the contamination of the extraction procedure. Method blank spike (MBS) and duplicate (MBSD) analysis were also performed similarly but in addition to the 100 µL surrogate, 100 µL of the 1000 ppm spike mix (all of the aliphatic and aromatic compounds) was added before fractionation.

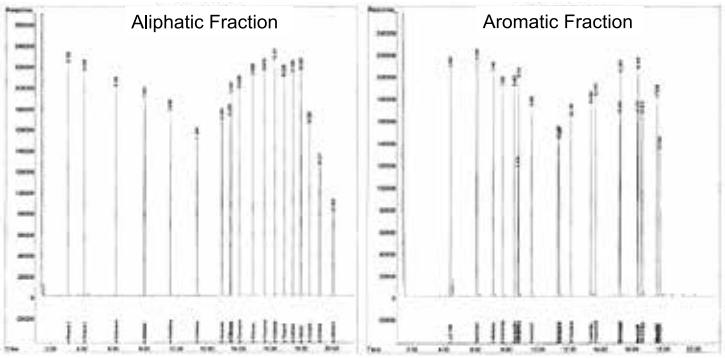
B. SPE-10 automated workstation

SPE-10 can process up to 6 samples per batch sequentially with automatic 2-fraction collection. The system employs positive pressure delivering constant flow with high precision and good reproducibility. In operation, the introduction of sample does not go through pump and thus prevents clogging with unfiltered samples. The benefits of system include:
-Automatic operation, saves up to 90% cost of labor and solvent.
-Independent channel design, eliminates cross contaminations.
-Combinatorial workstation, provides parallel control for up to 10 units to maximize throughput.

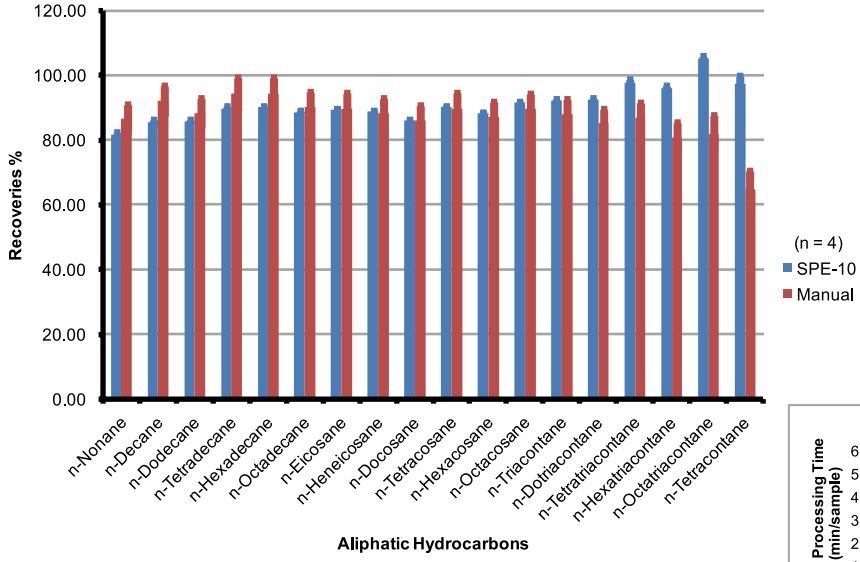
Results

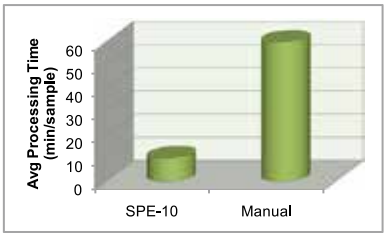
200 ppm Fractionating Check Solution (FCS) 1 was used to monitor the fractionation efficiency of the silica gel cartridge as well as the SPE-10 unit and establish the optimum hexane volume required to efficiently elute the aliphatic fraction without significant aromatic breakthrough (naphthalene and 2-methylnaphthalene were not detected in aliphatic fraction, data now shown).

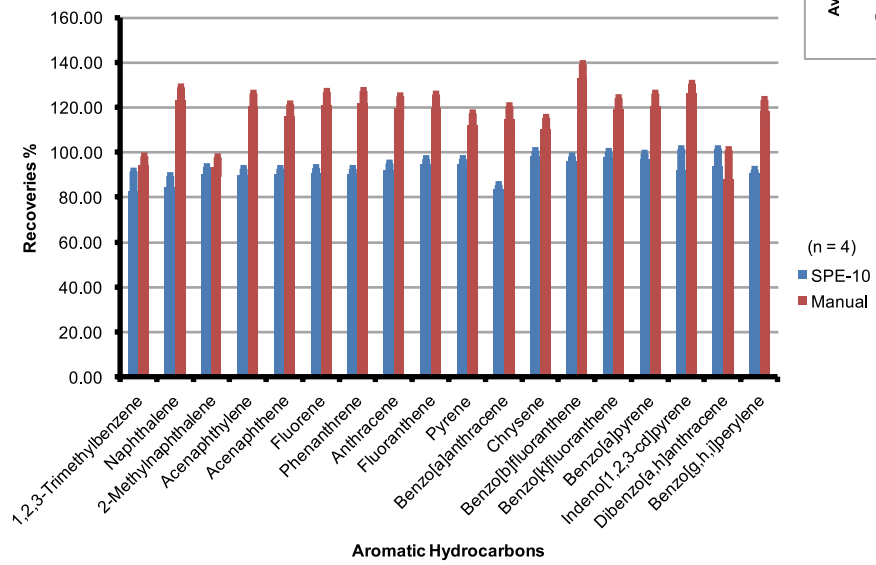
D. Representative FCS gas chromatogramsF.



E. Fractionation efficiency







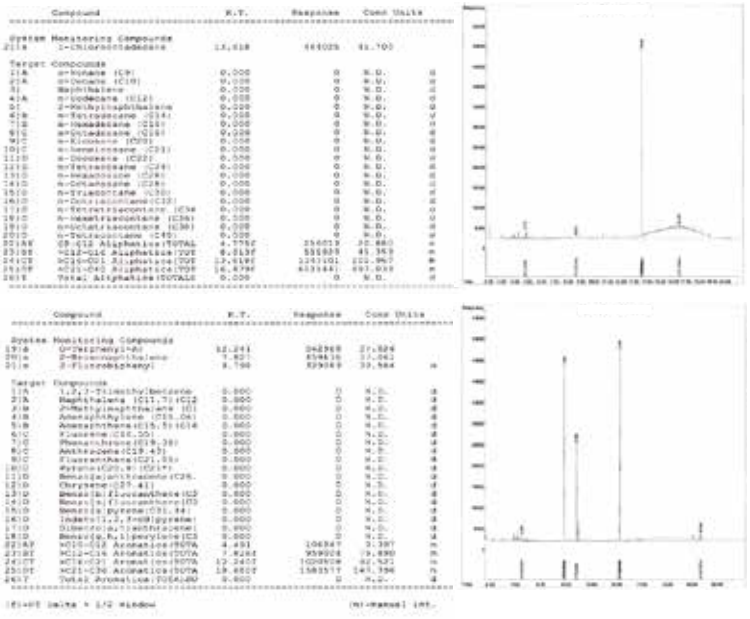
Comparing with manual fractionation on the same batch of silica cartridges, SPE-10 provides comparable recoveries, if not better, with improved reproducibility. Error bars in plots represent one standard deviations.

Average sample processing time by SPE-10, including adding samples and rinsing channels in between different batches of samples, is about 10 min/sample; while during manual fractionation, one would have to monitor the whole procedure for 60 min/sample.

F. Optimized SPE-10 method

Step	Action	Flow Rate (mL/min)	Volume (mL)	Description
0	Elute 1 (hexane)	10	4.5	Add 4.5 mL hexane to silica cartridge.
1	Elute 4 (air)	10	4.5	Blow dry silica cartridge. Step 0-1 is used to minimize the possible differences between silica cartridge packings.
2	Elute 1 (hexane)	20	9.0	Rewet the silica cartridge with 9.0 mL hexane. Cartridge needs to be wet before adding samples for best aliphatic recoveries.
3	Add sample	10	1.0	Add 1.0 mL of sample onto cartridge.
4	Collect 1 (hexane)	5	15.0	Elute and collect aliphatic fraction with 15.0mLhexane. Flow rate and hexane volume is optimized. If fractionation is done manually, flow needs to be controlled as “drop-by-drop” for best aliphatic recoveries.
5	Collect 2 (methylene chloride)	10	30.0	Elute and collect aromatic fraction with 30.0 mL methylene chloride.
6	Elute 4 (air)	10	4.5	Push out any remaining aromatic fraction on the cartridge with 4.5 mL air.

G. Quantitationreport of a real soil sample



Summary

SPE-10 automated processing station provides high fractionation capability with improved reproducibility comparing with manual fractionation.
Processing time per sample was reduced to 1/6 of the manual fractionation time and this relieves the laboratory labor of babysitting the whole process.

Selected Reference

Analysis of Extractable Petroleum Hydrocarbon Compounds (EPH) in Aqueous and Soil/Sediment/Sludge Matrices, New Jersey Department of Environmental Protection, Office of Data Quality, August 2010, Revision 3.

Detection of Phenols in Water (Cleanert® PEP, P/N: PE0603)

EME10002

Material

Cleanert® PEP 60mg / 3mL
7 types of phenols: phenol, 4-nitrophenol, metacresol, 2-chlorophenol, 2,4-bitin, 2,4,6- trichlorophenol, pentachlorophenol

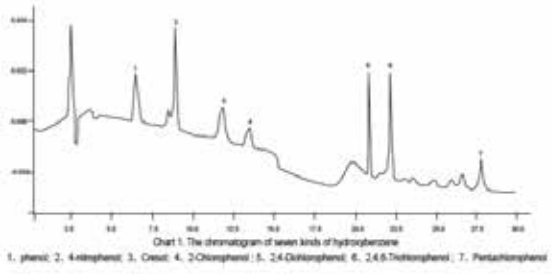
Experimental

Cleanert® PEP procedure

(1) Activation: activate the Cleaner® PEP cartridge sequentially with methyl tertiary butyl ether of 3 mL (10:90, V/V), methanol of 3 mL
and deionized water of 3 mL at 5 mL/min
(2) Wash: wash the cartridge with deionized water of 10 mL at 5 mL/min and dry it for 20 min by vaccum
(3) Elution: elute the methanol of 2 mL and methy tertiary butyl ether (10:90, V/V) by two steps and collect the elution to the fine tip flask
(4) Concentration: concentrate the collected elution of 2 mL with a stream of nitrogen to 1 mL

HPLC conditions

Column: Unisol C18, 4.6×150 mm, 5 μm, (PN: UO051505-0)
Mobile phase: A: 1% acetic acid
B: 1% acetic methyl alcohol
Detector: UV



Time	Mobile phase ratio	Flow rate(mL/min)	Detection wavelength(nm)
0-15 min	A:B = 50:50	1	275
15-30 min	A:B = 15:85	1.8	295

Results

Recovery of the 7 types of phenol seen in Table 1

Table 1 Result of recovery of the phenols

	Mark			Average value	Standard deviation	Average recovery (%)
	1	2	3			
phenol	1.367	1.541	1.524	1.477	0.096	100.3
4-nitrophenol	1.229	1.308	1.430	1.322	0.101	90.0
metacresol	1.294	1.540	1.548	1.461	0.144	106.3
2-chlorophenol	0.527	0.684	0.641	0.617	0.081	100.6
2,4- bitin	1.305	1.613	1.621	1.513	0.180	92.8
2,4,6- trichlorophenol	1.365	1.609	1.511	1.495	0.123	90.3
pentachlorophenol	1.259	1.487	1.472	1.406	0.128	95.6

Detection of Polycyclic Aromatic Hydrocarbons (PAHs) in Water (Cleanert® PEP, P/N: PE0603)

EME10003

Material

Cleanert® PEP, 60mg / 3mL

Target components

Naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo (a) anthracene, benzo (k) fluoranthene, benzo (a, h) fluoranthene, Benzo (a) pyrene, Benzo (g, h, i) pyrene and indeno (1, 2, 3-cd) pyrene.

Experimental

Sample preparation

Add 20 mL of 10% nitric acid into 1 L of water

PEP cleanup

Activation: isopropanol 5 mL, water 5 mL, sequentially
Sample Loading: Load the prepared sample on PEP cartridge
Washing: eluant (water 300 mL + methanol 700 mL + Na_2HPO_4 2.1 g + KH_2PO_4 2.04 g) 5 mL
Drying: dry the cartridge by vacuum pump for 30 min.
Elution: eluant (isopropanol 90 mL + acetic acid 10 mL + toluene 200 mL + petroleum ether 1 L)
Reconstitute sample to 4 mL with methanol after evaporation of the collected eluant.

HPLC conditions

Column:Venusil® PAH, 4.6×250 mm, 5 μm, 200 Å(PN: VP952505-L)
Sample: soluble in methanol: dichloromethane (1:1) 16PAHs sample, dilute 10times with methanol and dichloromethane (1:1)
Flow Rate: 1.2 mL/min
Injection: 10 μL
Temperature: 30°C
Wavelength: 254 nm
Gradient:

Time (min)	Methanol (%)	Water (%)
0	85	15
2	85	15
7	95	5
40	95	5

Detection of Nitrobenzene in Water Samples (Cleanert PEP, P/N: PE5006)

EME10004

Material

Cleanert® PEP, (500 mg/6 mL)

Experiment

Sample preparation

Adjust pH of water sample to neutral. Add methanol to each sample to obtain solution containing 0.5% methanol.

PEP SPE method

- PEP cartridge activation: Set PEP cartridge on SPE system. Wash the cartridge with 3 mL of n-hexane; add 5 mL of methanol; load 10 mL of water onto the cartridge before it is dry. Keep the cartridge wet and activated.
- Sample enrichment: Transfer definite amount of water sample to a separatory funnel connected with PEP cartridge. Turn on the SPE vacuum system; let the water sample flow through the activated cartridge at the flow rate of below 5 mL/min. Keep the liquid level at least 1 cm above the adsorption bed during the extraction process. After all the sample passes the cartridge, wash the internal wall of separating funnel with 10 mL of ultra pure water and keep vacuum for 20 min.
- Elution of SPE cartridge: Put test tube rack and receiving tubes in the extraction cylinder of vacuum multiple tube system. Elute the cartridge with n-hexane of 10 mL/acetone (90:10, V/V) and collect the outflow into the receiving tubes, concentrate to 1.0 mL by nitrogen evaporators at 40°C for analysis.
- Drying cartridge pretreatment: Add 5 g of anhydrous sodium sulfate to a drying cartridge with sieve plate. Wash the cartridge with 10 mL of acetone, n-hexane, and acetone sequentially to purify the drying cartridge before use.
- Elution: Put test tube rack and receiving tubes in the extraction cylinder of vacuum multiple tube system. Connect the drying cartridge between cartridge holding part of SPE system and extraction cartridge. Load 1 mL of acetone on the cartridge (do not let the cartridge dry in this procedure and let the acetone and packing material balance for 2 minutes). Elute the extraction cartridge with 10 mL of hexane/acetone (90/10, V/V) and let the flow pass the drying cartridge (connected to PEP cartridge). Collect the eluate in receiving tubes. Concentrate the eluate under nitrogen stream at 40°C to 1.0 mL for further analysis. (separated layers caused by residual water can affect the efficiency of evaporation under nitrogen stream).

Detection of Bentazone in Water Samples (Cleanert® PEP, P/N: PE5006)

EME10005

Material

Cleanert® PEP, 500mg / 6mL

Experimental

Sample preparation

Adjust the pH of sample with 0.5 mL of H_2SO_4

PEP cleanup

- Activation: Wash the PEP cartridge with 5 mL of furanidine, 5 mL of methanol and 5 mL of water sequentially.
- Sample Loading: Load 500 mL of water sample onto the cartridge at a flow rate of below 5 mL/min.
- Washing: Wash the cartridge with 5 mL of pure water and dry the cartridge under nitrogen stream for 20 min. Then wash the cartridge with 0.9 mL of methanol and discard the eluate.
- Elution: Elute the cartridge with 3 mL of furanidine at the flow rate of below 1 mL/min. Collect the eluate and concentrate to 3 mL for HPLC determination. Or dehydrate by anhydrous sodium sulfate cartridge and evaporate under a stream of nitrogen to 1 mL for HPLC determination.

Results

Recovery of spiked tap water sample is beyond 85 %.

Detection of 2, 4-D in Water (Cleanert® PEP, P/N:PE5006)

EME10006

Material

Cleanert® PEP, 500mg / 6mL.

Experimental

Sample preparation

Adjust the pH of water sample with 0.5 mL H_2SO_4 to 1.5~2.0

Cleanert® PEP cleanup

- Activation: activate the PEP cartridge with 5 mL of methanol and 5 mL of water sequentially.
- Sample loading: Load 500 mL of water sample on the cartridge at a flow rate of below 5 mL/min.
- Washing: Wash the cartridge with 5 mL of pure water and dry the cartridge under nitrogen stream for 20 min. Wash the cartridge with 0.8 mL of methanol (stand for 2 min to ensure methanol to roak packing material thoroughly) and discard the eluate.
- Elution: Elute the cartridge with 3 mL of furanidine at a flow rate of below 1 mL/min. Collect the eluate and concentrate to 3 mL for HPLCdetermination. Or dehydrate with anhydrous sodium sulfate cartridge and evaporate under a stream of nitrogen to 1 mL for HPLC determination.

Results

Recovery of spiked tap water sample is above 80%.

Detection of Chlorophenol in the Water (Cleanert® PEP, P/N: PE5006)

EME10007

Material

Cleanert® PEP, (500mg / 6mL)

Experimental

Sample preparation

Adjust the pH of 500 mL of water sample with 0.5 mol/L H_2SO_4 to 1.5~2.0

PEP method

- Activation: activate the PEP cartridge with 5 mL of methanol and 5 mL of water sequentially.
- Sample loading: load water sample onto the cartridge at a flow rate of below 5 mL/min.
- Washing: Wash the cartridge with 5 mL of pure water and dry the cartridge under nitrogen stream for 20 min. Wash the cartridge with 0.8 mL of methanol (stand for 2 min to ensure methanol to soak packing material thoroughly) and discard the eluate.
- Elution: Elute the cartridge with 5 mL of furanidine. Collect the eluate and concentrate under nitrogen stream at 30°C to 1 mL for HPLC determination.

Results

Recovery of chlorophenol is between 75-90%.

Pharmaceutical (Bioanalytical/DMPK/Clinical)

Detection of Oleic Acid and Its Metabolites in Blood Plasma by LC-MS (Cleanert® PAX, P/N: AX0301)

EMB10001

Material

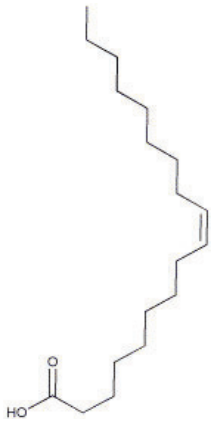


Figure 1. Oleic acid structure

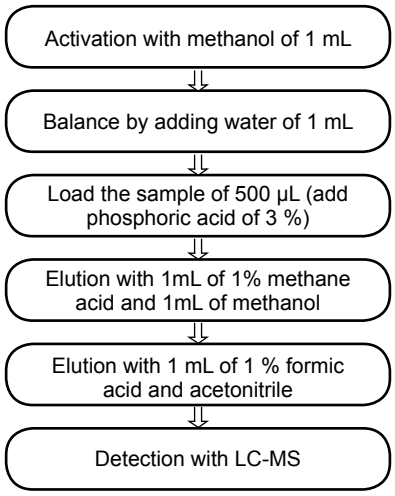


Figure 2. Sample preparation using Cleanert® PAX cleanup

Experimental

Instrument: API Qtrap 3200 (from Applied Biosystem in USA), LC-20A HPLC (from Shimadzu Corporation in Japan).
Mass spectra conditions: electric ion spray sources; detection of anion model; multiple reaction monitoring.
Ionic reaction for quantitative analysis are m/z 281.2→ m/z 281.2 (oleic acid), m/z 315.2→ m/z 315.2 (oleic acid metabolite) and m/z 269.2→m/z 269.2 (internal label C17)
Mobile phase: ACN: 3 mmol/L ammonium acetate = 85:15

Results

Density	10 ng/mL (n = 3)	100 ng/mL (n = 3)	2500 ng/mL (n = 3)
Recovery of oleic acid(%)	77	87	91
RSD	2.9	0.9	0.2

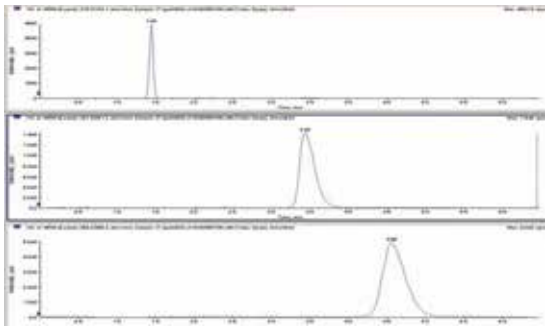


Figure 3. Chromatographic profiles of oleic acid

Detection of Pseudoephedrine in Human Plasma Using LC-MS (Cleanert® PCX, P/N: CX0301)

EMB10002

Material

Cleanup (Cleanert® PCX, 30mg / 1mL)

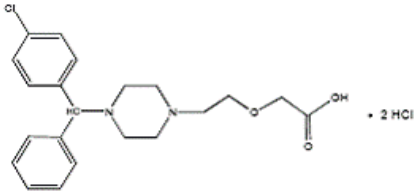


Figure 1. Pseudoephedrine formula

Activation: first add 2 mL methanol followed by 2 mL water.
Sample loading: load the serum diluted with formic acid of 2%.
Washing: first with 1 mL water and then with 1 mL methanol.
Elution: elute targeted sample with 1 mL of 5% ammonia water and methanol and collect.

Experimental

Instrument: API Qtrap 3200 (Applied Biosystems, USA); LC-20A HPLC (Shimadzu Corporation, Japan)
Mass spectrum conditions: electric ion spray sources; detection of anion model; multiple reaction monitoring.
Ionic reaction for quantitative analysis are m/z 166.0 → m/z 148.1 (Pseudoephedrine) and m/z 235.3 → m/z 86.1 (internal label, lidocaine)

Results

Concertration	10 ng/mL (n = 3)	100 ng/mL (n = 3)	2500 ng/mL (n = 3)
Recovery of Pseudoephedrine (%)	77	79	85
RSD	3.5	1.9	0.5
Recovery of Lidocaine (%)	88	92	87
RSD	4.9	2.1	0.3

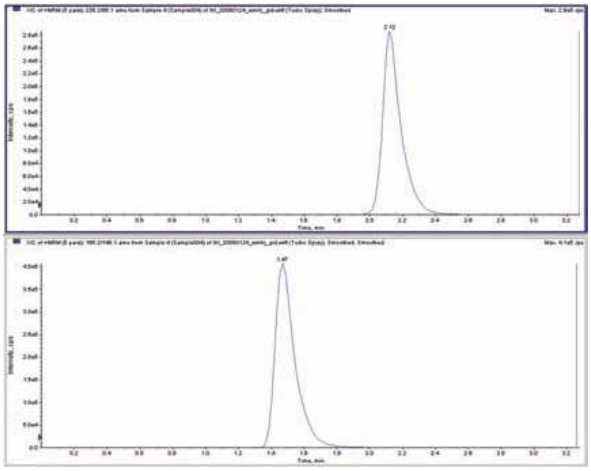


Figure 2. Chromatogram map of pseudoephedrine and internal label of lidocaine

Detection of Evodianmine and Rutaecarpine in Human Serum (Cleanert® C18, P/N: S182003)

EMB10003

Material

Cleanert® C18, 200mg / 3mL, P/N: S182003
Sample: human serum, sample concentration: 50 ng/mL (diluted 2 fold with water)

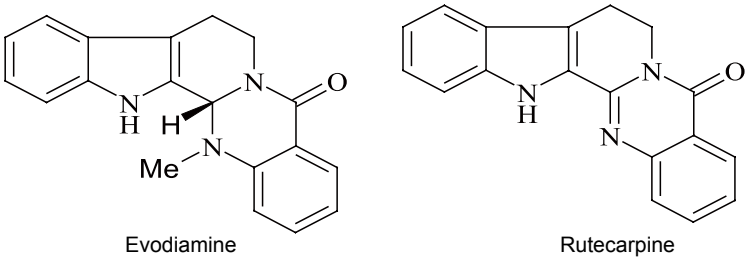


Figure 1. Structural formula of two extracts from Tetradium ruticarpum (evodiamine and rutecarpine)

Experimental

C18 SPE Cleanup

- Cartridge activation: activate the SPE cartridge with 2 mL of methanol followed by 2 mL of water.
- Sample loading: load 2 mL of sample at the flow rate of 0.5 mL/min.
- Washing: wash the SPE cartridge twice with 1 mL of water, then dry the cartridge under nitroge.
- Elution: elute with 1 mL of acetic ether twice, and collect the eluate.

Evaporate the eluate to dryness under nitrogen at 40°C. Dilute with 1mL of methanol : water (85:15). Filter the solution through 0.45 μm filter membrane for LC-MS analysis.

LC-MS conditions

Instrument: Agilent 1100
Column: Venusil® XBP C18, 4.6 x 150 mm, 5 μm, P/N: VX951505-0;
Mobile phase: methanol:water = 85:15;
Flow rate: 0.5 mL/min; Column temperature: 25°C;
Injection volume: 10 μL; MS: ESI;

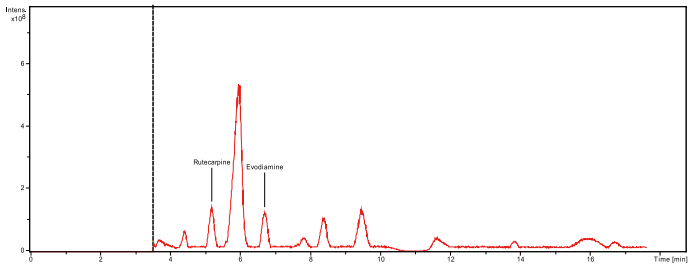


Figure 2. Total ion chromatogram of Evodiamine and Rutecarpine in human serum

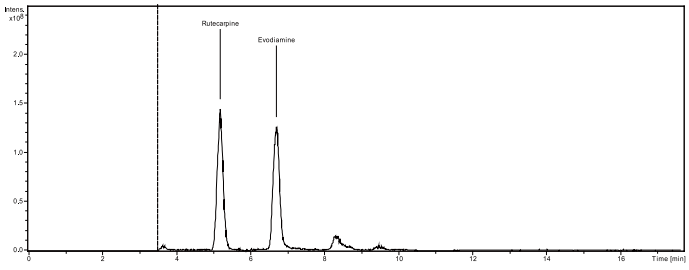


Figure 3. Total ion chromatogram of Evodiamine and Rutecarpine in human serum

Detection of Sulpiride in Human Plasma by SPE and HPLC (Cleanert® C18, P/N: S181001)

EMB10004

Material

Cleanert® C18, 100mg / 1mL, P/N: S181001

Experimental

Sample preparation

Transfer 0.75 mL of blood plasma accurately to a 2 mL centrifuge tube. Add 10 μL of internal standard solution of metoclopramide and vortex mix.

C18 SPE procedure

- Activation: activate with 2 mL of methanol, equilibrate with 2 mL of water
- Sample loading: load the prepared blood plasma sample onto the cartridge
- Washing: wash the cartridge with 1mL of water after all sample solution flows out
- Elution: methanol 2 mL, collect the eluate and dry under nitrogen in water bath at 55°C. Dissolve the residue in 100μL of methanol.

Centrifugate at 3000 r/min for 5 min and take 20 μL of the supernatant for analysis.

Detection of IFO in Serum by SPE and HPLC (Cleanert® C18, P/N: S181001)

EMB10005

Material

(1) Cleanert® C18, 100mg / 1mL.
(2) Cyclophosphamide solution as internal standard: add 0.2 g of cyclophosphamide to a 100 mL volumetric flask. Dissolve and dilute to
volume with mobile phase solution. Store this internal standard solution (2 mg/mL) in refrigerator at 4°C.

Experimental

Pretreatment of Blood Sample

Add 12.5 μL of internal standard solution to 0.5 mL of serum sample for later use.

C18 cleanup

- Activation: Activate SPE cartridge with 2 mL of acetonitrile, followed by 2 mL of physiological saline.
- Sample loading: Load the prepared sample onto SPE cartridge.
- Washing: Wash the cartridge with 1 mL of physiological saline followed by 1 mL of 5% acetonitrile solution. Dry the cartridge after all eluant flows out.
- Elution: Elute with 0.5 mL of acetonitrile. Collect the eluate and take 20 μL of eluate for further determination.

Figure 1 shows the chromatogram of ifosfamide (retention time: 11.6 min) and cyclophosphamide (retention time: 12.8 min). Both peaks are in good shape without interference of impurities.

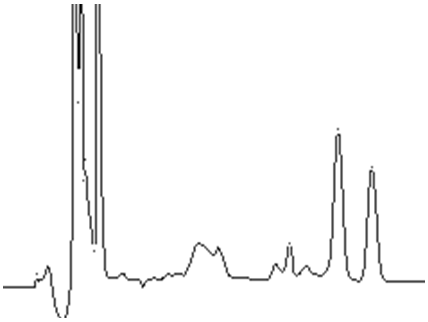


Figure 1. Chromatogram of ifosfamide and cyclophosphamide spiked in blank serum

HPLC Conditions

Column: Venusil® ASB C18, 4.6 mm×250 mm, 5 μm (PN: VS052505-0);
Mobile phase: acetonitrile: water (25:75); Flow rate: 1 mL/min;
UV: 200 nm; Injection volume: 20 μL; Column temperature: room temperature.

Results

Table 1. Recovery and precision of ifosfamide (n = 5)

Spiked concentration	Recovery of extraction	Recovery of method	RSD (%)	
(μg·mL-1)	(%)	(%)	Inter-day	Intra-day
100	92.0±1.8	101.2±3.5	1.1	2.1
50	93.6±5.6	107.2±1.5	2.9	4.1
5	89.3±1.3	95.3±4.3	2.2	2.4

Please download the details of the article at the website of Bonna Agela: www.bonnaagela.com

Detection of Uretic Residues in Animal Urine (Cleanert® PAX, P/N: AX0603)

EMB10006

Material

Cleanert® PAX, 60mg / 3mL

Experimental

Sample pretreatment

Transfer 2 mL of sample accurately to a 50 mL centrifuge tube. Adjust pH with 5 mol/L hydrogen chloride solution to 3.5 ± 0.5. Add 1 mL of 5% lead acetate solution and 5 mL of water-saturated acetic ether. Vortex mix and vibrate on shaker for 10 min. Centrifugate at 5000 rpm for 5 min and transfer the supernatant to another 50 mL centrifuge tube. Add 5 mL of water-saturated acetic ether to the aqueous underlayer. Vortex mix and vibrate on shaker for 10 min. Centrifuge at 5000 rpm for 5 min and combine the supernatants. Dry the solution under nitrogen at 50°C. Dissolve the residue in 3 mL of acetonitrile 2% ammonia (10:90, V/V) for later use.

Cleanert® PAX cleanup

Load the sample onto Cleanert® PAX cartridge, which is already activated first with 3 mL methanol and then followed with 3 mL of 2% ammonia solution. Wash the SPE cartridge with 3 mL of 2% ammonia, methanol and 5% formic acid, sequentially. Dry the cartridge. Elute the cartridge with 3 mL of methanol containing 5% formic acid. Dry the eluate under nitrogen. Dissolve the residue in 1.00 mL of acetonitrile 0.3% ammonia (10:90, V/V). Vortex mix and filter the solution through 0.22 μm membrane for LC-MS/MS analysis.

Results

Table 1 Recovery of eight diuretics spiked in bovine urine (n = 5)

Spiked concentration (μg/L)	Measured parameter	Drugs							
		Chlorath-iazide	Dihydro-chlorot-hiazide	Hydrofl-umethia-zide	Chlort-alido-ne	Trichlor-methiaz-ide	Methy-clothi-azide	Furose-mide	Etacrynic Acid
20	Mean recovery (%)	88.9	80.0	91.2	71.1	86.6	89.9	95.3	99.8
	Intra-batch RSD (%)	6.7	7.9	5.3	6.9	10.1	7.6	8.8	7.1
	Inter-batch RSD (%)	9.3	10.3	13.5	7.7	15.6	9.9	12.7	13.3
50	Mean recovery (%)	87.2	86.7	86.0	78.6	93.3	91.5	97.7	90.8
	Intra-batch RSD (%)	9.5	6.7	9.8	4.6	7.2	6.5	10.0	8.3
	Inter-batch RSD (%)	11.3	8.5	13.8	7.7	9.8	7.4	13.9	9.8
100	Mean recovery (%)	86.3	93.6	95.6	81.2	101.1	82.6	106.5	109.0
	Intra-batch RSD (%)	6.6	7.9	4.7	8.6	5.2	3.9	5.8	9.3
	Inter-batch RSD (%)	8.9	10.7	6.4	11.8	9.6	7.7	13.0	12.8

Application of Qdaura® automatic SPE system for the determination of hypnotic drugs in plasma by GC/MS (Cleanert® PEP-2 200mg/6mL,PN: PE2006-2)

EAF10005

Experimental Details

Apparatus

Qdaura® Automated SPE System
Agilent 7890-5975 GC-MS

Chromatographic conditions

Chromatographic column: DA-5MS (30 m×0.25 mm×0.25 μm); carrier gas: He (99.99%); flow rate: 1mL/min; inlet temperature: 270℃ . The oven temperature was programmed at 130℃ , and then raised to 280 at 10℃ /min for 10min.

Mass spectrometry conditions

Ion source: EI; MS quad temperature:150℃ ; EM voltage: 500 V; Mass range: 40~500 m/z; Scan mode: SIM.

Experimental

(1) Materials and reagents
Methanol, dichloromethane, ethanol, acetic acid, Na_2HPO_4, NaH_2PO_4, purified water.
The concentration of the seven hypnotics were 400 ng/mL and were dissolved in ethanol.
0.45 μm filter membrane, Cleanert® PEP-2 Cartridge (200 mg/6mL).
(2) Methods

Table1. The method of SPE-40 SPE system

Procedure	Order	Solvent	Flow Rate(mL/min)	Volume(mL)
1	Conditioning	Methanol	3	6
2	Conditioning	Water	3	6
3	Loading	Air	2	10
4	Washing	acetic solution(pH=6)	3	6
5	Washing	Air	5	10
6	Washing	Air	3	30
7	Elution/Collecting	dichloromethane	2	8
8	Elution/Collecting	Air	3	10
9	End	-	-	-

The eluates were evaporated to dryness under gentle nitrogen stream at 35°C and were redissolved with ethanol (250 μL). The final samples were filtered with 0.45 μm membrane for GC-MS analysis.

Results

Table2. The detection of hypnotic drugs in plasma by GC-MS (n=3)

Compound	RT/min	Recovery 1#/%	Recovery 2#/%	Recovery 3#/%	Average recovery /%	RSD/%
Barbital	6.117	98.59	97.28	94.22	96.69	2.31
Phenobarbital	11.231	105.69	112.54	93.57	103.93	9.24
Chlorpromazine	16.050	71.94	78.31	71.03	73.76	5.37
Clozapine	20.207	88.86	97.41	86.46	90.91	6.33
Estazolam	20.751	93.81	110.87	91.89	98.85	10.57
Aprobarbital	7.521	91.04	111.44	90.37	95.62	8.90
Alprazolam	21.338	98.60	96.28	91.55	95.48	3.76

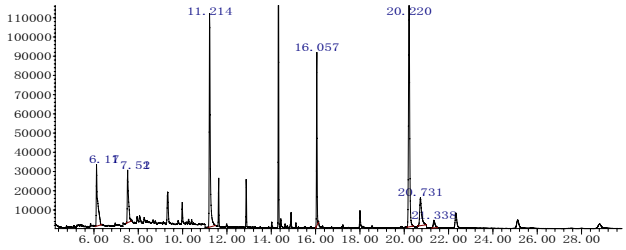


Figure 1. GC-MS chromatogram of seven hypnotic drugs

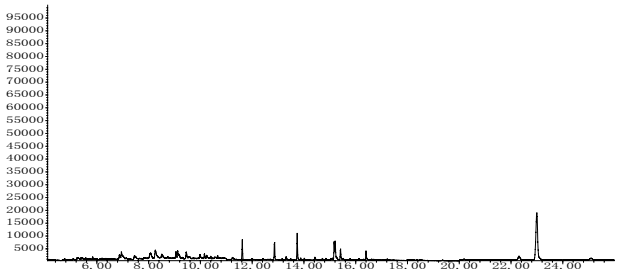


Figure 2. GC-MS chromatogram of blank plasma

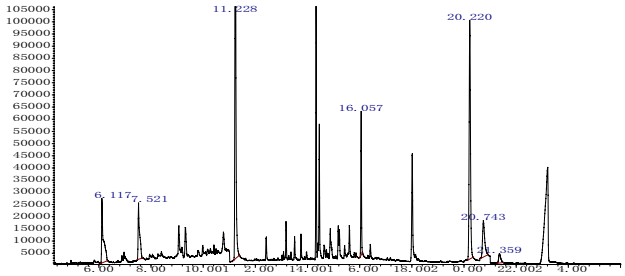


Figure3. GC-MS chromatogram of hypnotic drugs spiked (400 ng/mL) sample

Detection of Hydrochlorothiazide in Plasma — PPT Method

EAB10002

Experimental Details

Sample Preparation

(1) Put Cleanert® PPT and collection plate onto the SPE positive pressure device (PN: SPE-M96)
(2) Add 50 µL of 20 ng/mL hydrochlorothiazide into each well
(3) Quickly add 250 µL ACN and stand for 3 min
(4) Suction by vacuum and dry with N_2, then resolve with 200 µL mobile phase.

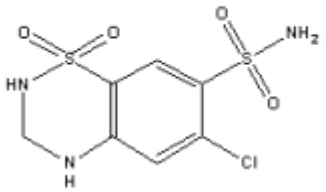


Figure 1 Chemical structure of Hydrochlorothiazide

Instrumentation

Instrumentation: LC-MS/MS, API 4000
Column: Venusil® ASB C18, 2.1×150 mm, 5 µm (PN: VS051502-0)
Column temperature: 25℃
Mobile phase: 90% ACN, 10% 0.01 mol/L ammonium acetate aq (0.1% formic contained)
Flow rate: 0.2 mL/min
Sample injection: 5 µL
Ion source: ESI - Negative
Scan mode: MRM

Table 1 MS/MS transitions information of Hydrochlorothiazide

Compound	Parent ion	Daughter ion
Hydrochlorothiazide	295.7	269.0

Results

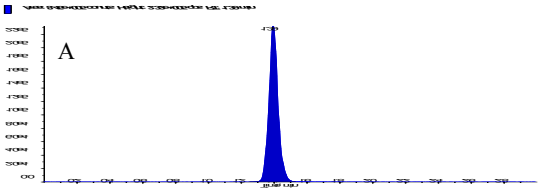


Figure 2 Chromatogram of Hydrochlorothiazide standard solution

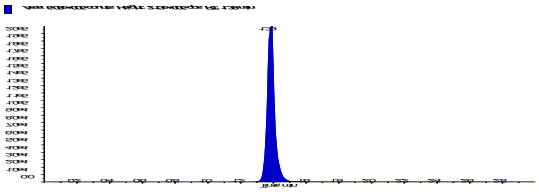


Figure 3 Chromatogram of plasma sample spiked with Hydrochlorothiazide

Table 2 Recovery data of spiked sample

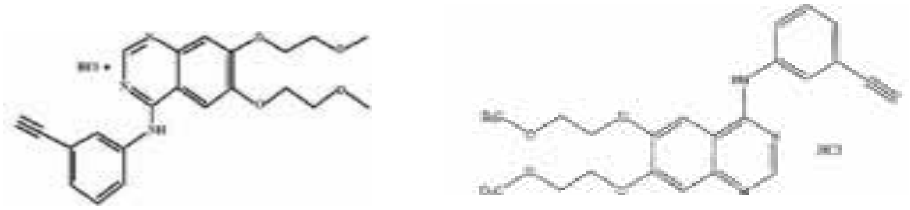
Concentration (ng/mL)	Recovery (%)						Average recovery (%)	RSD (%)
	1	2	3	4	5	6		
20	79.4	75.8	76.5	74.9	75.1	78.4	76.7	2.39

Ordering Information

Products	Specification	Cat.No.
Cleanert® PPT Plate	2 mL/well	96CD2025-Q
Venusil® ASB C18	2.1×150 mm, 5 μm, 150 Å	VS951502-0
96-well collection plate	2.2 mL Squaral well	96SP2036-2
96-well positive pressure device	adapt to 96 well plate	SPE-M96
Nitrogen evaporator	adapt to 96 well plate	NV96-G
1.5 mL vials	1.5 mL short thread vial, amber glass, label and filling lines	AV1111-0
1.5 mL vials caps	9 mm screw neck cap, center hole; red silicone/ white PTFE septa	AV2100-0
Micro-insert clear glass	300 μL micro-insert, 31x6 mm	AV1132-6

Erlotinib Hydrochloride in Plasma (Cleanert® SLE plate 200 µL; PN:HC2002SQ-9W)

EAB10003



Erlotinib　　Erlotinib -d6(IS)

Figure 1 Chemical structure of target compounds

Experimental Details

Sample Preparation

SLE plate procedure

(1) Put the Cleanert SLE plate and 96 well collection plate on the 96-well vacuum manifold

(2) Mix 100 µL of plasma, 10 µL of erlotinib hydrochloride standard aq, 10 µL internal standard solution and 100 µL 10% ammonia together, load the mixture into cartridge

(3) Stand for 5min

(4) Elute with 1400 µL of Methyl-t-butyl ether by gravity, dry with N_2, resolve the eluate with 200 µL mobile phase;

LLE procedure

Mix 100 µL of plasma, 10 µL oferlotinib hydrochloride standard aq, 10 µL internal standard solution and 100 µL 10% ammonia together, add the solution into centrifuge tube, then add 1.5mL of Methyl-t-butyl ether into the tube for extraction. The layers were left to separate and the organic aliquot removed, dry with N_2, resolve the eluate with 200 µL mobile phase;

Instrumentation

Instrumentation: LC-MS/MS, API 4000
Column: Venusil® XBP Silica, 2.1×50 mm, 3 µm
Column temperature: 25℃
Mobile phase: 80% ACN aq with 0.1% formic acid, 20% water with 0.1% formic acid
Flow rate: 1 mL/min
Sample Injection: 5 µL
Ion source: ESI - Positive
Scan mode: MRM

Table 1 MS/MS transitions information of Erlotinib hydrochloride and Erlotinib hydrochloride-d6

Compound	Parent ion	Daughter ion
Erlotinib hydrochloride	394.2	278.2
Erlotinib hydrochloride -d6 (IS)	400.2	278.2

Results

(1) Recovery

Same quantity of Methyl-t-butyl is used in the experiment. The follow result shows that the recovery of SLE is higher than that of LLE.

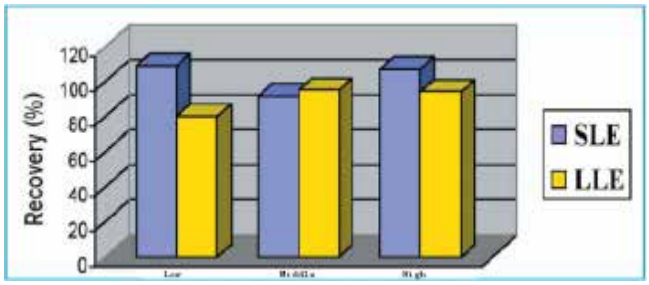


Figure 2 The comparison of recovery between SLE and LLE

(2) Phospholipids Removal

The abundance of phospholipids (496.0/184.0) in plasma sample is detected with LC-MS method. Sample processed with SLE contain 1/100 of phospholipids compared with that processed with LLE.

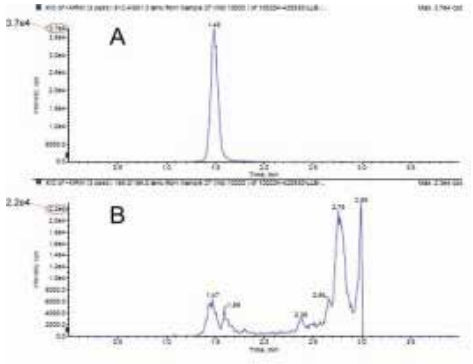


Figure 3 Peak of drug (A) and phospholipids (B) by SLE method

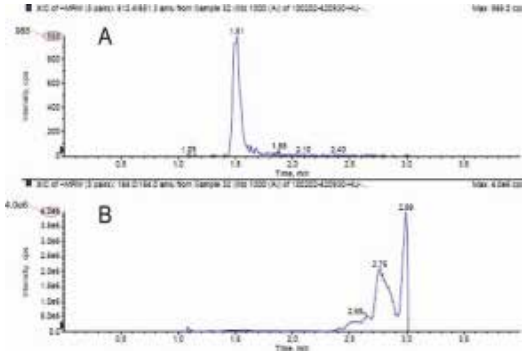


Figure 4 Peak of drug (A) and phospholipids (B) by SPE method

Ordering Information

Products	Specification	Cat.No.
Cleanert® SLE plate	Max sample loading volume 200 µL	HC2002SQ-9W
Venusil® XBP Silica	2.1×50 mm, 3 µm	VSi930502-0
96-well collection plate	2.2 mL Squaral well	96SP2036-2
96-well vacuum manifold	adapt to 96 well plate	VM96
Nitrogen evaporator	adapt to 96 well plate	NV96-G
Acetonitrile	HPLC, 4 L	AH015-4
1.5 mL vials	1.5 mL short thread vial, amber glass, label and filling lines	AV1111-0
1.5 mL vials caps	9 mm screw neck cap, center hole; red silicone/ white PTFE septa	AV2100-0
Micro-insert clear glass	300 µL micro-insert, 31×6 mm	AV1132-6

Estrogens and Estrogens Metabolites analysis in Human Plasma (Cleanert® SLE Plate 400 µL; PN:HC4002SQ-9W)

EAB10004

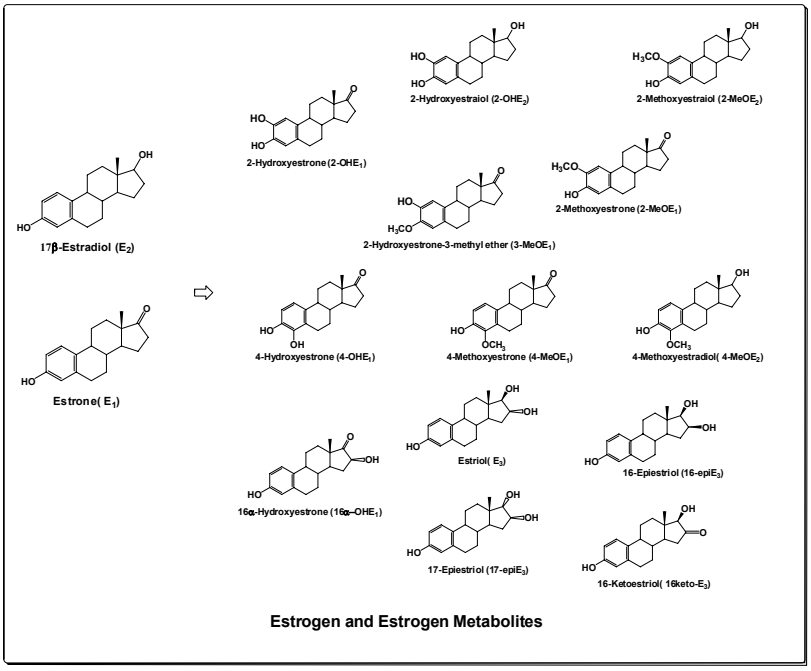
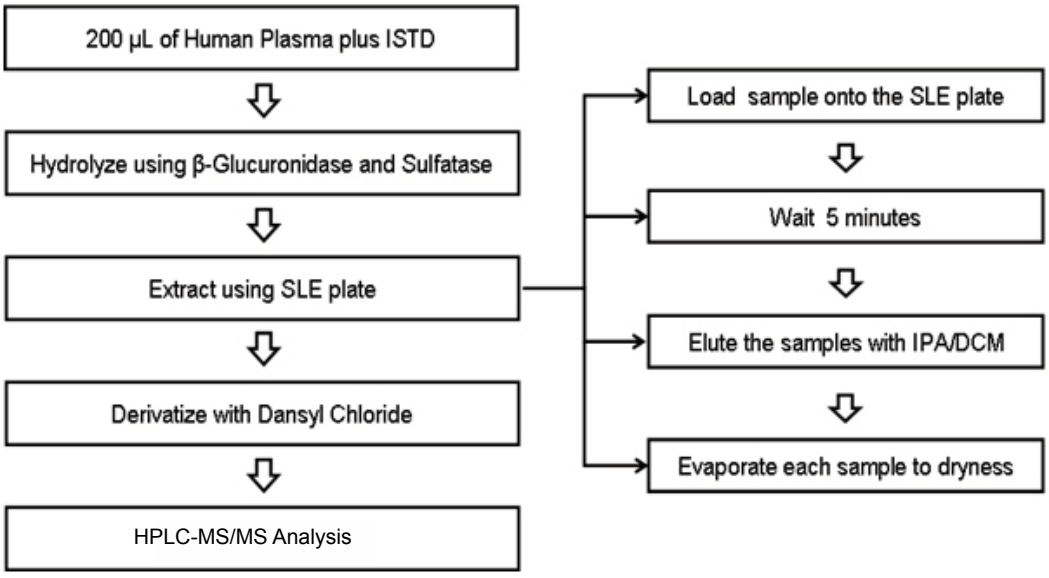


Figure 1 Chemical structure of Estrogens and Estrogens Metabolites

Experimental Details

Sample Preparation



Instrumentation

Instrumentation: LC-MS/MS, API 4000
HPLC column: Synergi Hydro-RP, 2×150 mm, 2.4 μm, 80 Å
Mobile Phase A: 0.1% FA in water;
Mobile Phase B: Methanol
Flow rate: 0.4 mL/min MS/MS Detection
Ionization mode: ESI Positive
Scan mode: MRM

Table 1 The gradient conditions of HPLC

Time (minute)	Module	Function	Value (%)
5.00	Pumps	Pump B Conc.	30
41.00	Pumps	Pump B Conc.	98
46.00	Pumps	Pump B Conc.	98
47.00	Pumps	Pump B Conc.	30
57.00	Controller	STOP	-

Results

(1) Chromatograms of estrogens and Estrogens Metabolites

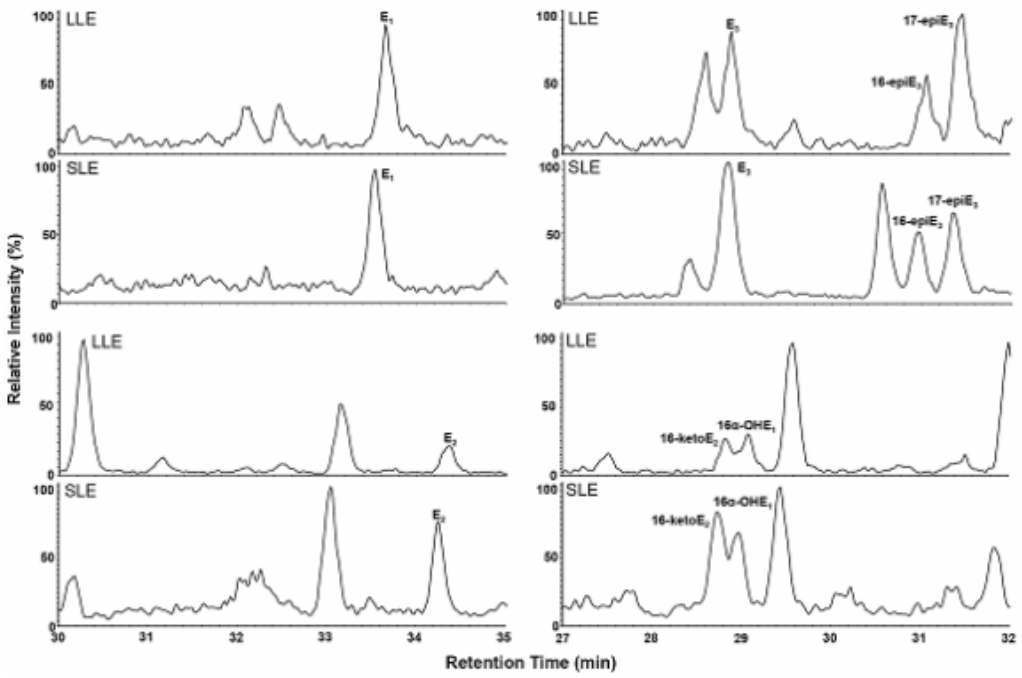


Figure 2 LLE and SLE method comparison at 10 pg/mL (LLOQ)

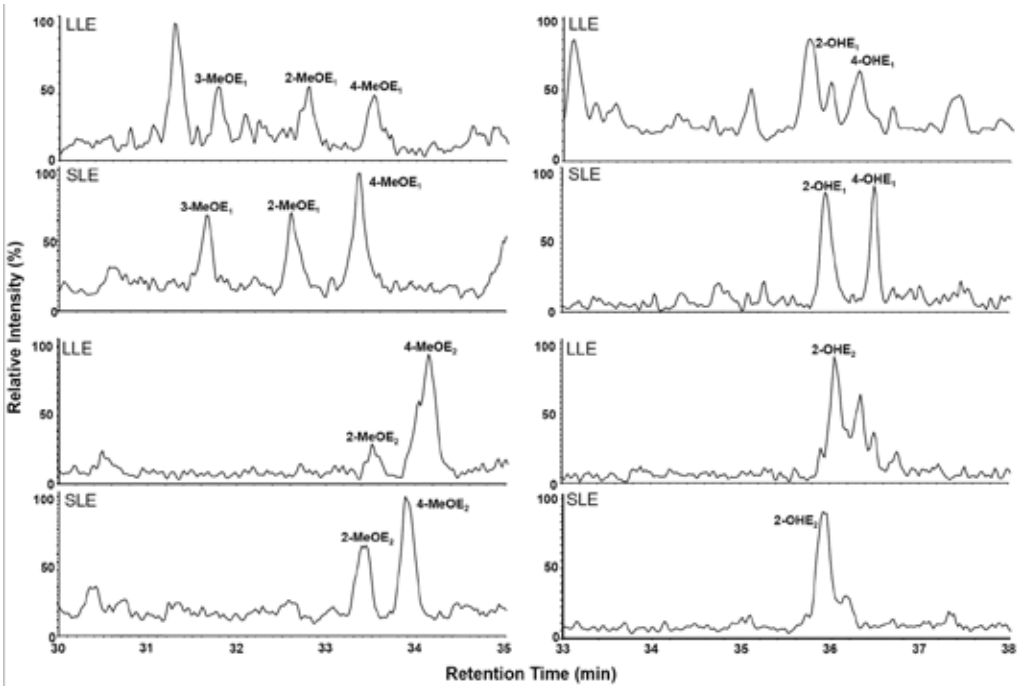


Figure 3 LLE and SLE method comparison at 10 pg/mL (LLOQ).

Table 2 Short-term Stability and Dilution Integrity Results

Time (minute)	Module	Function	Value (%)
5.00	Pumps	Pump B Conc.	30
41.00	Pumps	Pump B Conc.	98
46.00	Pumps	Pump B Conc.	98
47.00	Pumps	Pump B Conc.	30
57.00	Controller	STOP	-

Nominal Conc. (pg/mL)	E1	E2	E3	16-keto	16a-OH	16-epiE3
Freeze and Thaw (n=6)						
30.0	Conc.	31.7	31.5	31.7	30.2	30.8
	Accuracy (%)	105.6	105.1	105.7	100.7	102.5
	CV (%)	4.6	5.8	8.3	7.8	7.5
7000	Conc.	6985	6750	6845	6665	6523
	Accuracy (%)	99.8	96.4	97.8	95.2	93.2
	CV (%)	0.7	2.8	2.3	5.6	3.2
Dilution (n=6)						
20000	Conc.	19225	18675	19400	18217	18300
	Accuracy (%)	96.1	93.4	97.0	91.1	91.5
	CV (%)	3.1	2.4	2.4	5.7	4.2

Nominal Conc. (pg/mL)		17-epiE3	3-MeOE1	2-MeOE1	4-MeOE1	2-MeOE2	4-MeOE2	2-OHE1	4-OHE1	2-OHE2
Freeze and Thaw (n=6)										
30.0	Conc.	30.5	28.3	29.9	32.2	30.9	32.2	34.2	32.4	33.7
	Accuracy (%)	101.5	94.4	99.8	107.4	103.0	107.2	113.8	108.1	112.3
	CV (%)	6.7	7.4	6.6	4.3	6.0	5.8	2.6	5.6	1.1
7000	Conc.	7128	7382	7315	6485	6270	6938	7963	7667	8075
	Accuracy (%)	101.8	105.5	104.5	92.6	89.6	99.1	113.8	109.5	115.4
	CV (%)	2.3	1.7	3.8	7.8	5.6	0.4	3.5	5.2	3.0
Dilution (n=6)										
20000	Conc.	30.5	28.3	29.9	32.2	30.9	32.2	34.2	32.4	33.7
	Accuracy (%)	101.5	94.4	99.8	107.4	103.0	107.2	113.8	108.1	112.3
	CV (%)	6.7	7.4	6.6	4.3	6.0	5.8	2.6	5.6	1.1

Ordering Information

Products	Specification	Cat.No.
Cleanert® SLE Plate	300mg / 2mL / well	HC4002SQ-9W
96-well collection plate	2.2 mL Squaral well	96SP2036-2
96-well vacuum manifold	adapt to 96 well plate	VM96
NV-96G for 96 Well Plates	adapt to 96 well plate	NV96-G
Methanol	HPLC, 4 L	AH-230-4
1.5 mL vials	1.5 mL short thread vial, amber glass, label and filling lines	AV1111-0
1.5 mL vials caps	9 mm screw neck cap, center hole; red silicone/ white PTFE septa	AV2100-0
Micro-insert clear glass	300 µL micro-insert, 31×6 mm	AV1132-6

A Rapid and Sensitive Solid Liquid Extraction Method to Eliminate the Matrix Effects of Liquid Chromatograpy-tandem Mass Spectrometry for the Determination of Levonorgestrel in Plasma (Cleanert® SLE Plate 400 µL; PN:HC4002SQ-9W)

RAB10010

Experimental Details

Sample Preparation

200 µL of plasma was diluted to 400 µL with 30 mM potassium phosphate buffer. 50 µL of working solution of Levonorgestrel with specific concentration was spiked into the plasma at first if needed.

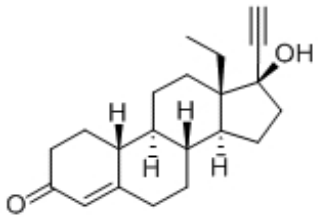


Figure 1 Chemical structure of levonorgestrel

Steps of SLE

Sample loading: Added 400 µL of the sample into each well of SLE plate.
Elution: After 10 minutes, eluted each well with 1.4 mL acetic ether:Hexane (20:80) by twice with a time gap of 30 seconds.
The collected eluant was dried with nitrogen blowing at 45℃ and reconstituted with 0.1ml of 50% acetonitrile in water for further determination by LC-MS/MS

Steps of LLE

A process of LLE was carried out as a contrast. 1.4 mL acetic ether:Hexane (20:80) was applied to extract Levonorgestrel twice from 400 µL sample solution by vortex. Then the combined extracted solution was treated as those processes of SLE.

Instrumentation

Instrumentation: LC-MS/MS, API 4000+
Mobile phase: water with 0.1% formic acid/methanol (20/80, v/v) - for the determination of Levonorgestrel
water with 0.1% formic acid/methanol (15/85, v/v) - for the determination of phospholipids
Column: Venusil ASB C18, 2.1 mm×150 mm, 3 µm, 150 Å
Flow rate: 200 µL/min (for the detection of Levonorgestrel)
400 µL/min (for the detection of phospholipids)
Injection: 5 µL
Temperature: 30℃

MS Conditions

Ion source: ESI - Positive
Scan mode: MRM

Table 1: MS/MS transitions of Levonorgestrel and Phospholipids

Analyte	Q1	Q3
levonorgestrel	313.3	109.1
	313.3	184.9
	313.3	295.1
phospholipids	496.3	184.4
	524.3	184.3

Results

(1) Chromatogram

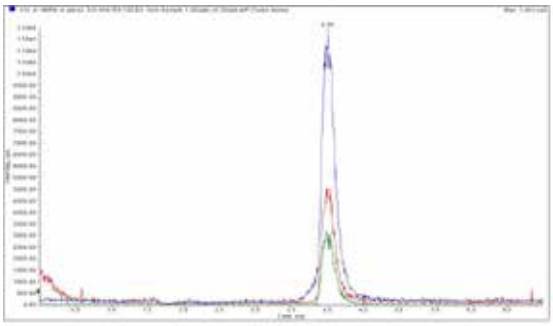


Figure 2 Chromatogram of 20 ng/mL lenovorgestrel standard solution

Comparison of Phospholipids elimination between SLE and LLE

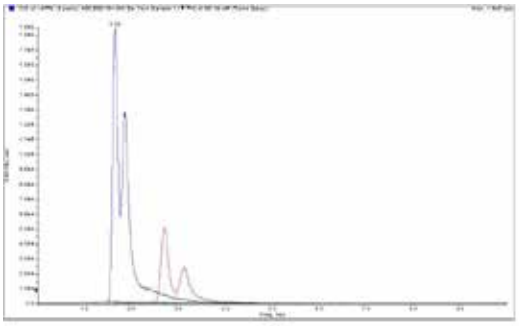


Figure 3 Chromatogram of phospholipids in the sample treated by LLE

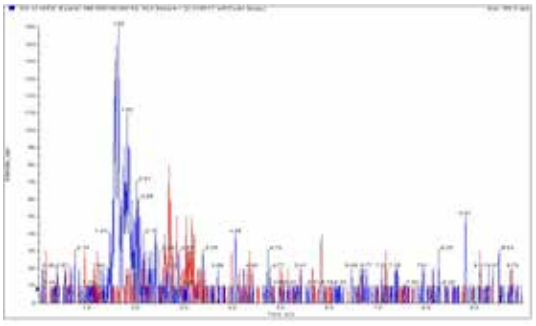


Figure 4 Chromatogram of phospholipids in the sample treated by SLE

(2) Recovery

Table 2 Recoveries of levonorgestrel treated by SLE

Analyte	Concentration (ng/mL)	Recovery (%)					Mean(%)	RSD (%)
		1	2	3	4	5		
levonorgestrel	5 ng/mL	91.82	101.18	101.74	99.80	97.21	98.35	4.10
	10 g/mL	94.37	97.27	94.18	99.46	92.13	95.48	3.02

Table 3 Comparison with LLE (Recoveries of spiked samples at the level of 50 ng/mL)

Name		LLE-1	LLE-2	SLE-1	SLE-2
Area of phospholipids	m/z496.350-184.300	1.6908e6	2.2190e6	11559	16500
	m/z524.37-184.300	4.835e5	5.8900e5	9040	10358
Recoveries of levonorgestrel		81.13%	87.85%	95.13%	93.28%

Ordering Information

Products	Specification	Cat.No.
Cleanert® SLE plate	Max sample loading volume 400 µL	HC4002SQ-9W
Venusil® ASB C18	2.1 mm×150 mm, 3 µm, 150 Å	VS931502-0
96-well collection plate	2.2 mL Squaral well	96SP2036-2
96-well vacuum manifold	adapt to 96well plate	VM96
NV-96G for 96 Well Plates	adapt to 96well plate	NV96-G
1.5 mL vials	1.5 mL short thread vial, amber glass, label and filling lines	AV1111-0
1.5 mL vials caps	9 mm screw neck cap, center hole; red silicone/ white PTFE septa	AV2100-0
Micro-insert clear glass	300 µL micro-insert, 31x6 mm	AV1132-6

Analysis of Telmisartan in Plasma by SLE Pre-treatment and LC-MS/MS (Cleanert® SLE Plate 400 µL; PN:HC4002SQ-9W)

RAB10001

Experimental Details

Sample Preparation

The experiment employed Cleanert® SLE 96 well plate to process plasma sample.

a) Aliquot 100 µL human EDTA plasma sample;
b) Add 200 µL of buffer;
c) Mix well;
d) Load the sample and buffer mixture onto a Cleanert SLE 96 well plate
e) Apply low vacuum (0.2MPa) for 5 seconds to initiate loading;
f) Wait for 5 min;
g) Elute with 700 µL of MTBE twice by gravity;
h) Evaporation;
i) Reconstitution in 500 µL of mobile phase.

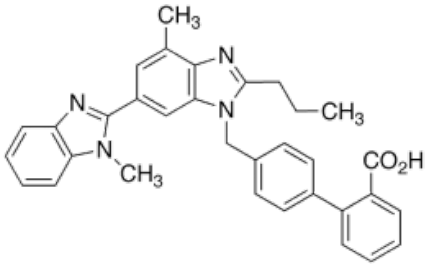


Figure 1 Chemical Structure of Telmisartan

Instrumentation

Instrumentation: LC-MS/MS, API 4000+
Column: Venusil® XBP C18, 2.1×150mm, 5µm, 120Å
Mobile phase: Acetonitrile:water (75:25)
Flow rate: 0.2 mL/min
Column temperature: 30℃
Injection volume: 3 µL
Ion source: ESI - Positive
Scan mode: MRM

Table 1 MS/MS transitions information of Telmisartan

Compound	Q1	Q3
Telmisartan	516.2	306.3
		276.2
		211.1

Results

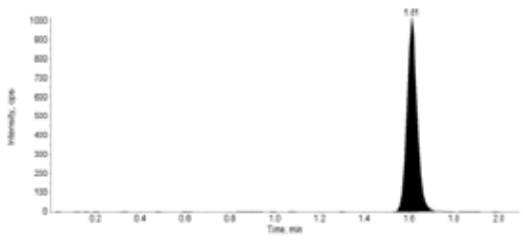


Figure 2 Lower limit of quantization human EDTA plasma sample (2 ng/mL)

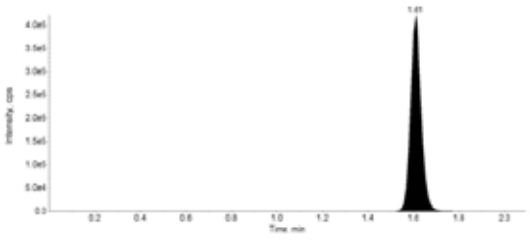


Figure 3 Higher limit of quantitation human EDTA plasma sample (1000 ng/mL)

Recovery of Telmisartan spiked in plasma (n=5)

Spiked Concentration	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Average	RSD
2ng/mL	88.3%	81.6%	87.8%	85.5%	90.6%	86.8%	3.93%
1000ng/mL	92.1%	83.9%	89.7%	88.5%	86.5%	88.1%	3.54%

Ordering Information

Products	Specification	Cat.No.
Cleanert® SLE plate	Max sample loading volume 400 µL	HC4002SQ-9W
Venusil® XBP C18	2.1 mm×150 mm, 5 µm, 120 Å	VX951502-A
96-well collection plate	2.2 mL Squaral well	96SP2036-2
96-well vacuum manifold	adapt to 96well plate	VM96
NV-96G for 96 Well Plates	adapt to 96well plate	NV96-G
Acetonitrile	HPLC, 4 L	AH015-4
1.5 mL vials	1.5 mL short thread vial, amber glass, label and filling lines	AV1111-0
1.5 mL vials caps	9 mm screw neck cap, center hole; red silicone/ white PTFE septa	AV2100-0

Comparison of SLE and MAS Method in Dexamethasone Analysis in Plasma (Cleanert® SLE Plate 200 µL; PN:HC2002SQ-9W)

EAB10005

Experimental Details

Sample Preparation

(1) Traditional PPT Method

Add 250 µL of ACN-methanol solvent (9/1, v/v) into 50 µL plasma sample (proper amount of dexamethasone is contained), take the supernatant, dry with N_2, resolve with mobile phase.

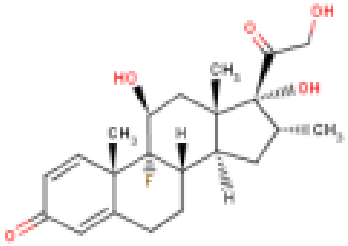


Figure 1 Chemical structure of dexamethasone

(2) Cleanert® PPT

- Put Cleanert® PPT plate and 96-well collection plate on 96-well negative SPE manifold.
- Add 50 µL plasma sample (proper amount of dexamethasone is contained) into each well
- quickly add 250 µL of solvent (ACN:methanol=9:1), stand for 3 min
- suction by vacuum, dry with N_2, resolve with 200 µL mobile phase

(3) SLE method

- Put Cleanert® SLE plate and 96-well plate on 96-well negative SPE manifold.
- Add 50 µL of water in 50 µL plasma sample (proper amount of dexamethasone is contained), load the diluted sample on Cleanert SLE plate
- Stand for 10min
- Add 1400 µL methyl tertiary butyl ether in each well, elute by gravity, dry with N_2, resolve with 200 µL

(4) MAS method

- Put Cleanert® MAS and 96-well collection plate on the 96-well negative SPE manifold. Condition each well with 1 mL of ACN, suction by vacuum
- Add 50 µL plasma sample (proper amount of dexamethasone is contained) into each well
- Quickly add 600 µL of solvent (ACN: methanol=9:1), stand for 3 min
- Suction by vacuum, dry with N2, resolve with 200 µL mobile phase

Instrumentation

Instrumentation: LC-MS/MS, API 4000
Column: Venusil® ASB C18 (2.1×150 mm, 3 µm,150 Å)
Column Temperature: 25℃
Mobile phase: 38% ACN, 62% 0.01 mol/L ammonium acetate aq
Flow rate: 0.25 mL/min
Sample injection: 5 µL

Table 1 MS/MS transitions information of dexamethasone

Compound	Parent ion	Daughter ion
dexamethasone	393.4	373.3

Results

(1) Chromatogram

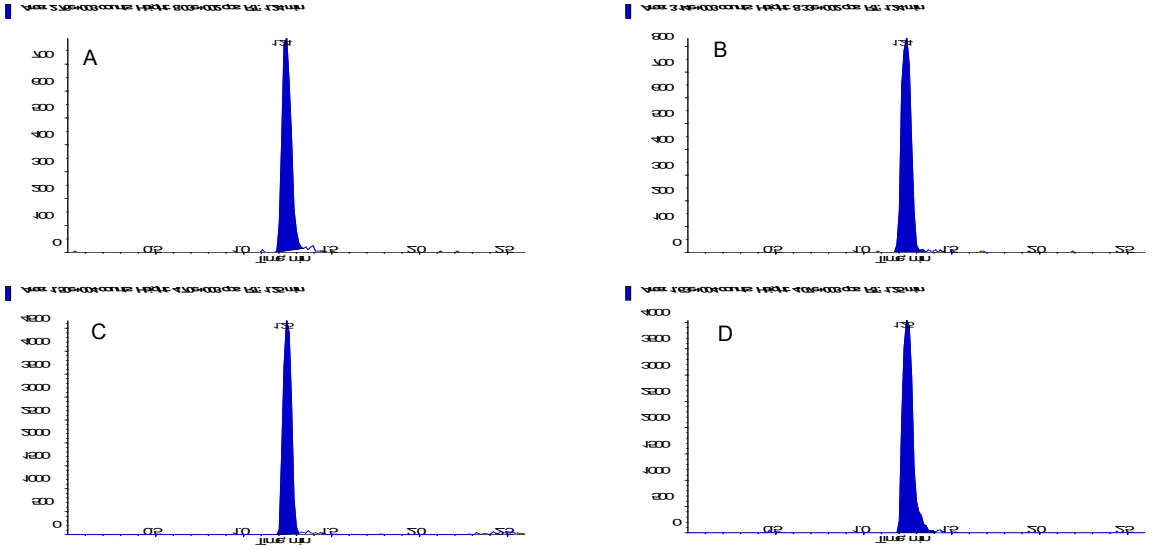


Figure 2 The MRM spectra of sample processed by traditional PPT method (A), Cleanert PPT(B), SLE (C) and MAS (D)

(2) Recovery

Table 2 Peak area of dexamethasone treat by 4 methods

Concentration (ng/mL)	Recovery data			
	Traditional PPT	Cleanert® PPT	SLE	MAS
10	2.38E+02	2.91E+02	1.32E+03	1.46E+03
100	2.76E+03	3.14E+03	1.50E+04	1.63E+04

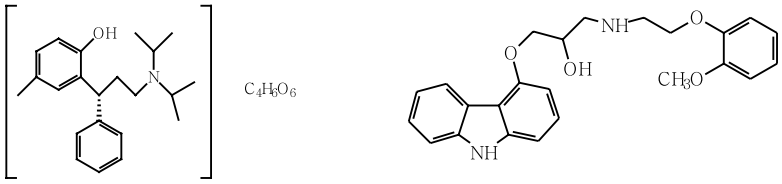
SLE and MAS method perform excellent in phospholipids and protein removing, which raise the signal by 5 times.

Ordering Information

Products	Specification	Cat.No.
Cleanert® PPT	2mL / well	96CD2025-Q
Cleanert® SLE plate	Max sample loading volume 200 µL	HC2002SQ-9W
Cleanert® MAS-B Plate	50mg / well	MS-B-0502W
Venusil® ASB C18	2.1 mm×150 mm, 3 µm, 150 Å	VS931502-0
96-well collection plate	2.2 mL Squaral well	96SP2036-2
96-well vacuum manifold	adapt to 96well plate	VM96
NV-96G for 96 Well Plates	adapt to 96well plate	NV96-G
Acetonitrile	HPLC, 4 L	AH015-4
Methanol	HPLC, 4 L	AH-230-4
1.5 mL vials	1.5 mL short thread vial, amber glass, label and filling lines	AV1111-0
1.5 mL vials caps	9 mm screw neck cap, center hole; red silicone/ white PTFE septa	AV2100-0
Micro-insert clear glass	300 µL micro-insert, 31x6 mm	AV1132-6

Tolterodine Tartrate in Plasma (Cleanert® MAS-B Plate 30mg/2mL/well; PN:MS-B-0302W)

EAB10006



Tolterodine tartrate　　Carvedilol (IS)

Figure 1 Chemical structure of Tolterodine Tartrate and carvedilol

Experimental Details

Sample Preparation

(1) Put the Cleanert® MAS plate and 96-well collection plate on the 96-well SPE negative manifold, condition with 1 mL of ACN for each well, suction by vacuum;
(2) Add 40 μL of plasma in each well, 20 μL of tolterodine standard solution and 20 μL internal standard;
(3) Quickly add 600 μL of ACN in each well, stand for 3 min;
(4) Filter by vacuum, dry with N2, resolve with 200 μL of mobile phase.

Result

(1) Chromatogram

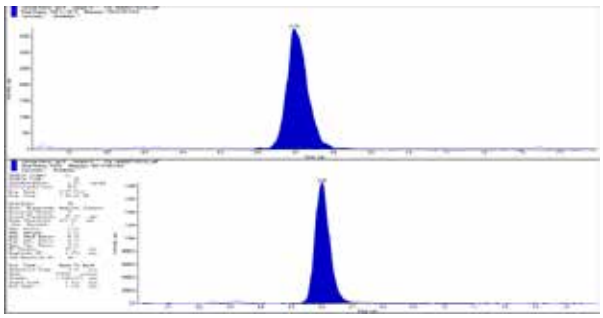


Figure 2 MRM gram for internal standard of tolterodine (A) and carvedilol (B)

(2) Method validation
LOQ: 0.1 ng/mL
linearity range: 0.1 ng/mL ~20 ng/mL
Regression equation: Y = 0.954X + 0.098, r = 0. 9961 (weight = $1/X^2$)m

Table 1 Recovery data of tolterodine spiked in plasma sample

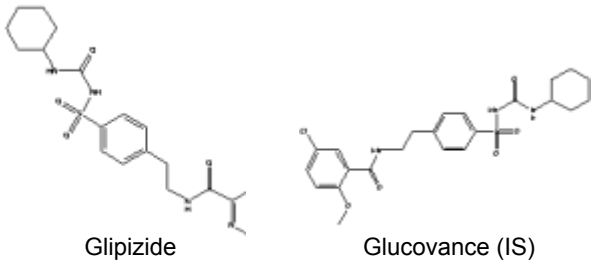
Concentration (ng/mL)	Recovery (%)						Average(%)	RSD (%)
	1	2	3	4	5	6		
0.3	87.1	91.0	94.0	91.5	93.1	90.3	91.1	2.65
8	90.5	90.1	91.2	90.1	90.2	91.4	90.6	0.63
16	90.1	85.7	84.8	85.9	86.4	82.6	85.9	2.85

Ordering Information

Products	Specification	Cat.No.
Cleanert® MAS-B Plate	30mg / well	MS-B-0302W
96-well collection plate	2.2 mL Squaral well	96SP2036-2
96-well vacuum manifold	adapt to 96 well plate	VM96
NV-96G for 96 Well Plates	adapt to 96 well plate	NV96-G
1.5 mL vials	1.5 mL short thread vial, amber glass, label and filling lines	AV1111-0
1.5 mL vials caps	9 mm screw neck cap, center hole; red silicone/ white PTFE septa	AV2100-0
Micro-insert clear glass	300 µL micro-insert, 31×6 mm	AV1132-6

Detection of Glipizide in Plasma (Cleanert® MAS-A Plate 30mg/2mL/well; PN:MS-A-0502W)

RAB10002



Glipizide Glucovance (IS)

Figure 1 Chemical structure of glipizidein and glucovance

Experimental Details

Sample Preparation

(1) Put Cleanert® MAS plate and 96-well collection plate on 96-well negative SPE manifold. Condition each well with 2 mL of ACN, suction by vacuum.
(2) Add 100 µL of plasma in each well, 50 µL of Glipizide standard and 20 µL internal standard
(3) Quickly add 1 mL of ACN aq mixed with 5% ammonia, stand for 3 min
(4) Suction by vacuum, dry with N_2, resolve with 200 µL mobile phase

Instrumentation

Instrumentation: LC-MS/MS, API 5000
Column: Venusil® AQ C18, 2.1×150 mm, 5 µm (PN: VA051502-0)
Column temperature: 25℃
Mobile phase: 60 % ACN, 40 % 0.01 mol/L ammonium acetate aq (formic acid contained)
Flow rate: 0.4 mL/min
Sample injection: 5 µL
Ion source: ESI - Positive
Scan mode: MRM

Table 1 MS/MS transitions information of Glipizide and Glucovance

Compound	Parent ion	Daughter ion
Glipizide	446.3	321.1
Glucovance (IS)	464.2	369.0

Result

(1) Chromatogram

Figure 2 Chromatogram of Glipizide standard solution

(2) Method validation
LOQ: 1.23 ng/mL
linearity range:1.23 ng/mL ~ 300 ng/mL
Regression equation: Y = 0.0269X + 0.0394, r = 0.9936 (weight = $1/X^2$)m

Table 2 Recovery data of glipizide spiked in plasma

Concentration (ng/mL)	Recovery (%)						Average(%)	RSD (%)
	1	2	3	4	5	6		
1.23	62.8	51.8	59.8	57.7	54.2	64.3	58.4	13.7
33.3	68.3	68.9	63.6	66.7	62.9	54.6	64.1	5.3
300	85.8	85.5	84.3	84.9	86.4	78.4	84.2	4.3

Ordering Information

Products	Specification	Cat.No.
Cleanert® MAS-A Plate	50 mg/well	MS-A-0502W
Venusil® AQ C18	2.1 mm×150 mm, 5 μm, 150 Å	VA951502-0
96-well collection plate	2.2 mL Squaral well	96SP2036-2
96-well vacuum manifold	adapt to 96 well plate	VM96
NV-96G for 96 Well Plates	adapt to 96 well plate	NV96-G
1.5 mL vials	1.5 mL short thread vial, amber glass, label and filling lines	AV1111-0
1.5 mL vials caps	9 mm screw neck cap, center hole; red silicone/ white PTFE septa	AV2100-0
Micro-insert clear glass	300 μL micro-insert, 31×6 mm	AV1132-6

Comparison of MAS and PPT Method in Propranolol Detection in Plasmas Sample (Cleanert® MAS-B Plate 30mg/2mL/well; PN:MS-B-0302W)

EAB10007

Experimental Details

Sample Preparation

(1) PPT Method

Add 50 µL of plasma into 20 µL 20 ng/mL propranolol standard solution followed by 250 µL ACN, shake for 3 min, centrifuge at 10000 rpm for 15 min. Take the supernatant and dry with N_2, resolve with 200 µL mobile phase.

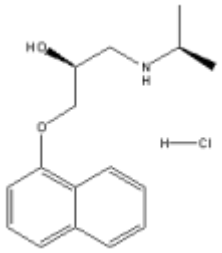


Figure 1 Structure of propranolol

(2) MAS Method

This experiment employed Cleanert® MAS-B to purify the plasma sample.

a) Put Cleanert® MAS plateand 96-well collection plate on 96-well negative SPE manifold, condition each well with 2 mL of ACN, suction by vacuum;

b) Add 50 µL of blood plasma in each well followed by 20µL 20 ng/mL propranolol standard solution;

c) Add 800 µL of ACN in each well and stand for 3 min;

d) Suction by vacuum, dry with N_2, resolve with 200 µL mobile phase.

Instrumentation

Instrumentation: LC-MS/MS, API 5000;
Column: Venusil® ASB C18, 2.1×150 mm, 5 µm
Column Temperature: 25℃
Mobile Phase: 60% ACN, 40% 0.01 mol/L ammonium acetate aq (formic acid)
Flow rate: 0.2 mL/min
Sample injection: 5 µL
Ion source: ESI - Positive
Scan mode: MRM

Table 1 MS/MS transitions information of propranolol

Compound	Parent ion	Daughter ion
Propranolol	260.1	183.0

Result

(1) Chromatogram

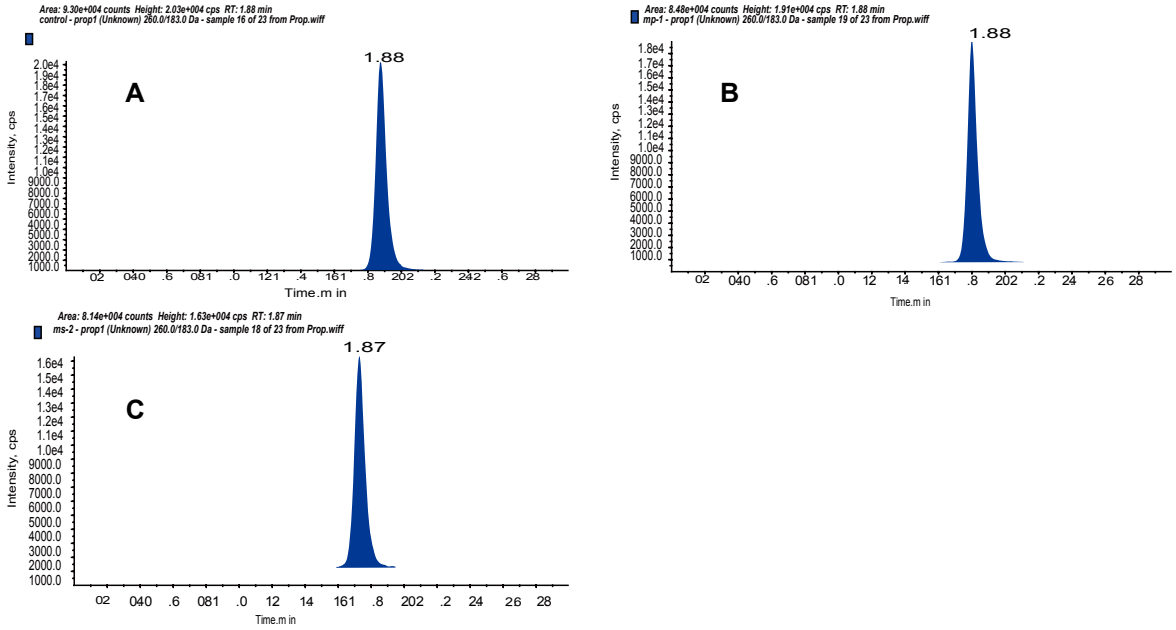


Figure 2 MRM Spectra of Propranolol Standard Solution (A) and Spiked Standard Processed by MAS(B) and PPT(C)

(2) Recovery

Table 2 Recovery data of propranolol spiked in plasma

Method	Average recovery(%)	RSD (%)
MAS	90.0	2.7
PPT	82.9	5.4

The experimental result show that MAS method perform better than PPT method.

Ordering Information

Products	Specification	Cat.No.
Cleanert® MAS-B Plate	30mg/well	MS-B-0302W
Venusil® ASB C18	2.1 mm×150 mm, 5 μm, 150 Å	VS951502-0
96-well collection plate	2.2 mL Squaral well	96SP2036-2
96-well vacuum manifold	adapt to 96well plate	VM96
NV-96G for 96 Well Plates	adapt to 96well plate	NV96-G
1.5 mL vials	1.5mL short thread vial, amber glass, label and filling lines	AV1111-0
1.5 mL vials caps	9 mm screw neck cap, center hole; red silicone/ white PTFE septa	AV2100-0
Micro-insert clear glass	300 μL micro-insert, 31×6 mm	AV1132-6

Comparison between Different Methods for Analysis of Arachidonic Acid in Plasma (Cleanert® MAS-M Plate 50mg/2mL/well; PN:MS-M-0502W)

RAB10003

The plasma components are complex, containing protein and phospholipid which will interfere the detection. Therefore the sample pre-treatment method is crucial. This experiment presents 3 kinds of methods to extract Arachidonic Acid (AA) from plasma, which involve Cleanert® MAS-M 96-well plate, protein precipitation 96-well plate and Brand W 96-well plate.

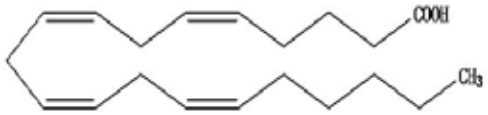


Figure 1 Chemical structure of arachidonic acid

Experimental Details

Solution preparation

AA standard substance was diluted by methanol to required concentration.

Sample Preparation

(1) Cleanert® PPT plate

100 µL of plasma was placed into the well of protein precipitation plate and mixed with 400 µL ACN on vortex shaker, and then centrifuged under 6000r/min for 5 min. The elution was collected and then analyzed by LC-MS/MS.

(2) Brand W 96-well plate

100 µL of plasma was placed into the well of Brand W 96-well plate which was claimed to be packed with Zirconia and then mixed with 300 µL of ACN containing 1 % formic acid. Then the mixture was drove through the plate by vacuum under 60 psi and collected analysis on LC-MS/MS.

(3) Cleanert MAS-M 96-well plate

A mixed-phase sorbent of RP adsorption, cation exchange interaction and anion exchange interaction was packed into the well of Cleanert® MAS-M 96-well plate.
Activation: 600 µL of Methanol and 600 µL of Water were added into the well of Cleanert MAS-M plate successively.
Sample Loading: 100 µL of plasma sample diluted with 100 µL of 3 % ammonium hydroxide solution was added into the activated well.
Washing: 600 µL of water and then 600µL of methanol was used to wash the well.
Elution: 600 µL of ACN with 3% formic acid was used to eluted the well.
Then, the elution was collected for further analysis on LC-MS/MS.

Instrumentation

Instrumentation: LC-MS/MS, API 4000+
HPLC Column: Venusil® ASB C18, 2.1 mm×150 mm, 3 µm, 150 Å
Mobile Phase: Acetonitrile:Water = 70:30(v/v)
Flow rate: 0.2 mL/min
Injection: 5 µL
Ion source: ESI Negative
Scan mode: MRM

Table 1 Precursor/Product Ions of AA

Analyte	Q1	Q3
AA	303.0	259.1
		230.9
		205.1
		177.1

Result

Table 2 Recoveries and Precision

Spiked concentration	Protein precipitation plate		Brand W plate		Cleanert® MAS-M plate	
	Ave. Recovery(n=5)	RSD(n=5)	Ave. Recovery(n=5)	RSD(n=5)	Ave. Recovery(n=5)	RSD(n=5)
100ppb	149.02%	9.24%	5.45%	8.46%	99.19%	5.17%
200ppb	129.32%	11.14%	44.09%	7.40%	103.21%	5.34%
1ppm	130.42%	2.06%	70.15%	11.51%	106.38%	4.29%

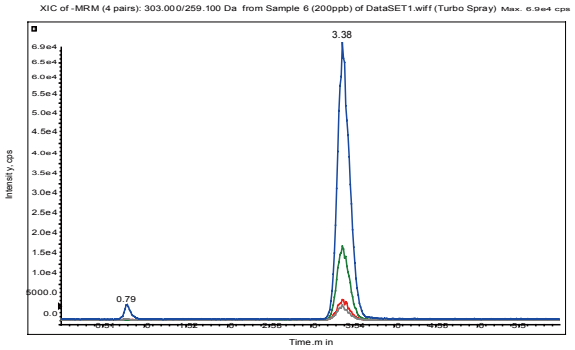


Figure 2 Chromatogram of 1ppm AA standard solution

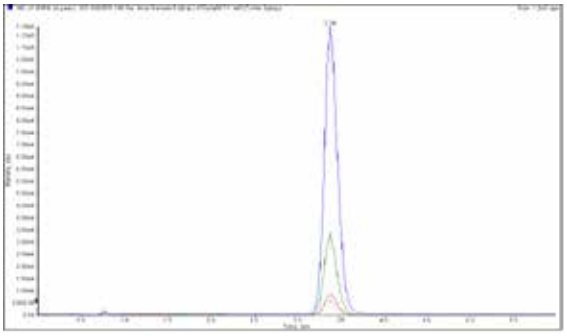


Figure 3 Chromatogram of plasma sample

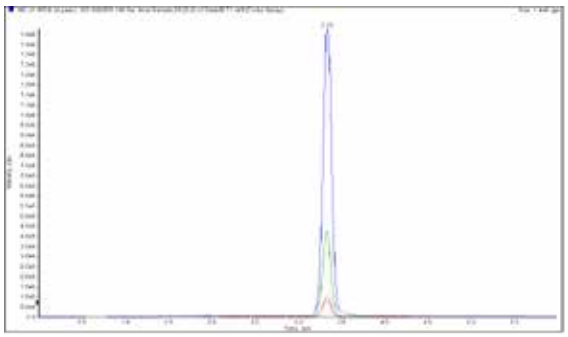


Figure 4 Chromatogram of plasma sample spiked with 1ppm AA standard (Protein precipitation plate)

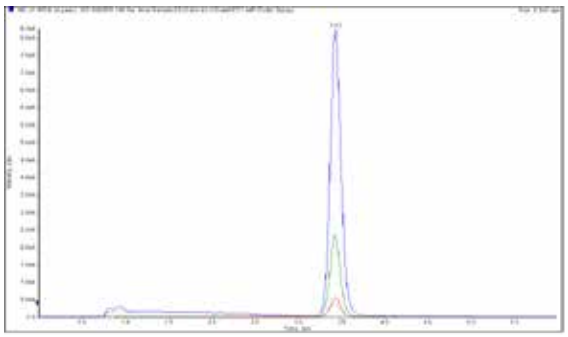


Figure 5 Chromatogram of plasma sample spiked with 1ppm AA standard (Brand W plate)

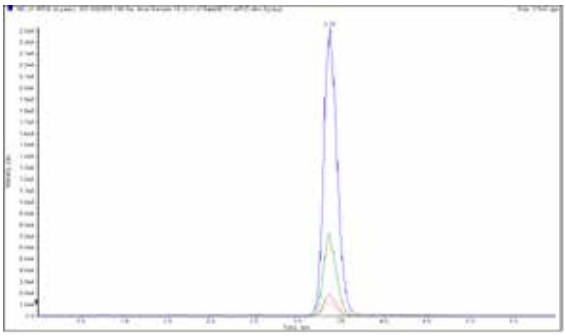


Figure 6 Chromatogram of plasma sample spiked with 1ppm AA standard (Cleanert® MAS-M plate)

The experiment employed 3 kinds of sample pre-treatment to extract AA from plasma. Protein precipitation method enjoyed a convenience due to its minimum procedures, but its recoveries of AA were 129.32%~149.02%, implying the worst purification effect which caused the matrix enhancement on mass spectrum. The recoveries of AA on Brand W 96-well plate were 5.45%~70.15%, while the recoveries of Cleanert® MAS-M 96-well plate were 99.19 %~106.38 which ensured an extraction procedure without reconstitution to support a rapid, high throughput assay of AA in plasma.

Ordering Information

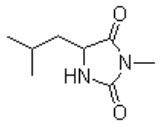
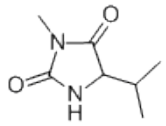
Products	Specification	Cat.No.
Cleanert® PPT plate	2mL / well	96CD2025-Q
Cleanert® MAS-M plate	50mg / 2mL / well	MS-M-0502W
Venusil® ASB C18	2.1 mm×150 mm, 3 μm, 150 Å	VS931502-0
96-well collection plate	2.2 mL Squaral well	96SP2036-2
96-well vacuum manifold	adapt to 96well plate	VM96
1.5 mL vials	1.5 mL short thread vial, amber glass, label and filling lines	AV1111-0
1.5 mL vials caps	9 mm screw neck cap, center hole; red silicone/ white PTFE septa	AV2100-0
Filtration membrane(Nylon)	φ13; 0.22 μm	AS021320-T
Disposable Syringe	2 mL, needless	ZSQ-2ML

A Rapid Clean-up Procedure for Monitoring the Biomarker of Dimethylformamide in Hemoglobin by LC-MS/MS (Cleanert® MAS-B Plate 30mg/2mL/well; PN:MS-B-0302W)

RAB10004

3-Methyl-5-Isopropylhydantoin (MVH), a metabolite of DMF was selected as the biomarker to monitor occupational exposure to DMF. A rapid sample preparation method to extract MVH from hemoglobin was established utilizing Cleanert® MAS-B 96-well Plate (25mg/well). 3-Methy-5-isobutylhydantion (MIH) was used as internal standard which had similar structure with MVH.

Table 1 Information of MIH and MVH

Compound	Name	CAS	Molecular weight	Structure
MIH	3-Methy-5-isobutylhydantion	675854-31-6	170.10	
MVH	3-Methyl-5-Isopropylhydantoin	74310-99-9	156.18	

Experimental Details

Solution preparation

Diluted the stock solution of MVH and MIH with methanol/water(1:1,v/v) to required concentrations.

Sample Preparation

0.1g hemoglobin was weighed accurately and placed in 15mL tube. 0.25 mL of 20 μg/mL MIH and 4.75 mL of hydrochloric acid/ acetic acid (1:1,v/v) was added into the tube and vortexed for 3 min. Then the tube was heated in boiling water to degrade hemoglobin for 1 h. There might be bubbles emerged during the degradation process which was a spontaneous phenomenon. The tube was cooled to room temperature and brought the total volume of the sample to 5 mL by adding methanol/water (1:1,v/v). Then 200 μL of the treated sample was transferred into Cleanert® MAS-B 96-well Plate and mixed with 600 μL of 1% formic acid in acetonitrile. Finally, the treated sample was passed through the plate under vacuum for 2 min to 4 min. The fractions were collected and analyzed by LC-MS/MS.

Instrumentation

Instrumentation: LC-MS/MS, API 4000+
LC Instrument: Shimadzu LC-20A
HPLC Column: Venusil® ASB C18, 150mm×2.1 mm, 5 μm, 100 Å
Mobile Phase: Acetonitrile/0.1 % formic acid-Water, 35:65(v/v)
Flow rate: 0.2 mL/min
Injection volume: 2 μL
Ion source: ESI Positive
Scan mode: MRM

Table 2 Precursor/Product Ions of MVH and MIH

Compounds	Retention time (min)	Q1	Q3
MVH	2.8	157.2	129.1
			71.9
			55.2
MIH	3.4	171.1	143.1
			86
			69

Result

(1) Chromatogram

Table 3 LOD of MVH and MIH standard

Analyte	LOD
MVH	1.0 ng/mL
MIH	1.0 ng/mL

(2) Recoveries and Precision

Standard solutions of MVH and MIH were added in to hemoglobin samples accurately. The recoveries of the spiked samples were listed in Table 4.

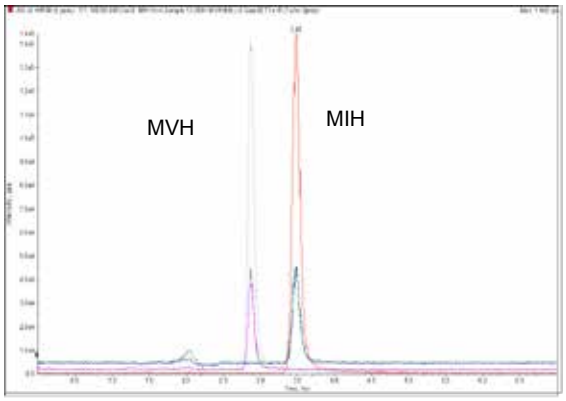


Figure 1 Chromatogram of MVH and MIH (250ng/mL)

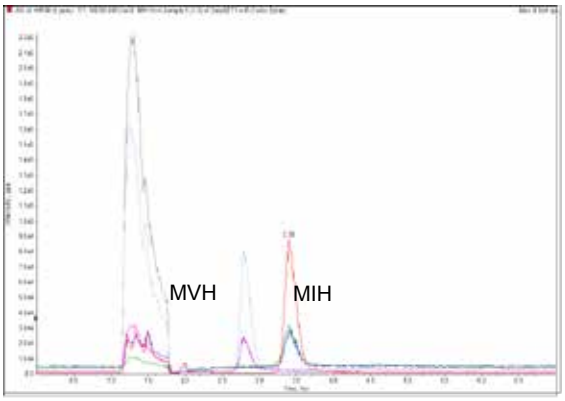


Figure 2 Chromatogram of hemoglobin spiked with 50mg/kg MVH

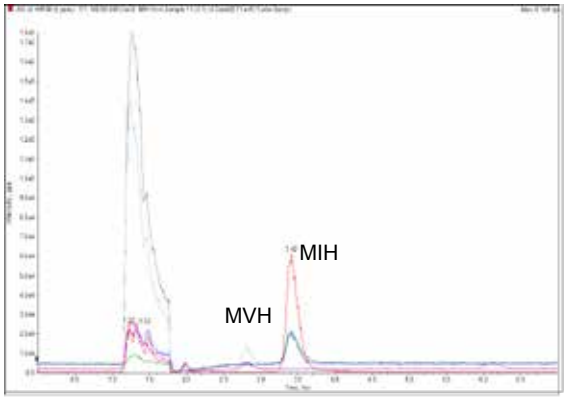


Figure 3 Chromatogram of actual hemoglobin

Table 4 Recoveries and Precisions

Compounds	Dosage(mg/kg)	Measurement(mg/kg)	Mean recoveries (n=5)	RSD(n=5)
MVH	10	10.5	105.0%	4.6%
	50	50.2	100.4%	3.5%
	100	101.9	101.9%	2.9%

Cleanert® MAS-B 96-well Plate is suitable to remove the matrix of hemoglobin which might interfere the analysis of MVH by LC-MS/MS. The usage of the 96-well plate is sufficient for high throughput sample clean-up procedure prior to LC-MS/MS. This experiment provided a solution for monitoring cumulative exposure to DMF.

Ordering Information

Products	Specification	Cat.No.
Cleanert® MAS-B Plate	30 mg/well	MS-B-0302W
Venusil® AQ C18	2.1 mm×150 mm, 5 μm, 100 Å	VA951502-0
96-well collection plate	2.2 mL Squaral well	96SP2036-2
96-well vacuum manifold	adapt to 96well plate	VM96
1.5 mL vials	1.5 mL short thread vial, amber glass, label and filling lines	AV1111-0
1.5 mL vials caps	9 mm screw neck cap, center hole; red silicone/ white PTFE septa	AV2100-0
Filtration membrane(Nylon)	φ13; 0.22 μm	AS021320-T
Disposable Syringe	2 mL,needless	ZSQ-2ML

Extraction of β-blockers From Small Volume Biological Fluid Samples Using a New Versatile SPE 96-well Plate Format (Cleanert® PEP Micro Plate 5mg/1mL/well; PN: PE00501-MW)

RAB10005

The Cleanert® PEP MicroPlate is optimised for the sample processing and extraction of small volume biological fluids.

The Cleanert® PEP MicroPlate is a modular design, allowing for flexibility in sample numbers. Full or partially populated plates can be processed using vacuum or positive pressure. In addition, the well outlet design ensures good collection plate penetration, removing any possibility of well to well cross contamination.

The Cleanert® PEP MicroPlate (Polar-modified Reversed Phase) is a water-wettable, non-polar SPE sorbent utilising a proprietary polymeric backbone. The surface chemistry has been optimised to provide the necessary balance of non-polar interactions for retaining compounds of varying polarity, from polar metabolites to higher molecular weight analytes.

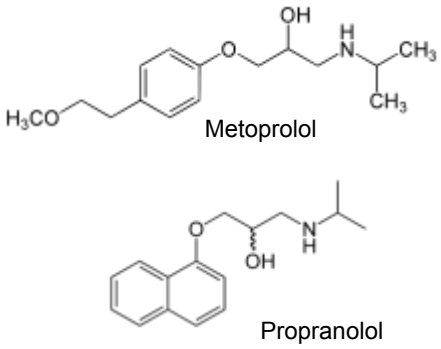


Figure 1 Structures of Metoprolol and Propranolol

β-blockers, Metoprolol and Propranolol, was used for the investigation work. The two analytes were spiked at the desired concentrations into human plasma and extracted using a Cleanert® PEP MicroPlate.

Experimental Details

Sample Preparation

Human plasma samples were spiked with the two analytes in the concentration range 1 to 5 ng/mL.
The SPE Step
Equilibration: 200 µL Methanol; 200µL deionized water;
Sample Loading:100 µLhuman plasma diluted with 100 µL water
Washing: 200 µL deionized Water/Methanol (95/5, v/v)
Elution: 50 µL Acetonitrile/IPA(4/6, v/v) containing 2 % formic acid, 150 µL of water was added into the collected fractions for further determination by LC-MS/MS

Instrumentation

Instrumentation: LC-MS/MS, API 4000+
Column: Venusil® AQ C18, 2.1×150 mm, 5 µm, 100 Å
Mobile Phase: 0.1 % formic acid in Acetonitrile/0.1 % formic acid in Water (22/78, v/v)
Flow rate: 300 µL /min
Injection: 10 µL
Temperature: 30 ℃

Table 1 Precursor Ion and Product Ion

Analyte	Q1	Q3
Metoprolol	268.3	191.0
Propranolol	260.1	157.1
	260.1	183.1

Result

(1) Chromatogram

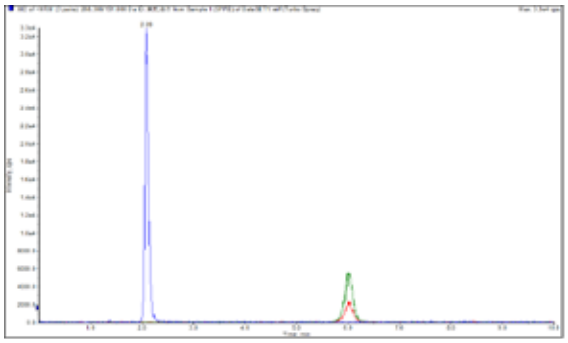


Figure 2 Chromatogram of 5ppb Metoprolol and Propranolol

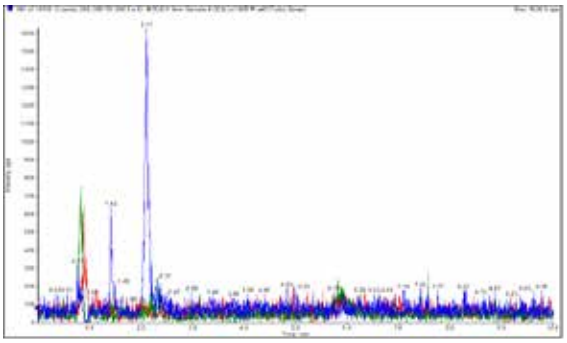


Figure 3 Chromatogram of blank sample

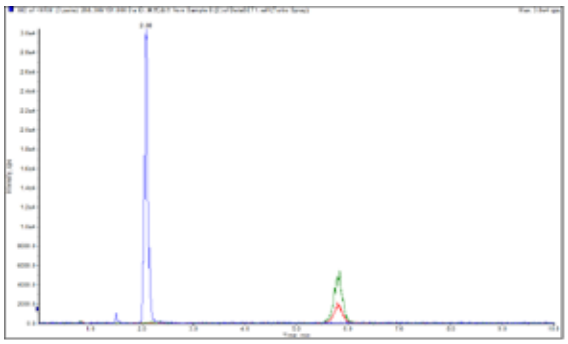


Figure 4 Chromatogram of spiked plasma sample with 5ppb Metoprolol and Propranolol

(2) Recovery data

LODs for Metoprolol and Propranolol were 0.02 and 0.1 ng/mL respectively

Table 2 Recoveries of Metoprolol and Propranolol

Analyte	Concentration (ng/mL)	Recovery (%)					Mean(%)	RSD (%)
		1	2	3	4	5		
Metoprolol	1 ng/mL	83.2	88.5	85.2	81.6	82.5	84.2	3.26
	5 ng/mL	90.6	86.6	91.6	94.2	89.4	90.5	3.09
Propranolol	1 ng/mL	86.2	92.3	87.7	84.2	83.9	86.9	3.92
	5 ng/mL	95.4	92.6	93.5	90.8	88.6	92.2	2.81

Ordering Information

Products	Specification	Cat.No.
Cleanert® PEP Micro Plate	5mg / well	PE00501-MW
Venusil® AQ C18	2.1 mm×150 mm, 5 μm, 100 Å	VA951502-0
96-well collection plate	2.2 mL Squaral well	96SP2036-2
96-well vacuum manifold	adapt to 96 well plate	VM96
1.5 mL vials	1.5 mL short thread vial, amber glass, label and filling lines	AV1111-0
1.5 mL vials caps	9 mm screw neck cap, center hole; red silicone/ white PTFE septa	AV2100-0
Micro-insert clear glass	300 μL micro-insert,31x6 mm	AV1132-6

Extraction of Indomethacin and Ibuprofen from Small Volume Biological Fluid Samples Using a New Versatile µElution SPE 96-well Plate Format (Cleanert® PEP Micro Plate 5mg/1mL/well, PN: PE00501-MW)

RAB10006

Developments in LC-MS/MS detection levels have seen biological fluid sample volumes reduced to such an extent that conventional SPE formats are not always suitable, or provide considerable analyte dilution and extended evaporation times. When extracting from sample volumes of less than 100 µL, it is important elution volumes and the sample preparation format are fully compatible with the original sample volumes being processed.

This poster summarises the solid phase extraction of two drugs from human plasma, Indomethacin and Ibuprofen using Cleanert® PEP, a water-wettable polymer-based SPE sorbent. Analyte concentrations range from 5 to 50 ng/mL. The data highlights the minimum elution volumes that can be achieved from the 5 mg sorbent mass and the versatile nature of the modular plate design.

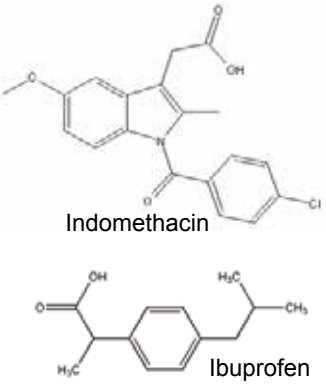


Figure 1 Chemical structure of Indomethacin and Ibuprofen

Experimental Details

Sample Preparation

Human plasma samples were spiked with the two analytes in the concentrations range 0.25 to 25 ng/mL.
SPE Method using Cleanert® PEP MicroPlate, 5 mg.
Conditioning: 200 µL Methanol.
Equilibration: 200 µL Deionised Water
Sample Application: 50 µL Human plasma diluted by 50 µL 2% formic acid aqueous solution
Interference Elution: 200 µL Deionised Water/Methanol (95/5, v/v)
Analyte Elution: 100µL Acetonitrile
The Cleanert® PEP MicroPlate was processed using a vacuum manifold.

Instrumentation

Instrumentation: LC-MS/MS, API 4000+
HPLC Column: Venusil® XBP C18, 150×2.1 mm, 5 µm, 120 Å
Mobile Phase: Acetonitrile/Water(75/25, v/v)
Flow rate: 0.2 mL/min
Injection: 3 µL
Ion source: ESI Negative
Scan mode: MRM

Table 1 Precursor/Product Ions

Analyte	Q1	Q3	DP/V	CE/V
Indomethacin	356.0	312	-67	-14
		296.7	-65	-25
		270.1	-63	-23
Ibuprofen	205.2	160.6	-60	-10
		159.1	-65	-9.6

Result

(1) Chromatogram

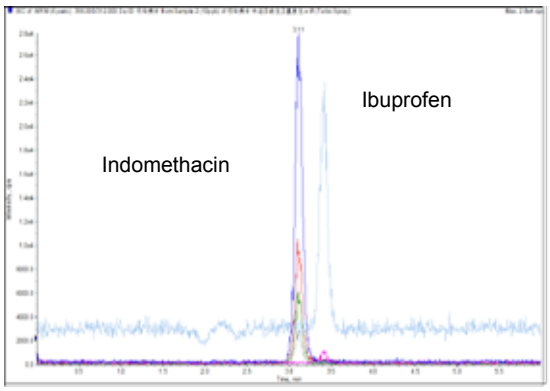


Figure 1 Chromatogram of spiked plasma sample with 10 ng/mL Indomethacin and Ibuprofen

(2) LOD and Recovery data

Table 2 LOD of Indomethacin and Ibuprofen

Analyte	LOD
Indomethacin	0.5ng/mL
Ibuprofen	1.0ng/mL

Table 3 Recoveries of Indomethacin and Ibuprofen spiked in plasma

Analyte	Concentration (ng/mL)	Recovery (%)					Mean(%)	RSD (%)
		1	2	3	4	5		
Indomethacin	5	87.60	85.90	90.30	85.10	89.90	87.80	2.60
	20	102.00	100.10	98.90	94.30	93.20	97.70	3.90
	50	95.10	89.30	101.10	93.10	97.40	95.20	4.70
Ibuprofen	5	100.90	104.50	102.30	99.50	96.70	100.80	2.90
	20	100.10	98.10	103.90	90.80	98.00	98.20	4.90
	50	92.50	94.10	98.10	96.50	99.90	96.22	3.10

Extraction procedure using Cleanert® PEP MicroPlate assure the number of wells to match sample numbers being processed. Only 100 µL of elution solvent ensured elute Indomethacin and Ibuprofen completely which were spiked into 50µL of plasma. The SPE method included an organic solvent/water combination as the interference elution was to remove polar interferences from the sorbent. This ensured a short analytical run time of less than 10 min, supporting a high throughput application.

Ordering Information

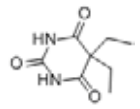
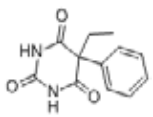
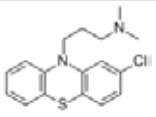
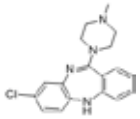
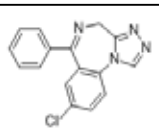
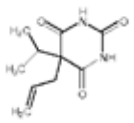
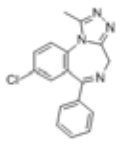
Products	Specification	Cat.No.
Cleanert® PEP Micro Plate	5mg/well	PE00501-MW
Venusil® XBP C18	2.1 mm×150 mm, 5 µm, 120 Å	VX951502-A
96-well collection plate	2.2 mL Squaral well	96SP2036-2
96-well vacuum manifold	adapt to 96well plate	VM96
1.5 mL vials	1.5 mL short thread vial, amber glass, label and filling lines	AV1111-0
1.5 mL vials caps	9 mm screw neck cap, center hole; red silicone/ white PTFE septa	AV2100-0
Micro-insert clear glass	300 µL micro-insert, 31×6 mm	AV1132-6
Filtration membrane(Nylon)	φ13; 0.22 µm	AS021320-T
Disposable Syringe	2 mL, needless	ZSQ-2ML

Hypnotic Drugs Detection in Blood Sample (Cleanert® PEP-2 cartridge 200mg/6mL, PN: PE2006-2)

EAB10008

This experiment used Qdaura® automated station to do the pre-treatment experiments of three different types hypnotic drugs,barbiturates, tricyclic and benzodiazepine class,etc. and achieved relative ideal results.

Table 1 Hypnotics sample information

Sample Name	Sample Name	CAS	Molecular weight	Structure
Barbital	$C_8H_{12}N_2O_3$	57-44-3	184.19	
Phenobarbital	$C_{12}H_{12}N_2O_3$	50-06-6	232.24	
Chlorpromazine	$C_{17}H_{19}ClN_2S$	50-53-3	318.86	
Clozapine	$C_{18}H_{19}ClN_4$	5786-21-0	326.82	
Estazolam	$C_{16}H_{11}ClN_4$	29975-16-4	294.74	
Aprobarbital	$C_{10}H_{14}N_2O_3$	77-02-1	210.23	
Alprazolam	$C_{17}H_{13}ClN_4$	28981-97-7	308.76	

Experimental Details

Reagent material

Methanol, dichloromethane, ethanol, acetic acid were HPLC grade; disodium hydrogen phosphate, sodium dihydrogen phosphate were of analytical grade; ultra-pure water; blood;

Acetic acid - solution: Take 50 mL of deionized water with acetic acid adjusted to pH = 6;

Disodium hydrogen phosphate solution: 0.1 mol/L, weighed 35.81 g disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$), dissolved in water and dilute to 1 L;

Sodium dihydrogen phosphate solution: 0.1 mol/L, weighed 15.60 g disodium hydrogen phosphate ($Na_2HPO_4 \cdot 2H_2O$), dissolved in water and dilute to 1 L;

0.1 mol/L phosphate buffer solution (pH = 6): The 1.70 g of dibasic sodium phosphate and 12.14 g sodium dihydrogen phosphate was dissolved in 800 mL of deionized water, and then diluted with deionized water to 1 L, mix well. PH adjusted to 6.0 (with 0.1 mol/L sodium dihydrogen phosphate to lower pH; with 0.1 mol/L disodium hydrogen phosphate to increase the pH);

Hypnotic standard stock solution: mixed to standard 1 mg/mL, including barbiturates, phenobarbital, chlorpromazine, clozapine, estazolam, Appleton ratio properly, alprazolam, solvents for dry ethanol;

Hypnotic standard products working solution: take hypnotic standard stock solution 50 μL, diluted with ethanol to mix standard solution of 400 ppb;

Sample Preparation

Sample Extraction

Measured 2.0 mL blood sample, added 2 mL pH=6 phosphate buffer solution, vortex mixed 1min, then ultrasound 10 min, mixed and to be purified.

Sample purification

(1) Activation: 6 mL methanol and 6 mL of water were added into the Cleanert® PEP-2 cartridge in sequence.
(2) Sample Loading: 4 mL of the pre-treament plasma sample was load into the cartridge.
(3) Washing: 6 mL of acetic acid aqueous solution (pH = 6), discard the eluent.
Suction the cartridge by pump for 10 min.
(4) Elution: Eluted the target compounds by 8mL of dichloromethane
Concentrated the elution to dryness by nitrogen and reconstituted it by 250 μL of ethanol, filtrated the solution by filter and detected by GC-MS.

Table 2 Qdaura® SPE-40 automated extraction procedure

Step	Instruction	Solvent	Flow rate (mL/min)	Flow volu (mL)
1	Activation	Methanol	3	6
2	Activation	Water	3	6
3	Adding sample	Air	2	10
4	Washing sample tube	Acetic acid - water	3	6
5	Washing sample tube	Air	5	10
6	Washing extraction column	Air	3	30
7	Collecting an extraction column	Dichloromethane	2	8
8	Collecting an extraction column	Air	3	10

Instrumentation

Chromatographic conditions

Instrumentation: GC-MS; Qdaura® (automated solid phase extraction device)

HPLC Column: DA-5MS capillary column (30 m × 0.25 mm × 0.25 μm), the carrier gas is helium with the purity of 99.99%

Flow rate: 1 mL / min;

Initial column temperature: 130℃ , 10℃ /min heat up to 280℃ ,then keep 10 min; injector temperature is 270℃ .

MS conditions

Ion source temperature: 230℃ , MS quadrupole temperature: 150℃ , the scan mode is SIM.

Result

(1) Chromatogram

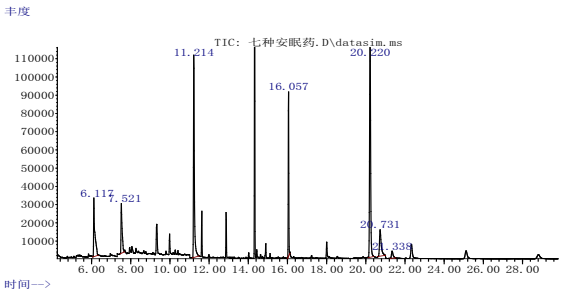


Figure 1 Chromatogram of hypnotic drugs standard solution

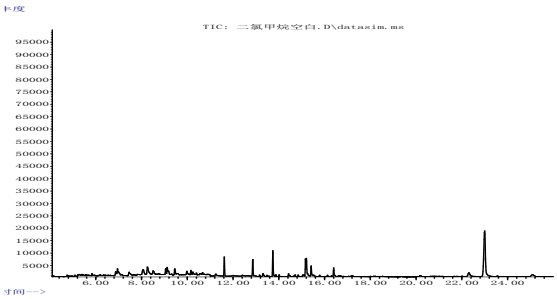


Figure 2 Chromatogram of blood sample

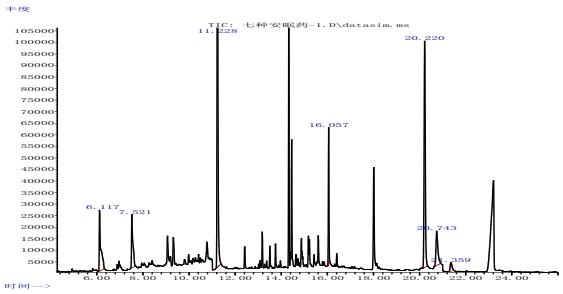


Figure 3 Chromatogram of hypnotic drugs spiked to blood sample

(2) Recovery data

Table 3 Recovery data of 7 kinds of hypnotic spiked in the plasma sample (400ng/mL)

Compounds	Retention Time/min	Average Recovery	RSD/% (n=5)
Barbital	6.117	96.69	2.31
Phenobarbital	11.231	103.93	9.24
Chlorpromazine	16.050	73.76	5.37
Clozapine	20.207	90.91	6.33
Estazolam	20.751	98.85	10.57
Aprobarbital	7.521	95.62	8.90
Alprazolam	21.338	95.48	3.76

The experiment using Cleanert® PEP-2 established pre-treatment methods of seven kinds of psychotropic drugs in blood, and combined Qdaura® automated SPE station to measure the psychotropic drugs in blood samples . Experiments tested the amount of 400 ng/mL samples,and the results show that the recovery rate of this method is good, between 70% -110%, can be used to detect psychotropic drugs in the blood.

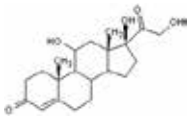
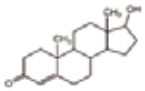
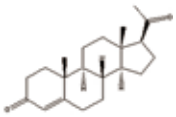
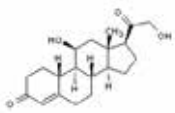
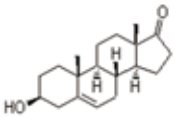
Ordering Information

Products	Specification	Cat.No.
Cleanert® PEP-2	200mg / 6mL	PE2006-2
DA-5MS	30 m×0.25 mm×0.25 μm	1525-3002
Qdaura® automated solid-phase extraction device	4-channel 24	SPE-40
1.5 mL vials	1.5 mL short thread vial, amber glass, label and filling lines	AV1111-0
1.5 mL vials caps	9 mm screw neck cap, center hole; red silicone/ white PTFE septa	AV2100-0
Filtration membrane(Nylon)	φ13;0.22 μm	AS021320-T
Disposable Syringe	2 mL,needless	ZSQ-2ML

Cleanert® PEP microplate for the Extraction of Steroid Hormones from Serum (Cleanert® PEP Micro Plate 5mg/1mL/well, PN: PE00501-MW)

RAB10007

Table 1 Information of the analytes

Sample Name	CAS	Structure
Hydrocortisone	50-23-7	
Testosterone	58-22-0	
Progesterone	57-83-0	
Aldosterone	52-39-1	
Dehydroepiandrosterone	53-43-0	

Experimental Details

Standard solution

The standards were dissolved by methanol to get stock solutions at the concentration of 1 mg/mL. Then the stock solutions were diluted to required concentration by methanol.

Sample Preparation

This experiment employed Cleanert® PEP 96-well plate (5 mg/well) for sample purification.
Column condition: 200 µL of Methanol was added into each well of Cleanert® PEP, followed by 200 µL of Water to condition the packing material.
Sample loading: 100 µL of serum was diluted with 100 µL solvent which contained 1% formic acid in methanol/ water (50:50,v/v), then loaded the sample onto the plate.
Washing: 400 µL of methanol/water (35/65, v/v) was used to wash the plate.
Elution: 200 µL of methanol with 1% formic acid was used to elute the plate.
Then, the elution was collected and analyzed by LC-MS/MS. (The elution could be evaporated and reconstituted to obtain lower LOD)

Instrumentation

Instrumentation: LC-MS/MS, API 4000+
Column: Venusil ASB C18, 2.1×50 mm, 3 μm, 150 Å;
Mobile phase: 0.1% FA in acetonitrile / 0.1%FA in Water (60:40, v/v)
Flow rate: 0.2 mL/min
Column temperature: 30℃
Injection volume: 3 μL
Ion source: ESI - Positive
Scan mode: MRM

Table 2 MS/MS transitions and Retention time of target compounds

Compounds	tR/min	Q1	Q3
Hydrocortisone	0.98	363.2	120.9
			327.2
Testosterone	1.66	289.4	97.1
			109.1
Aldosterone	0.98	361.3	325.3
			315.4
Dehydroepiandrosterone	1.66	289.4	253.4
			213.1
Progesterone	3.15	315.4	97.1
			109.1

Result

(1) Chromatogram

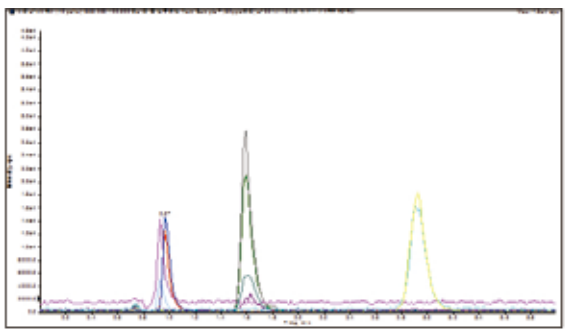


Figure 1 Chromatogram of 30 ppb Steroid hormones standard solution

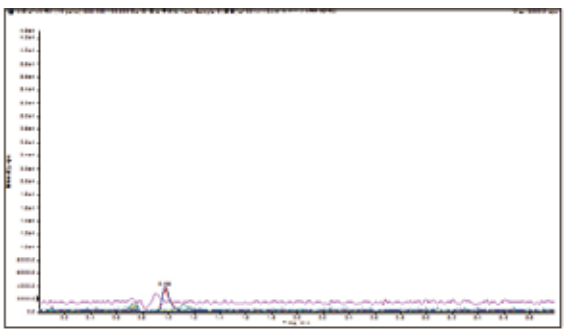


Figure 2 Chromatogram of serum sample

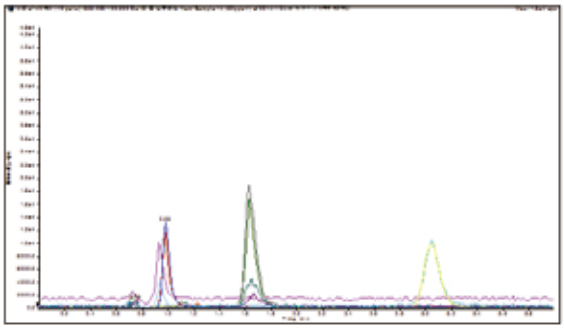


Figure 3 Chromatogram of Serum sample spiked with 30 ppb Steroid hormones standard solution

(2) Recovery data

Table 3 Recovery data

Spiked concentration	Aldosterone	Hydrocortisone	Testosterone	Dehydroepiandrosterone	Progesterone
30 ppb	85.9%	87.0%	105.5%	110.6%	97.7%

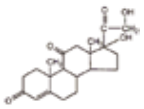
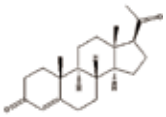
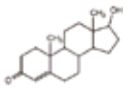
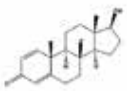
Ordering Information

Products	Specification	Cat.No.
Cleanert® PEP Micro Plate	5 mg/well	PE00501-MW
Venusil® ASB C18	2.1×50 mm, 3 μm, 150 Å	VS930502-0
96-well collection plate	2.2 mL Squaral well	96SP2036-2
96-well vacuum manifold	adapt to 96well plate	VM96
NV-96G for 96 Well Plates	adapt to 96well plate	NV96-G
1.5 mL vials	1.5 mL short thread vial, amber glass, label and filling lines	AV1111-0
1.5 mL vials caps	9 mm screw neck cap, center hole; red silicone/ white PTFE septa	AV2100-0
Micro-insert clear glass	300 μL micro-insert, 31×6 mm	AV1132-6
Filtration membrane(PTFE)	φ13; 0.22 μm	AS041320-T
Disposable Syringe	2 mL,needless	ZSQ-2ML

Cleanert® SLE for the Extraction of Steroid Hormones From Serum(Cleanert® SLE cartridge 200 µL, PN: HC2003Q-9)

RAB10008

Table 1 Information of the analytes

Sample Name	CAS	Structure
Cortisone	53-06-5	
Progesterone	57-83-0	
Testosterone	58-22-0	
Boldenone	846-48-0	

Experimental Details

Standard solution

The standards were dissolved by methanol to get stock solutions at the concentration of 1 mg/mL. Then stock solutions were diluted to required concentration by methanol.

Sample Preparation

This experiment employed Cleanert® SLE (200 µL, PN:HC2003Q-9) for sample purification.

Sample loading: Appropriate volume of methanol was added to 200 µL of serum sample, adjusted the content of methanol to 5%. Shook the sample and loaded onto the cartridge, then drew through the top frit under low vacuum (< -0.04 MPa) and stood for 10 min.

Elute analytes: 600 µL MTBE was used to elute the cartridge, and then the elution was collected at 1~2mL/min, repeated the elute operation after standing 1min, repeated twice. Then the elution was combined together for concentrate.

The elute was evaporated to dryness at 40℃ and reconstitute the residue by 200 µL of Acetonitrile:Water (3:7, v/v), and then analyzed by LC-MS/MS

Instrumentation

Instrumentation: LC-MS/MS, API 4000+
Column: Venusil® ASB C18, 2.1×50 mm, 3 μm, 150 Å
Mobile phase: Acetonitrile:Water (55:45, v/v) for analysis of progesterone, testosterone and boldenone
Acetonitrile:Water (30:70, v/v) for analysis of cortisone
Flow rate: 0.2 mL/min
Column temperature: 30℃
Injection volume: 5 μL
Scan mode: MRM
Two ionization modes were employed on the basis of compound structure. Analyzed progesterone, testosterone and boldenone with positive mode and analyzed cortisone with negative mode.

Table 2 MS/MS transitions and Retention time of target compounds

Compounds	tR/min	Q1	Q3
Boldenone	1.66	287.3	121.3
		287.3	135.3
Testosterone	1.95	289.3	97.1
		289.3	109.2
Progesterone	4.21	315.4	97.1
		315.4	109.1
Cortisone	4.21	405.3	359
		405.3	328.8
		405.3	300.9

Result

(1) Chromatogram

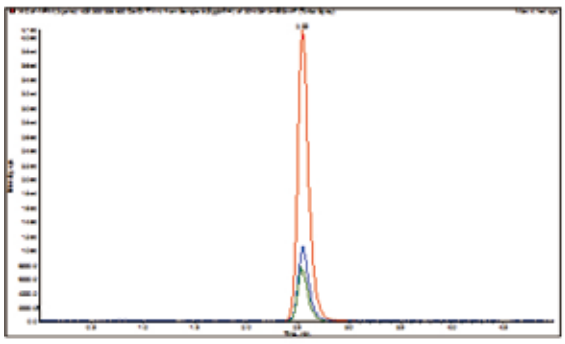


Figure 1 Chromatogram of Cortisone (5 ng/mL)

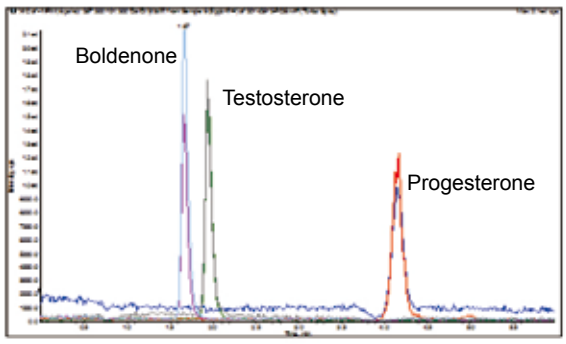


Figure 2 Chromatogram of boldenone, testosterone and progesterone (5 ng/mL)

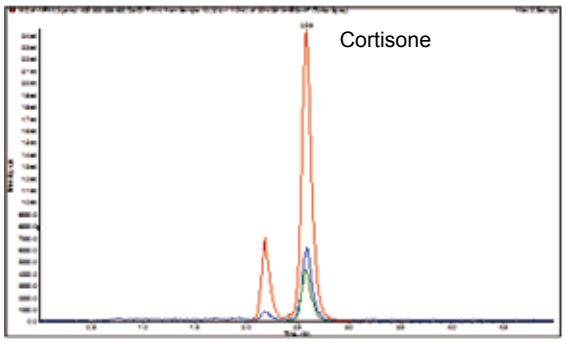


Figure 3 Chromatogram of actual serum sample (negative mode)

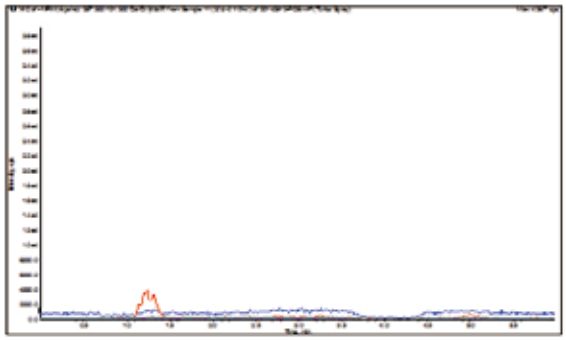


Figure 4 Chromatogram of actual serum sample (positive mode)

(2) Recovery data

Spiked two serum samples with the concentration of 5 ng/mL. Results of spiked recovery were showed in table 3. Actual serum samples were also extracted and analyzed by using the same procedures. Approximate 4ng/mL of cortisone was detected while the other three steroid hormones were free. Background should be subtracted from the response of cortisone in spiked samples to calculate the recovery data.

Table 3 Recovery data

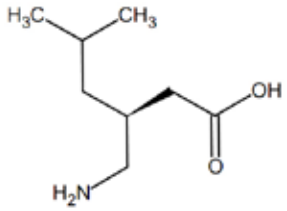
Compound	Sample 1	Sample 2
Progesterone	67.90%	63.48%
Testosterone	86.94%	78.57%
Boldenone	97.78%	94.07%
Cortisone	103.53%	107.69%

Ordering Information

Products	Specification	Cat.No.
Cleanert® SLE	Max sample loading volume 200 µL	HC2003Q-9
Venusil® ASB C18	2.1×50 mm, 3 µm, 150 Å	VS930502-0
96-well collection plate	2.2 mL Squaral well	96SP2036-2
96-well vacuum manifold	adapt to 96well plate	VM96
NV-96G for 96 Well Plates	adapt to 96well plate	NV96-G
1.5 mL vials	1.5 mL short thread vial, amber glass, label and filling lines	AV1111-0
1.5 mL vials caps	9 mm screw neck cap, center hole; red silicone/ white PTFE septa	AV2100-0
Micro-insert clear glass	300 µL micro-insert, 31×6 mm	AV1132-6
Filtration membrane(PTFE)	φ13; 0.22 µm	AS041320-T
Disposable Syringe	2 mL,needless	ZSQ-2ML

Detection of Pregabalin using Protein Precipitation Plates and followed by LC-MS/MS analysis

EMB10007



Experimental Details

Sample preparation

Protein precipitation process

1) Add 500 µL methanol into the Cleanert PPT Plate
2) Dilute the 50 µL plasma with 50µL methanol/water (1/1, v/v), and then add the diluted sample into each well, (blank group add with 50 µL methanol/water (1/1) instead)
3) Mixed the sample with pipette and put the plate with sample onto SPE-M96 positive pressure device, adjust the pressure to 3 psi, collect the elution into collection plate.
4) Add 400µL 0.1% formic acid in water into the sample, mix well and then for LC-MS/MS analysis.

HPLC Condition

Column: Unisol C18(2) (2.1x50mm, 5µm, 110 Å)
Flow rate: 200 µL/min
Column temperature: 30˚C
Injection volume: 5 µL
Mobile phase: A-0.1%formic acid in water; B-Methanol

Step	Total Time(min)	Flow Rate(µl/min)	A (%)	B (%)
0	0	200	80	20
1	4	200	80	20

MS Condition

Scanning mod: positive electrospray ionization mode
Acquisition mode: multiple reaction monitoring (MRM)
Electrospray voltage: 4500V
Curtain gas: 30
Atomizing gas (gas 1): 60
Auxiliary gas (gas 2): 30
Ion source temperature (TEM): 450˚C

Compound	Retention Time	Q1	Q3	DP	CE
pregabalin	1.98 min	160.2	142.1(quantitative)	50	15
			125	50	19
			97	50	21
			55	60	33

Chromatogram

Intensity: 4.3213 x 10^6

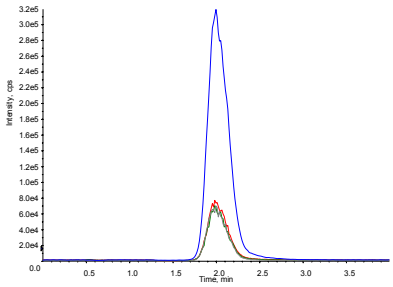


Figure 1. MRM spectra of standard solution(100 ng/mL)

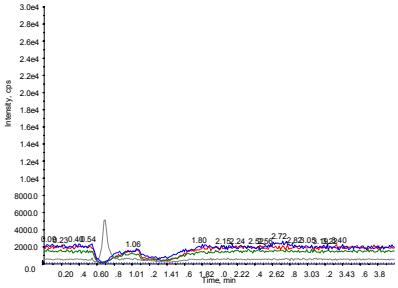


Figure 2. MRM spectra of blank plasma

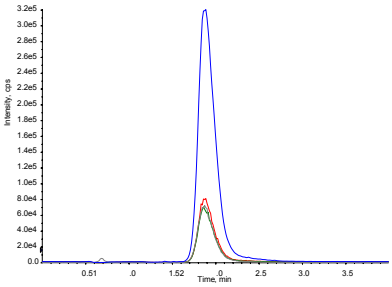


Figure 3. MRM spectra of spiked plasma

Intensity: 1.2562x 10^6

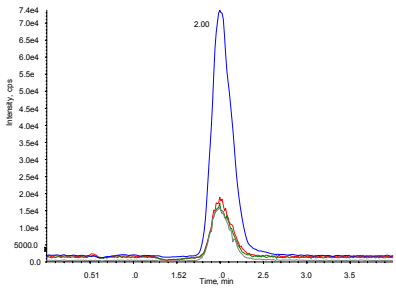


Figure 4.MRM spectra of standard solution (25 ng/mL)

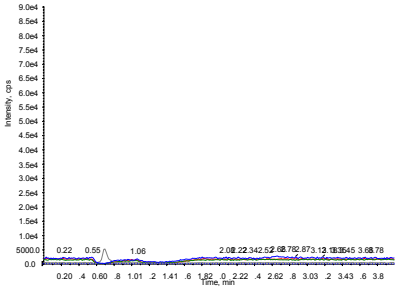


Figure 5.MRM spectra of blank plasma

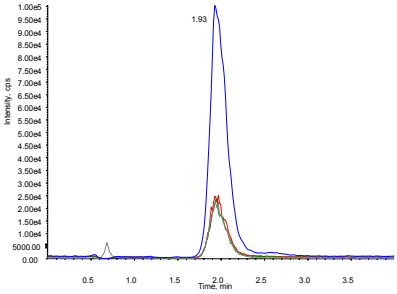


Figure 6. MRM spectra of spiked plasma

Table 1. The recovery rate of pregabalin in bovine plasma (Lot to Lot comparison)

Spiked concentration of pregabalin	Sample name	Lot: 150623 Recovery rate	Lot: 150701 Recovery rate	Lot: 150902 Recovery rate
2000 ng/mL	Parallel 1	92.58%	95.15%	95.00%
	Parallel 2	91.36%	100.69%	97.66%
	Parallel 3	93.04%	94.24%	99.25%
	Parallel 4	94.78%	99.76%	96.07%
	Parallel 5	91.56%	104.16%	95.46%
	Parallel 6	96.38%	101.57%	91.99%
	Parallel 7	97.54%	103.74%	102.11%
	Parallel 8	93.16%	105.67%	97.23%
	RSD%	RSD=2.39% (n=8)	RSD=4.11% (n=8)	RSD=3.12% (n=8)
500 ng/mL	Parallel 1	94.75%	96.23%	99.56%
	Parallel 2	94.70%	90.26%	88.02%
	Parallel 3	98.26%	97.37%	93.72%
	Parallel 4	99.98%	99.27%	100.71%
	Parallel 5	94.79%	98.65%	94.63%
	Parallel 6	99.59%	97.12%	94.91%
	Parallel 7	94.39%	88.84%	92.66%
	Parallel 8	96.44%	93.51%	102.79%
	RSD%	RSD=2.42% (n=8)	RSD=4.08% (n=8)	RSD=5.05% (n=8)

Table 2. The recovery rate of pregabalin in bovine plasma (Day to Day comparison)

Spiked concentration of pregabalin	Lot number	PPT Plate Lot number	Week 1	Week 2	Week 3
2000 ng/mL	150623	Ave.	93.80%	95.61%	96.38%
		RSD%	RSD=2.39%	RSD=4.52%	RSD=3.42%
	150701	Ave.	100.62%	106.22%	106.17%
		RSD%	RSD=4.11%	RSD=3.14%	RSD=1.30%
	150902	Ave.	96.85%	106.33%	102.70%
		RSD%	RSD=3.12%	RSD=3.14	RSD=3.81%
500 ng/mL	150623	Ave.	104.58%	91.69%	96.61%
		RSD%	RSD=5.62%	RSD=1.34%	RSD=2.42%
	150701	Ave.	111.70%	89.87%	83.87%
		RSD%	RSD=3.37%	RSD=4.67%	RSD=4.08
	150902	Ave.	97.11%	90.68%	95.88%
		RSD%	RSD=6.75%	RSD=4.10%	RSD=5.05%

Ordering Information

Products	Specification	Cat.No.
Cleanert® PPT plate	2mL/well	96CD2025-Q
Positive pressure device	Compatible with 96-well plate	SPE-M96
Unisol C18(2)	2.1x50mm,5μm,110 Å	UO950502-2
Collection Plate	2mL, square well	96SP2036-2
96 well Mat	Square well	96GP2036-2

Speciality Applications

Banned azo dyes in textiles (Cleanert® SLE Azo dyes Extraction Column, P/N: GB/T17592-2006)

EME10008

Reduce the textiles in citrate buffer solution by sodiumdithionate to obtain forbidden aromatic amines that possibly exist. Extract the aromatic amines by proper liquild-liquid partition cartridge. After concentration, dilute to volume with proper organic solvent for determination by GC-MS. If necessary, choose one or more other methods to confirm the existence of isomers. HPLC/DAD or GC/MS is employed for quantification.

Materials

(1) Cleanert® SLE extraction cartridge
20 cm × 2.5 cm (i.d.) polypropylene cartridge, packed with 20 g of diatomite.
(2) Citrate buffer (0.06 mol / L, pH = 6.0)
Dissolve 12.526 g of citric acid and 6.320 g of sodium hydroxide in water and dilute to 1000 mL.
(3) Sodiumdithionate solution
200 mg / mL sodiumdithionate in water, fresh prepared with solid sodiumdithionate ($Na_2S_2O_4$≥85%) before use.

Experimental

Sample Preparation

Cut representative sample into small pieces of 5 mm × 5 mm and mix. Transfer 1.0 (accurate to 0.01 g) of sample into reactor and add 16 mL of citrate buffer at 70 ± 2°C. Seal the reactor and shake up until all samples are soaked in liquid. Put the reactor in water bath at 70 ± 2°C for 30 min to soak the textiles thoroughly. Add 3.0 mL of sodiumdithionate solution, seal and shake up. After another 30 min in water bath, cool the reactor to room temperature in 2 min.

Extraction and Concentration

(1) Extraction:
Press the sample with a glass rod into the reactor and transfer the liquid into diatomite extraction cartridge. Allow to adsorb for 15 min. Elute the cartridge with ether four times (20 mL × 4). For each time, combine the ether and eluate, and load onto the cartridge. Control the flow rate. Collect the eluate in a round-bottom flask.
(2) Concentration:
Evaporate the eluate to 1 mL by rotary evaporator at 35°C and dry under a slow stream of nitrogen.

GC-MS Analysis

Capillary column: DA-5MS, 30 m × 0.25 mm × 0.25 μm (P/N: 1525-3002), or a corresponsive one.
Injection temperature: 250°C
Column temperature: 50°C (0.5 min) 20°C/min 150°C (8 min) 20°C/min 230°C (20 min) 20°C/min 260°C (5 min)
MS interface temperature: 270°C; MS scan range: 35~350 amu; Injection mode: splitless; Carrier gas: He(≥99.999%); Flow rate: 1.0 mL/min; Injection volume: 1 μL; Ionization source: EI; Ionization voltage: 70 eV

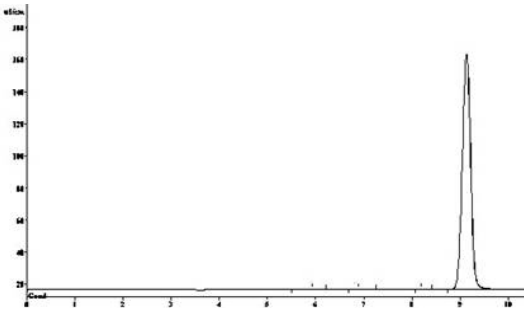
Nitrites in Food (Cleanert® IC-Ag and Na, P/N: IC-Ag10, IC-Na10)

EMF10037

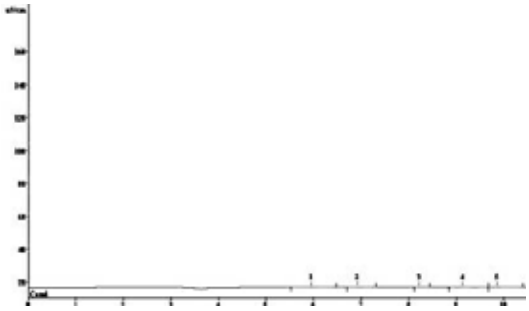
To monitor nitrites in food, ion chromatography is gradually replacing diazonium-coupled spectrophotometric analysis for its convenience and accuracy. During sample preparation process, impurities in extracted water solution such as particles, organic interference and cloride ions must be removed before analysis.

First particles are eliminated by MCM syringe filters. And then, Cleanert® IC-RP cartridge is employed to remove organic compounds in samples to avoid contamination of ion chromatography cartridge; Cleanert® IC-Ag and Na cartridges are combined to remove Cl^-, which can affect the peak shape of NO_3^-.

The comparison of chromatograms before and after sample pretreatment is shown in the following figures to demonstrate the elimination effect of Cl^- by Cleanert® IC-Ag and Na cartridge.



Chromatogram of untreated sample



Chromatogram of sample treated by Cleanert® IC-Ag and Na cartridge

Cleanup of Water Samples from Oilfield Using Cleanert IC Cartridges (Cleanert® IC-RP, P/N: IC-RP10)

EME10009

During oil exploration process, ion content in oil field water of different drilling depth must be monitored. MCM syringe filter can effectively trap unwanted particles in the water supernatant after centrifugation, and then Cleanert® IC-RP is followed to remove organic contaminants from oilfield.

SPE cartridges from Bonna-Agela can be easily used for batch sampling in a SPE manifold or workstation even though manual sampling might be still needed for random sampling.

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