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Bonna-Agela

Selection Guide for Bio-sample Preparation

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Innovation to Benefit Customers

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Innovation to Benefit Customers

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Sample Dilution

Sample Clean-up by Vacuum Manifold or Positive Pressure device

Sample Concentration

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Introduction

The objective of bioanalysis is to measure drugs, metabolites, poisons, chemicals of environmental exposure, endogenous substances, biological molecules, and biotics in body fluids and tissues. The quantitative and qualitative analysis of drugs and metabolites is extensively applied to pharmacokinetic studies. Nowadays, LC-MS/MS has become very useful means in bioanalysis. Therefore, high throughput sample preparation tools are the essential prior to the analysis. Sample preparation also are necessary in the situation, such as 1)matrix interfere with analysis; 2) matrix effects; 3) detection limit is getting lower and lower; 4)high throughput needs

Bonna-Agela Technologies is one of the leading companies in producing chromatographic related products for high throughput bio-sample separation, clean-up prior to characterization of drugs and their metabolites, bio-molecules. Nowadays, we have served many scientists around the world from academic institutions to renowned industrial companies in this field.

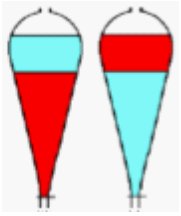
Today, we continue to develop new, innovative tools to support the scientists work in bioanalysis field. Our core technologies and synergies in chromatography, instrumentation, and customer service will continue to play an important role improving total solutions for you.

Methods and Products for Sample Clean-up

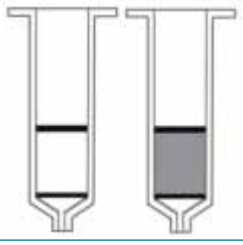
General Biosample Clean-up Methods

Traditional method used in biological sample prepareation

Protein Precipitation



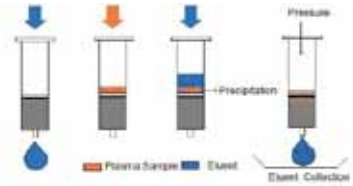
Liquid-Liquid Extraction



Solid Phase Extraction

Cleanert® Protein Precipitation Plate

Cleanert® Solid-Supported liquid Extraction



MAS Method

Sample Extraction and Clean-up Products

Filtration Series

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 clean-up result.
High purity raw materials are selected to manufacture high quality products. All the materials be used are inert to avoid adsorption of analysis. Each lot of the products has passed a strict examination.

Common Filters

Materials of the filters: PP, PTFE, PVDF, Cellulose mixed esters, regenerated cellulose and glass fiber with pore size of 0.22 μm, 0.45 μm, 1 μm and 5 μm, 0.22 μm, 0.45 μm, 1 μm, 5 μm…

MAS (Multi-functional Impurity Adsorption SPE)

MAS is a simple sample treatment method that applies multi-functional 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 sensitivity. Method development is much simpler by follow the method protocol, make lab work more effectively.

MAS Products

MAS-A screening method for acid compounds;
MAS-B screening method for basic compounds;
MAS-WA screening method for amphoteric compounds.

SPE Plates and Cartridges

OMM SPE Products (Optimized Molecular Modification)

PEP series, PAX, PCX, PWAX, PWCX and PS are based on polystyrene/divinylbenzene materials with different functionality and unique selectivity. They are highly recommended for the extraction of a wide range of compounds in pharmaceutical and clinical sample pre-treatment
Average particle diameter: 40-60μm / 30μm;
Average pore size: 70 Å;
Specific surface area: 600 m^2/g.

Traditional line

- PEP (polar enhanced polymer); pyrrolidone modified PS
- PCX ulfonic acid modified strong cation exchanger
- PAX tertiary amine modified strong anion exchanger
- PWCX weak cation exchanger
- PWAX weak anion exchanger

Unique product

- PEP-2, unique and more convenient than HLB; no need to adjust pH of sample; better removal of phospholipids; a better SPE for multiple analytes of neutral and ionic nature

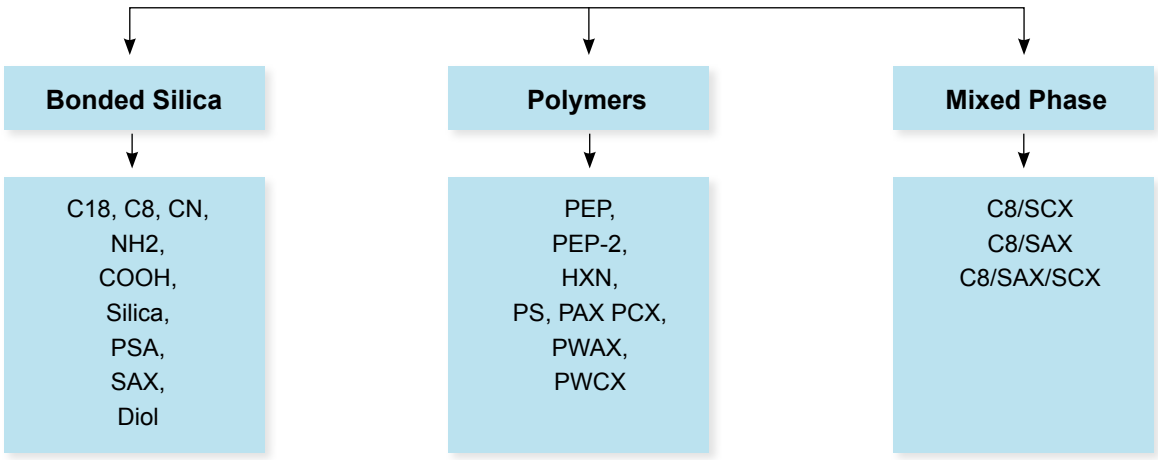
Bonded Silica SPE Cartridges

Silica based SPE series is made of high quality and low metal silica particles. Using the special surface modification methodology, the activity of silica surface is reduced largely, which in turn will reduce the tailing of compounds and will ensure high recovery and reproducibility.
Average particle diameter: 50 μm (spherical particles)
Average pore size: 60Å / 120Å
Volume of pore: 0.9 mL/g
Specific surface area: 600 m^2/g

Traditional line:

C18, C18-N, C8, NH_2, COOH, Silica, PSA, PRS, SCX, SAX;
Unique: AQ-C18, Amide, PEG / C18

Cleanert® SPE Product Lines



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

Cleanert® SLE can be used to replace most of the analysis using Liquid/Liquid extraction to reduce the solvent usage. Specially treated diatomite materials are packed in columns and well plates. The liquid/liquid extraction is run 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 application;
Surface modification and deactivated for sensitive compounds.

Product Formats

1. SPE Cartridges (Figure 1)

- The SPE cartridges consist of three parts: high purity polypropylene tube, porous PE frit and packing materials (40-60 μm).
- Cartridge size: 100mg / 1mL, 200mg / 3mL, 500mg / 6mL and 1g / 6mL etc. Where mg is the weight of the packings and mL is the volume of the SPE tube.
- SPE cartridges are disposable to avoid cross contamination.

Figure 1 SPE Cartridge
(General Cartridge, LRC, Glass Cartridge, LDC cartridge, Flangeless Cartridge)

2. 96-well SPE Plates (Figure 2)

96-well SPE plate is produced for high throughput applications in bio-analysis and clinical analysis. Each well contains a small amount of sorbents (10-100 mg). The maximum well volume is 2 mL. The plates are compatible with automatic systems for routine sample preparation efficiently.

Figure 2 96-well SPE plates
(Left: 1 mL-round well; Right: 2 mL-square well)

3. Integrated Micro SPE Plates (Figure 3)

The Integrated SPE plate is consisted with a plate block and individual cartridges which are assembled on the plate block. The benefit of the Integrated SPE plates is the flexibility that allows the user integrates various SPE cartridges on the same plate for various applications. The plate is suitable for method development.
The cartridges of the Micro plates are packed with small amount of the SPE material to treat the samples in microliters level.

Figure 3 Integrated micro SPE plates

4. Integrated Deep-well SPE Plates

The deep-well SPE plate packed with more sorbents is designed for larger volume sample loading. The capacity of each SPE cartridges is much great than normal SPE plate so that more sample can be loaded on the plate. Same as the integrated micro SPE plate, those plates are assembled on a plate block.

Figure 4 Integrated Deep-well SPE plates

Features and Benefits

Cleanert® PPT- Protein Precipitation Plate

Feature

Although protein precipitation with acetonitrile and flowed by centrifugation is a common method in bioanalysis to remove the proteins from the sample, It is no doubt that the process is laborious and time consuming. Moreover, the operation is difficult to automate. In contrast, Cleanert® PPT plates are designed for high throughput operation and it is easy to automate.

Cleanert® PPT plates come with 1 mL and 2 mL in format, the combination with hydrophobic frit with different pore size result in more effective performance to remove proteins from bio-sample matrix.

2 mL square well plate format

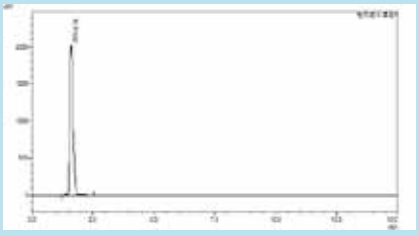
Advantages

Inert materials are stable in a wide range of pH from 0 to 14 without extra adsorption of analytes. No contaminations are introduced. The pore size of the frits is optimized for removing the proteins without blocking.

1 mL round well plate format

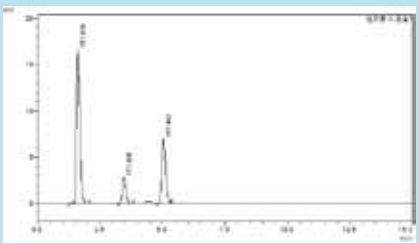
Benefits

Our PPT plates are special designed for high-throughput pretreatment of bio-samples. It eliminates time-consuming centrifuging step. After adding plasma and precipitation reagent into each well of the plate protein precipitation takes place while shaking the plate without any cover. There is no broken through until positive or negative pressure is applied.



Background Testing

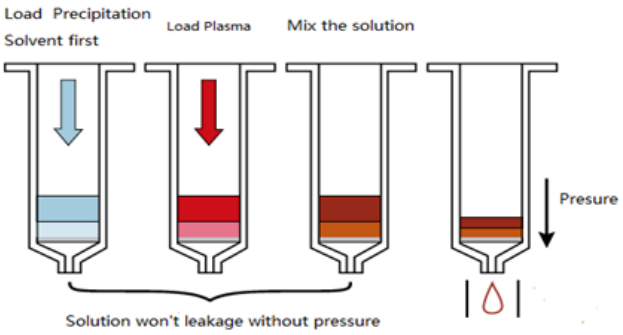
1 mL test solution (ACN:H_2O=1:1) contained Uracil was passed through the filter plate and subsequently analyzed by HPLC Specification: No peak area is > 1% of uracil



Recovery Testing

1 mL test solution (ACN:H_2O=1:1) contained uracil, aniline, phenol and benzophenone was passed through the filter and subsequently analyzed by HPLC Result: > 95% recovery

Hydrophobic frit is used to prevent leakage of organic solvent during precipitation, and avoid cross contamination, giving enough time for adequately protein precipitation.
Compatible with automatic and manual performance,such as vacuum manifold, positive pressure deviceand automatic workstation, saving time and labor.



	Time	Sample throughput	Centrifugation and transfer	Automation
Cleanert® PPT	10 min	96 per plate	No need	Yes
Classical PPT	30 min	One sample every tube	Yes	No

Procedure

PPT Plate

1. Place the Cleanert® PPT plate onto a vacuum manifold or positive pressure device;
2. Add precipitate solvent (such as acetonitrile and methanol) to the plate, 1:3 or 1:4 solvent to sample was suggested;
3. Add 20-300 µL plasma into each tube;
4. Mix the solvent and sample together by vortex mixer or pipette;
5. Amly pressure (0.04-0.08 MPa was suggested) on the plate to push the solvent through and collect the filtrate.

Cleanert® FAST—Filtration Plate Series

Feature

- High throughput filtration plate method can replace manual operation, and achieve a more efficient results.
- Filtration Plate can effectively remove most of the precipitated protein and particles.

2 mL Filtration plate- square well

Advantages

- Cleanert Filtration Plates are manufactured using super clean membranes offering greater porosity of the matrix.
- Membranes with different pore size and specification are available for 96 well plate format.
- Membranes: Glass fiber, PTFE, PP, Cellulose Acetate.
- Pore size: 0.22 μm, 0.45 μm, 1 μm, 5 μm are optional.

1 mL Filtration plate-round well

Benefits

Bonna-Agela provide vast selection of filter plates allow simple sample preparation of plasma, serum and other biological fluids. Optimazed membrance and frit avoid clogging and bringing in extra disturbing compounds.

Cleanert® MAS (Multi-function Impurity Adsorption SPE)

Feature

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

Advantages

In LC-MS/MS analysis, there are many interferences may cause unsatisfied result, such as proteins, phospholipids, fats and surfactants. Comparing with traditional sample preparation methods, The multi function of Cleanert® MAS appear more power to reduce the matrix effect especially for phospholipids.

	LLE	SPE	PPT	MAS
Interferences Removal	-	+	-	+
Operation	-	-	+	+

Formats

- Cleanert® MAS: 96-well plate for high throughput operation.
- Cleanert® MAS-C: Cartridges for more flexibility.

Benefits

Cleanert® MAS plate provides a convenient operation process, no method development is needed. As Figure 1 demonstrated this product results in more clean sample comparing with PPT and SPE.

Selection of Products and Solvents

Based on various matrix, Cleanert® MAS is packed with three kinds of sorbents, named MAS-A, MAS-B and MAS-WA. They are suitable for acid, basic and amphoteric compounds respectively. According to the diagram below you can choose an appropriate product and solvent. In some cases, mixing 10% of methanol with the eluant may improve the efficiency of protein precipitation. 9:1 to 1:1 is the general ratio range for ACN o methanol. Proper amount of water could be mixed with the eluant for the drugs with high polarity. However, the ratio should not be over 1/3 o make sufficient protein precipitation. Eluant mixed with a weak reagent (acetic ether, dichloromethane etc) is suitable for the samples with low polarity.

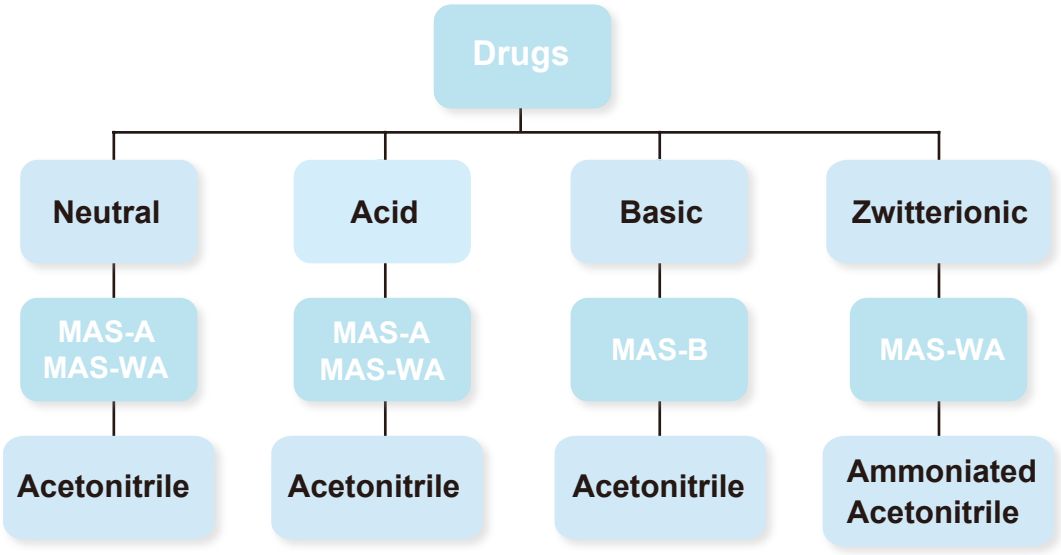
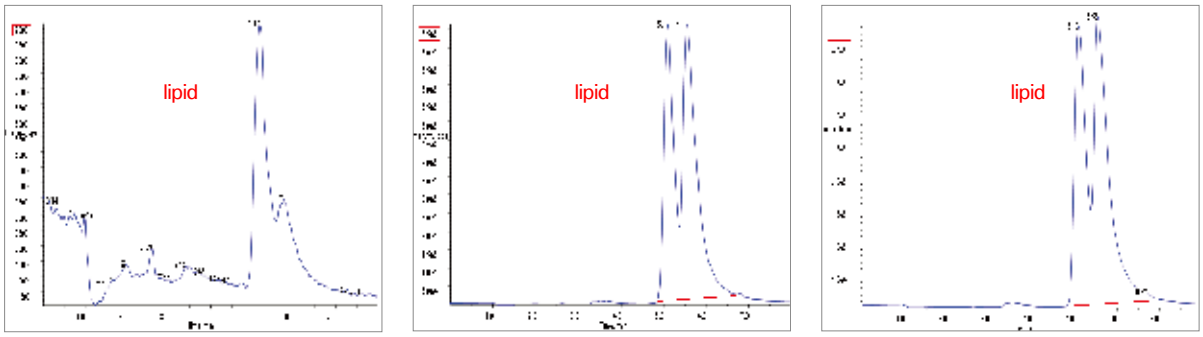


Figure 3 Flow chart of solvent and products selection

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

Perfect performance for phospholipids removal



Response : $9*10^3$ CPS
MAS

Response : $7.5*10^5$ CPS
PPT

Response : $4.3*10^5$ CPS
SPE

Figure 1 The comparison of phospholipids signal after processed by PPT, SPE and MAS method

The result showed that phospholipids signal deduced by 1-2 order magnitude compared with MAS compared with other method.

Perfect performance for protein removal

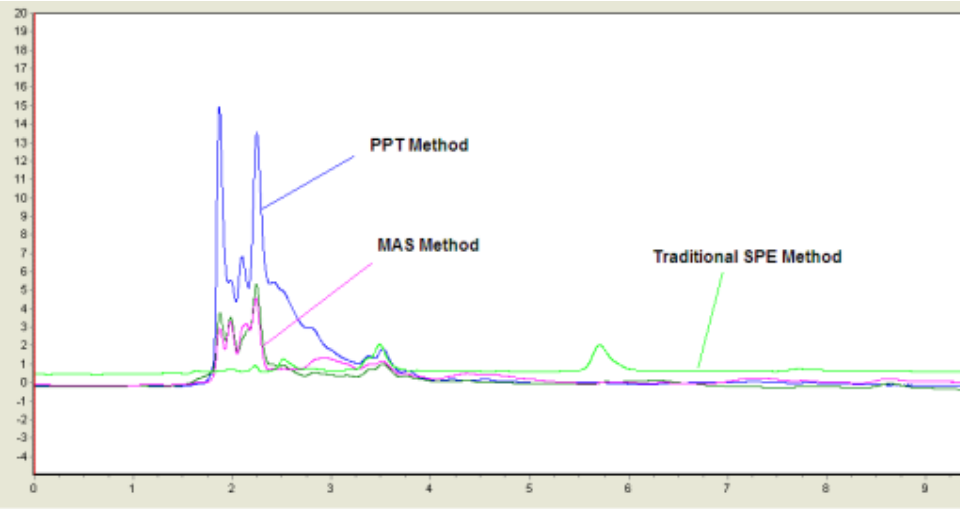
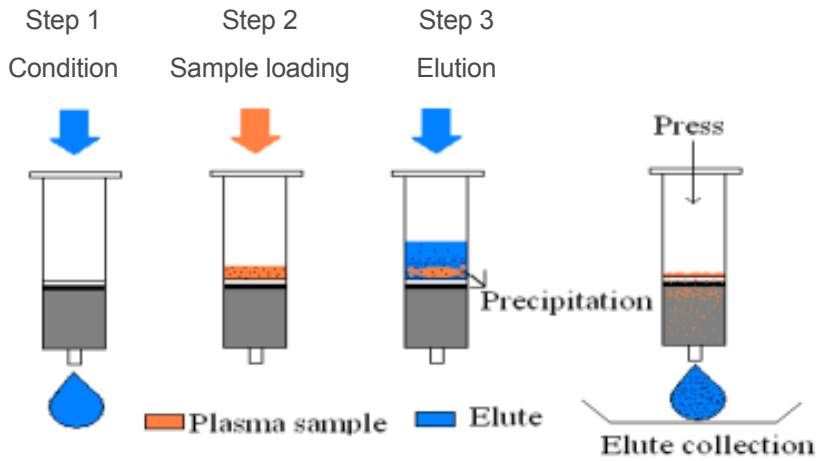


Figure 2 The comparison of protein signal after processed by PPT, SPE and MAS method

Procedure



- Protein precipitation and SPE clean-up in one step
- Adsorption of proteins and phospholipids in biological samples (plasma, tissue, and urine) and leave the analytes in liquid phase

Protocol of 96-well plate

Connect the M96 positive pressure processor to gas sources (nitrogen or compress air), rise the gas block by turning the UP/DOWN knob to "UP" positive.

Pull the platform out, stack the waste block and 96-well plate on the platform. Add condition solvents into each well of the plate and push the platform back. Introduce the gas on to 96-well plate by turning gas switch to "Low Flow" position to push liquid through the plate slowly.

Turn the "UP/DWON" knob to "UP" position to raise the Gas block and pull the platform out. Stack the 96-well plate onto the collection plate and set the stack onto the platform. Add finstand then elution solvent into each well of the plate.

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 for further analysis.

Cleanert® PEP-2 Polymer SPE Series

Feature

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.
Particle Characteristics Functionalized polymer sorbents; Average particle size: 40-60 µm. (30 µm are optional) Average pore size: 70 Å; Surface area: 600 m^2/g.

Advantages

Cleanert® 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.

Benefits

Special functional groups on PEP-2 allow to retain both neutral and ionized compounds so that the compounds with various properties can be extracted in one run. Because of the urea groups PEP-2 can absorb ionized compounds without pH adjustment so that the sample clean-up process can be more simple and faster.

Excellent retention of polar compounds

	PEP (Bonna-Agela)	PEP-2 (Bonna-Agela)	Strata-X (Phenomenex)	HLB (Waters)
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%

*Sample without pH adjustment

Recommended Method

	Neutral and Basic Compounds (metoprolol)	Acid Compounds (Salicylic acid)
Condition	MeOH or ACN; Water	MeOH or ACN; Water
Sample Loading	Diluted biological sample	Diluted biological sample
Washing	Water (or 5% organic solvent)	Water (or 5% organic solvent)
	Dry the cartridge by vacuum	Dry the cartridge by vacuum
Elution	MeOH or ACN	MeOH or ACN (add some acid in the solvent will helpful to elute acid compounds)

Cleanert® Micro Plate

Feature

The integrated SPE plates are built up with individual SPE cartridges and a 96-well plate block. The integrated SPE plates are sold with assembled cartridges or you can assembly yourself. Cartridges with various sorbents can be assembled on one plate block so that the plates can be used for method development. The common sorbents are: PEP, PEP-2, PCX, PAX, PWCX and PWAX. The particle size of the sorbents is 30 μm that can enhance the efficiency of the SPE plates.

Advantages

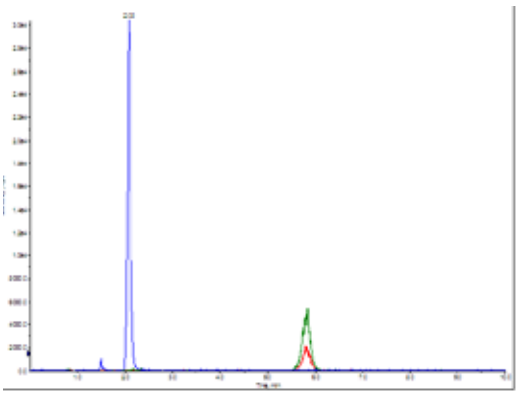
- Internally tapered well suitable for small volume of the samples
- High sorbent bed avoids breakthrough
- Removable cartridges allow flexible combination
- Low-elution volume saves time of concentration
- Various sorbents meet the requirement of method development

Benefits

Compared with the classical SPE methods, 96-well micro-plates uses less sorbents, loads smaller amount of samples and elutes smaller quantity of the solvent. Due to low-elution volume, it can save 1/3 time of evaporation and reconstitution steps. Reverse phase and ion-exchange polymer series provide different adsorption force for various analytes. Smaller particle size and internally tapered designs provide high column efficiency and avoid breakthrough. The modular designs, gave sensitive, robust, and reproducible products for complicated method development work.

Detection of Metoprolol and Propranolol in plasma

Cleanert® micro plate (5 mg/each well)
Condition: 200 μL methanol and 200 μL water;
Sample Loading: 100 μL plasma;
Wash: 200 μL 5% methanol in water
Elute: 50 μL ACN:IPA=4:6 (with 2% formic acid)
Then reconstitute it with 150 μL water to 200 μL for further LC-MS/MS analysis.



Extraction of Indomethacin and Ibuprofen from Small Volume Biological Fluids

Condition: Methanol (200 µL).
Equilibration: Deionized Water (200 µL)
Sample Loading: 50 µL Human plasma diluted with 50 µL 2% formic acid aqueous solution
Wash: Deionized Water/Methanol, 95/5, v/v (200 µL)
Elution: Acetonitrile (100 µL)

Analyte	Concentration	Recovery(%)	RSD (%)
Indomethacin	5 ng/mL	87.80	2.60
	20 ng/mL	97.70	3.90
	50 ng/mL	95.20	4.70
Ibuprofen	5 ng/mL	100.80	2.90
	20 ng/mL	98.20	4.90
	50 ng/mL	96.22	3.10

Cleanert® 96-well SPE Plate Series

Bonna-Agela provides a wide array of formats and sorbents of 96-well plates to meet the requirement of different compounds detection in biological sample.

OMM SPE Products (Optimized Molecular Modification)

Bonna-Agela SPE products have been developed based on a thorough understanding of interactive natures of chemical molecules. The OMM SPE products can thus better meet customer's needs. The results demonstrated that the adsorption/desorption property of the polymeric SPE materials is 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 with election drawing groups will 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.

Characteristics:

Average particle diameter: 40-60μm / 30μm;
Average pore size: 70 Å;
Specific surface area: 600 m^2/g.

Products:

- PEP (polar enhanced polymer); pyrrolidone modified PS;
- PCX sulfonic acid modified strong cation exchanger;
- PAX tertiary amine modified strong anion exchanger;
- PWCX weak cation exchanger;
- PWAX weak anion exchanger.

Unique product:

- PEP-2, unique and more convenient than HLB; no need to adjust pH of sample; better removal of phospholipids; a better SPE for multiple analytes of neutral and ionic nature.

Bonded Silica SPE Cartridges

Functionalities include C18, C18-N, C8, NH_2, COOH, Silica, PSA, PRS, SCX, SAX; All of them are made of high quality and low metal silica particles. Using the special surface modification methodology, the activity of silica surface is reduced largely, which in turn will reduce the tailing of compounds and will ensure high recovery and reproducibility.

Characteristics:

Average particle diameter: 50 μm (spherical particles);
Average pore size: 60Å / 120Å;
Volume of pore: 0.9 mL/g;
Specific surface area: 600 m^2/g;
Unique: AQ-C18; Amide; PEG/C18.

Integrated SPE Plate

Each loose cartridge could be removed from the base plate; this enables the cartridge to be used individually or to be assembled in the 96-well plates. Compared with the conventional fixed plate, it is much flexible in method development; it also allows various sorbents to be located on one plate as well to be customized.

Removable cartridge could solve the method transfer problem between cartridges and plates too. The cartridge volume is up to 3mL; it gives more room for sample loading and solvent storage. Besides this, the innovative design of the plates makes the job much quicker, more flexible limit unnecessary waste.

Cleanert® SLE (Supported Liquid Extraction) Technology and Sorbents

Features

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 and clinical, samples, it even can replace most of the Liquid/Liquid extraction (LLE).

Advantages

Different particle size: SLE 100-200 mesh, SLE 40-60 mesh.
Different particle size products provide option for different sample solution, bigger particle can effectively avoid the clogging of sticky samples.
Different surface modification: SLE-CM which is deactivated for sensitive chemical compounds.
Reduce the over adsorption of analytes and enhance the recovery.

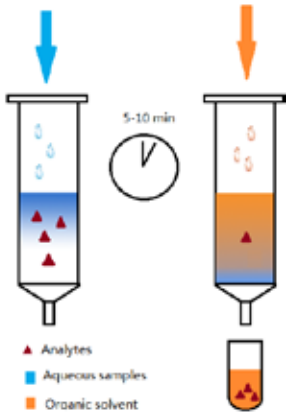
Benefits

Cleanert® SLE can be used to replace most of the analysis using Liquid/Liquid extraction It resuces the solvent usage.
It can also reduce matrix effect significantly by removal of most of the phospholipids and proteins from biological samples.
Further, the usage of Cleanert® SLE can effectively avoid emulsification; operation procedure can be easily automated.

	Time costing	Sample Qty	Emulsification	Automatic Instrument compatibility
Bonna-Agela SLE	15 min	96 per plate	No	Yes
LLE	30 min	One sample every tube	Yes	No

Mechanism

Highly purified graded diatomaceous earth packed in the Cleanert SLE is a kind of porous material with strong absorption ability for aqueous phase. When aqueous-based sample (for example diluted plasma or urine) was loaded onto the dry sorbent, the sample is adsorbed onto the micro-pore and forms a thin aqueous layer on the surface. A small volume of immiscible organic extraction solvent is added to the top of the SLE device and allowed to percolate by gravity (or sometimes with gentle vacuum or pressure) through the supported aqueous phase. The organic solvent has contact with the thin film of aqueous phase and rapid extraction (equilibration) occurs. The organic eluent containing the analytes of interest from the outlet of the SLE device is collected while matrix interferences such as phospholipids, proteins and salts are retained on the polar surface of SLE device. With SLE, there is no vigorous shaking and therefore eliminate emulsion formation.



Comparison of SLE and LLE —— determination of Levonorgestrel in Plasma

Steps of SLE (500 mg/well)

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 0.1 mL of 50% acetonitrile in water for further determination by LC-MS/MS.

Steps of LLE

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.

Conclusion:

SLE method get a 100 times lower response of phospholipids than ordinary LLE method;
By reduce the matrix effect, SLE method get a higher recovery and better stability.

Comparison results of LLE and SLE

Name	Concentration	LLE method	SLE method
Matrix (phospholipids)	m/z496.350-184.300	1.6908 e6	11559
	m/z524.37-184.300	4.835 e5	9040
Recoveries of levonorgestrel		81.13%	95.13%
RSD (n=5)		16.3%	6.8%

Analysis of Erlotinib in the human plasma

SLE Prodedure

Preatment: 100 µL plasma + 10 µL ISTD + 100 µL of 10 %NH_4OH in H_2O;
Sample loading: wait for 3-5 min; add 0.1 mL extra buffer to transfer completely the sample into the 96 well plate Cleanert® SLE (200mg / 2mL / well);
Elution: 400 µL MTBE twice (wait for 2 min after MTBE get into particles to get complete extraction before applying a vacuum);

Chromatographic Conditions

Column: Venusil® XBP Silica, 50×3 mm, 3 µm;
Column Tem: 25 ℃
Mobile Phase A: 0.1% Formic Acid in water;
Mobile Phase B: 0.1% Formic Acid in ACN;
Flow rate: 1.0 mL/min isocratic flow with 85% mobile phase B
Injector wash solution: 50% acetonitrile in water;

Mass Spectrometer

Positive ESI MRM on API 365;
Major Parameters

Compound ID	Compound Name	Transition Monitored	Collision Energy (eV)	Approximate Retention Time (min)
Analyte	Erlotinib	394.2-278.2	45	0.58
ISTD 1	Erlotinib-D6	400.2-278.2	45	0.58

Comparison of Recoveries

Preatment method	Recoveries	RSD (%)
SLE	96.8%	3.4%
LLE	81.2%	9.8%

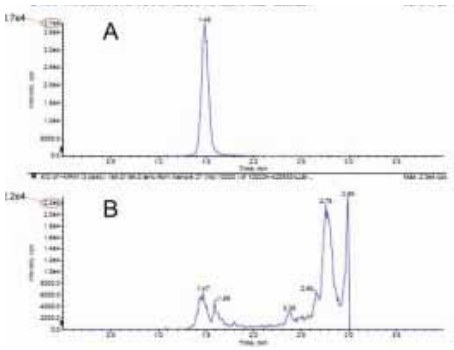


Figure 3 Peak of drug (A) and phospholipids (B) by SLE method

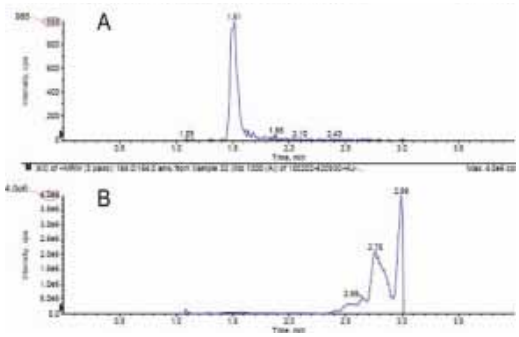


Figure 4 Peak of drug (A) and phospholipids (B) by SPE method

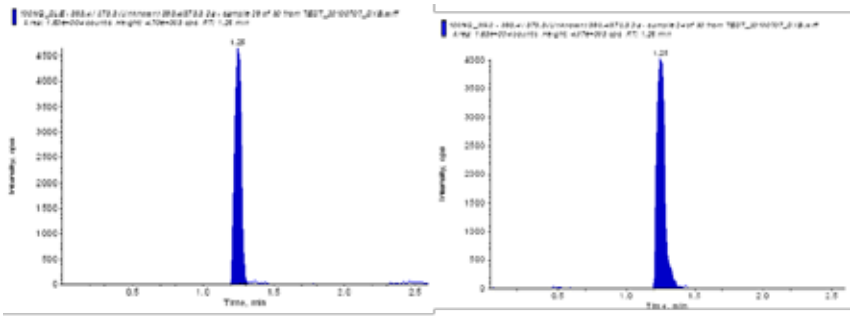
Determination of dexamethasone in plasma SLE procedure (200 mg/well)

50 μL plasma diluted by 50 μL water;
Load sample solution to each well, wait for 10 min;
1.5 mL MTBE elution in 2 times.

LC/MSMS Conditions

Column: Venusil® ASB C18 (2.1×150 mm, 3 μm)
Tempreture: 25℃
Mobile Phase: 38% ACN: 62%, 0.01 mol/L ammonium acetate
Flow rate: 0.25 mL/min
Injection volume: 5 μL
ESI, MRM Mode

Parent	Daughter
393.4	373.3



Cleanert® SLE | Other brand SLE product

Concentration of Dexamethasone in plasma (ng/mL)	Peak area of Dexamethasone	
	Cleanert® SLE	Other brand SLE product
10	1.45E+03	1.43E+03
100	1.68E+04	1.63E+04

Processing Method

Step 1: Apply the aqueous sample to the dry cartridge (No need to activated SLE product) Wait for 5-10 minutes;

Step 2: Add organic extraction solvent and collect the eluent. Ensure that the final eluent is water-immiscible.

Step 3: The collected eluent can be analyzed directly or dried and reconstituted.

SLE plates and cartridges usually are used under gravity; a pulse of vacuum or positive pressure is useful for the sample loading of the aqueous sample through the upper hydrophobic frit and for a final draw of organic solvent after elution. Hydrophilic frit is available for SLE-AQ series.

Figure 1 below shows the recommended procedure for plates and cartridges. Table 1 shows recommended buffer and elution volumes depending on sample volume for plate. Table 2 shows recommended buffer and elution volumes depending on sample volume for cartridge.

Tips: Thick samples is proposed to be diluted with an equal volume of buffer or water. 1M ammonium buffer (pH 9-10) are recommended for basic analytes and 1M phosphate buffers (pH 2-3) are recommended for acidic analytes. MTBE is widely used as elution solvent, and 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.

Table 1 Recommended buffer and elution volumns depending on initial sample volume (wellplates)

Sample Volume	< 100 µL	100-200 µL	200 µL	300 µL	400 µL	> 400 µL
Suggest to diluted the sample with water (1:1) when sample volume is below 200 µL;						
Recommended SLE product	200mg / 2mL; 96-well plate	200mg / 2mL 96-well plate	300mg / 2mL; 96-well plate	300mg / 2mL; 96-well plate	400mg / 2mL; 96-well plate	500mg / 3mL or 600mg / 3mL; 96-deep array well plate
Apply vacuum (0.5 bar) for seconds make the sample pass through the upper frit; Wait for 5-10 minutes for sample absorb;						
Extraction Solvent	1×1 mL or 2×600 µL	1×1 mL or 2×600 µL	1×1 mL or 3×700 µL	1×1 mL or 3×700 µL	3×700 µL or 4×550 µL	3×1 mL or 4×800 µL
Apply vacuum (0.5 bar) for 2 minutes to complete elution if necessary, then concentrate the elution and reconsititute as required; Don't make the sorbent bed dry between eluting.						

Table 2 Recommended buffer and elution volumns depending on initial sample volume (cartridges)

Sample Volume	< 200 µL	500 µL-1 mL	1-2 mL	2-4 mL	8-10 mL	10 mL-20 mL
Recommended SLE product	200mg / 3mL cartridge;	1g / 6mL cartridge;	2g / 12mL cartridge;	4g / 25mL cartridge;	10g / 60mL cartridge;	20g / 60mL cartridge;
Wait for 10 minutes for sample absorb;The suggested ratio of sample volume and sorbent amount between 0.8:1-1:1;						
Extraction Solvent	1×1 mL or 2×0.5 mL	1×6 mL or 2×3 mL	2×6 mL or 3×4 mL	2×6 mL or 3×6 mL	4×15 mL	4×20 mL
Apply vacuum (0.5 bar) for 2 minutes to complete elution, then concentrate the elution and reconsititute as required.						

Cleanert® Deep Well SLE Plate

Deep well SLE plate have a bigger room (3 mL) for sample and elution solution loading; All the cartridge can be removed from the base plate, and assemble by hand; Various sorbent can be used in one base plate, suitable for method development.

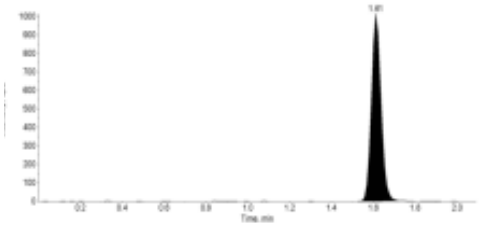
Analysis of Telmisartan in plasma by SLE pre-treatment and LC method

SLE Procedure

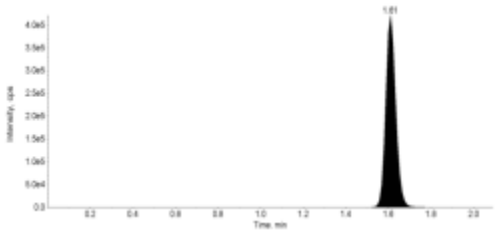
- Human plasma samples were spiked with the analyte in the concentrations range from 2 to 1000ng/mL.
- Diluted 300 µL human EDTA plasma with 300 µL of buffer
- Load the sample solution onto Cleanert SLE deep 96-well plate (600mg / 3mL / well)
- Apply low vacuum for 5 seconds to initiate loading, and wait for 5 minutes;
- Elute with 1 mL of MTBE twice by gravity;
- Evaporation and reconstitution in 500 µL of mobile phase.

LC Separation

Column: Unisol C18, 150×4.6 mm, 5 µm
Flow rate: 1.1 mL/min
Mobile phase: Acetonitrile/water (60 / 40), 10 mM ammonium acetate



Lower limit of quantitation human EDTA plasma sample (2 ng/ml)



Higher limit of quantitation human EDTA plasma sample (1000 ng/ml)

Spiked Concentration	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample6	RSD
2 ng/mL	88.3%	81.6%	87.8%	5.5%	90.6%	6.8%	3.93%
1000 ng/mL	92.1%	83.9%	89.7%	8.5%	86.5%	8.1%	3.54%

Sample Preparation Apparatus

Bonna-Agela also prove lab accessories for DMPK research which help you a lot in laboratory construction.

96-well Vacuum Manifold

P/N: 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

1. Use Silicon rubber vacuum tube to connect the suction port of VM96 and the inlet pot of the vacuum pump.
2. Remove the top cover of VM96, and put 96-well collection plate into the cavity of the bottom cover of VM96.
3. Put back the top cover.
4. Put the 96-well SPE plate exactly on the top cover of VM96.
5. Make sure the gas valve at vacuum state(perpendicular).Start the vacuum pump,adjust the needle-type vacuum valve to suitable degree of vacuum(i.e. SPE plate flow rate)
6. After elution,rotate the gas valve 90 degree to non-vacuum state.Then remove the 96-well SPE plate.
7. If the SPE 96-well plate is too long,add the metal elevation block to adjust.

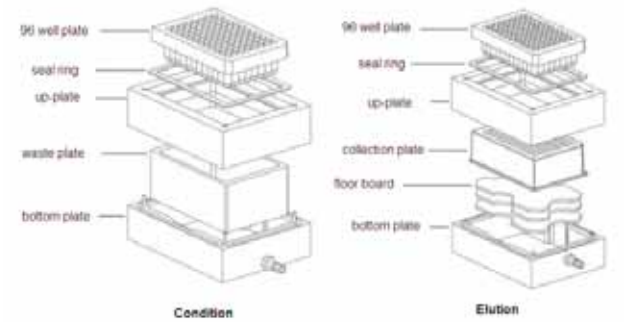
VM96 Specifications

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

VM96 Accessories

Si rubber vacuum tube	1(standard)
Collect.Plate Elev.Block	1(standard)
Metal Elevation block	1(optional)
Manual	1 copy

The structure of 96 well manifold



P/N: A01003

M96 Positive Pressure SPE Divice

MULTI-SPE M96 is a positive pressure manifold, specially designed for high-throughput sample preparation in research and testing laboratories. It can simultaneously process up to 96 samples by applying evenly gas pressure on each well to press liquid through 96-well SPE plates smoothly. It reduces largely the variation on the flow-rate between each well of 96-well plates, compared to vacuum SPE devices, thus improving sample-to-sample consistency. For some viscous samples, vacuum may not provide enough flow. However, MULTI-SPE M96 can provide up to 60 psi positive pressure to help the sample pass through 96-well plates smoothly.

P/N: SPE-M96

Features

- Low variation on the flow-rates between wells of 96-well plates; improved extraction consistency.
- High-throughput by processing up to 96 samples simultaneously.
- Good flexibility: can run any number of samples between 1-96.
- Dual-pressure control: separated coarse and fine tuning to get precise pressure control and quick tuning.
- Easy set-up and operation: ready to run; needs only purged gas source.
- The SPE stage indicator: manually switch the indicator position to remember the current stage of your operation.
- The whole SPE process can be completed in an inert (N_2) environment.
- The divice can also be used for PPT/SLE and filtration on 96-well plate fount

Specifications

Format of SPE	M96
SPE positions	Up to 96 (can choose any number less than 96)
Loading volume	1mL (sample/solvent)
Collection volume	1mL or 2mL (depending on collection plate types)
Pressure Control	Dual-pressure control (coarse and fine tuning), Max. 60psi
SPE step indication	Manual SPE step indicator(Condition\Load\Wash\Dry\Elute)
Gas	Nitrogen or air

MULTI-SPE M48 Solid Phase Extraction Manifold

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 that offers state-of-art operation for 48×1 mL SPE cartridges.

Positive pressure is applied for moving liquid through SPE cartridges smoothly. It eliminates the differences of the flow-rate between the cartridges that encountered frequently on vacuum SPE devices. Therefore, MULTI-SPE 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 processing power since the maximum pressure is limited to something less than atmospheric (-15 psi). In contrast to vacuum manifold, the Multi-SPE M48 allows for gas pressure up to 60 psi., supplying greater motive force for viscous sample flow. The flow through the cartridges response smoothly and immediately when the pressure is adjusted.

The user forgets which step has been done or not done occasionally when processing SPE manually. If it was a case, the user might has to re-process the extraction from the beginning. In order to avid it happens a step indicator is installed on the front panel of MULTI-SPE M48 so that the user can mark SPE process step by step.

Features and Advantages

- Low variation on the flow-rates between the cartridges; improved extraction consistency.
- High-throughput by processing up to 48 samples simultaneously. Good flexibility: can control on/off for gas by each 12 to save gas; can run any number of samples between 1-48.
- Dual-pressure control: separated coarse and fine tuning to get precise pressure control and quick tuning.
- Easy set-up and operation: ready to run; needs only purged gas source.
- The SPE stage indicator: manually switch the indicator position to remember the current stage of your operation.
- The whole SPE process can be completed in an inert (N_2) environment.

P/N: SPE-M48

Specifications

Format of SPE	1 mL cartridge
Max SPE positions	48 (can choose any number less than 48)
Max Loading volume	1 mL (sample / solvent)
Max Collection volume	2 mL or 5 mL (depending on test tube racks)
Pressure Control	Dual-pressure control (coarse and fine tuning), Max. 60psi
SPE step indication	Manual SPE step indicator(Condition / Load / Wash / Dry / Elute)
Gas	Nitrogen or air

NV-96G Evaporator for 96 Well Plates

P/N: NV-96G

Stable · Effective · Simple Operation

NV-96G meets the requirement of large volume concentration and preparation (drug screening, hormone assay, biochemical analysis and drug metabolism). Combined with a 96-well plate, it could process 1-96 samples without 2nd transfer. The nitrogen blew is controlled uniformLy, compared with the traditional water bath, NV-96G concentrator could avoid the contamination produced steam condensation.

Features

- Online temperature controlling
- Compatible with 96-well collection plate
- Adjustable height for blower holder
- Digital display and touch screen, simple and friendly operation
- Precisely controlled flow
- Double heating mode (through bottom plate and gas)

Specifications

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

NV-8G Evaporator

NV-8G Evaporator is widely used in both clinical and DMPK analysis for its simple operation. Its heating block for drying can heat the sample uniformly and would not pollute the samples by moisture like the thermostatic water bath, which can maintain the purity of the biological products.

P/N: NV-8G

Features

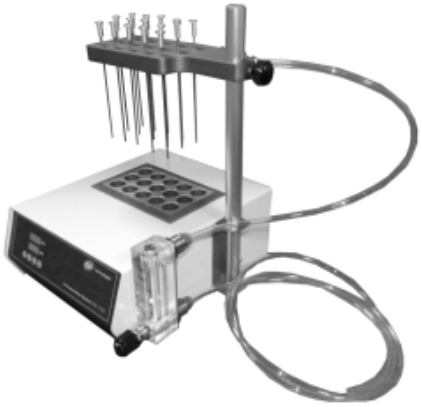
- Simple and flexible operation
  Convent temperature control
  Freely controllable concentration process at any time
- Uses 8 well sandblasting aluminized module with diameter of 19 mm and depth of 60 mm.
- Freely height adjustable venting needle holder
- Adjustable heating temperature through double digital display panel
- Distribution system of the venting needle can be separetly shut off, it can either handle multiple sample concentration or scavenging just one sample.
- Accurately adjust the purge of gas flow.

Specifications

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	220V / 50Hz
Temperature range	> Room temperature 5-80℃	Maximum power	240 W
Temperature error	Plus or minus 0.3℃	Instrument weight	3.5 kg
Work environment	Temperature 0 - 30℃ Relative humidity ≤ 85% RH		

NV-15G Evaporator

NV-15G Evaporator is widely used in various industries such as in pesticide residue analysis, food inspection, environmental, biological products for its simple operation, beautiful appearance, practical and timing etc. Its heating block for drying can heat the sample uniformly and would not pollute the samples by moisture like thermostatic water bath, which can maintain the purity of biological product.



P/N: NV-15G

Features

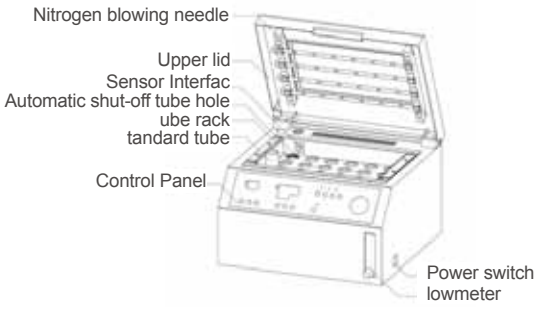
- Simple and flexible operation.
  Convent temperature control.
  Freely controllable concentration process at any time.
- Compatible test tube size and number: 19 mm × 60 mm (width × depth), 15 well.
- Blow holder could be adjusted freely.
- Double digital display touch screen.
- Blow needle can be shut off separately, suitable for single and multi-sample concentration.
- Gas flow could be adjusted accurately.

Specifications

Heating module	15 well	Well depth	60 mm
Adjustable of flow	Adjustable	Well Diameter	19 mm
Gas flow	0 - 10 L/min (0-100 psi)	Working power supply	220V / 50Hz
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 Evaperator

P/N: NV24A-11



NV24A-II automatic concentrated nitrogen blowing instrument has been used in various automated workstations with the advantages of large processing capacity, environmental protection, high degree of automation etc. It has been used in the fields of environmental protection, water supply, drug testing, food inspection, forensic identification etc. and can meet the laboratory requirements of high-end samples pre-treatment.

Features

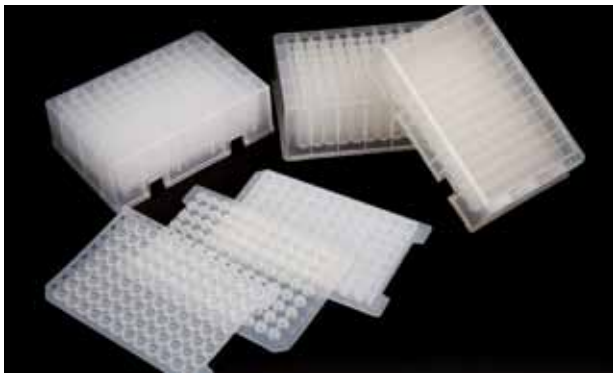
- Synchronous concentration of 24 samples;
- Separate control of each gas channel;
- Adjustable of gas flow and pressure and can be real-time displayed in the concentration process;
- Advanced optical fiber sensor can precisely control the automatic shutdown and alarm to the concentration of 0.5 mL and 1 mL;
- Uses PTC heating elements which can keep uniform heating of water bath temperature;
- Internal exhaust system which can effectively eliminate harmful gases in the system.

Specifications

Type	Performance index
Sample amount	Simultaneous processing of 24 samples
Water bath temperature range	Room temperature + 5℃ to 80℃
Temperature control accuracy	1℃
Quantitative concentration volume	0.5 mL、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

General Supplies

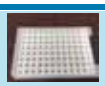
Collection Plate and Silica Mat



Bonna-Agela offers various 96-well collection plates and mats, the whole volume could be 2 mL, 1 mL and 0.5 mL.

Ordering Information

Cat. Number	Product	Specification	Picture
96SP2036-2	96well collection plate	2.2 mL, 8×12, Square well and round bottom	
96SP1036-Y		1.0mL, 8×12, round well and round bottom	
96SP2036-Y		2.0 mL, 8×12, round well and round bottom	
96SP1036		1.6 mL, 8×12, Square well and round bottom	
96SP0236-U		0.2 mL, 8×12, round well and round bottom	
96SP0236-V		0.2 mL, 8×12, round well and V-shape bottom	
08SP2036	8 well solution storage plate	20 mL	
12SP1436	12 well solution storage plate	14 mL	
48SP4036	48 well solution storage plate	4.6 mL	

Cat. Number	Product	Specification	Picture
96GP2036-1	96well mat	8×12, silica, square well	
96GP2036-2		8×12, silica, square well, piecible	
96GP2036-M		8×12, silica, round well, piecible	
A80001	8 collection tube	8well, round well, PP	
A80002	8 collection tube	12 pieces A80001 with rack	

Short Thread Vial

Bonna-Agela Technologies Inc. offers a number of high quality vials and seals. All of them meet the high standard of the chromatographic applications. The difference of our offering from many other brand name venders are the cost to you. We offer lower price for the same products as many other brands due to our lean cost structure and we provide personal assistant to you to select the right products.

Ordering Information

Protein Precipitation Plate

Product	Specification	Package	Cat.No
Cleanert® PPT cartridge	1 mL	100/PK	96CD01

Product	Specification	Cat.No(2/PK)	Cat.No(24/PK)
Cleanert® PPT Plate	1 mL	96CD1025	96CD1025D
	2 mL	96CD2025-Q	96CD2025D-Q

Filtration Plate

Product	Specification	Cat.No(2/PK)	Cat.No(24/PK)
Filtration Plate	2 mL; PP 0.22 μm	96GL2022	96GL2022D
	2 mL; PP 0.45 μm	96GL2024	96GL2024D
	2 mL; PTFE 0.22 μm	96GL0422	96GL0422D
	2 mL; PTFE 0.45 μm	96GL0424	96GL0424D
	2 mL; PVDF0.22 μm	96GL0322	96GL0322D
	2 mL; PVDF 0.45 μm	96GL0324	96GL0324D
	1 mL; PE 20 μm	96GL1025	96GL1025D
	2 mL; PE 20 μm	96GL2025	96GL2025D

Cleanert® Integrated Micro SPE Plates

Product	Specification	Loose cartridge (96/PK)	Assembled plate (2/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

Cleanert® MAS (Multi-function Impurity Adsorption SPE)

Cat. Number	Specification	Application	Package
MSC-B-0301	1 mL	Used for clean-up of basic and neutral compounds in plasma and biological samples (For vacuum or positive pressure)	100
MSC-B-0601	1 mL		100
MSC-A-0301	1 mL	Used for clean-up of acidic compounds in plasma and biological samples (For vacuum or positive pressure)	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 (For vacuum or positive pressure)	100
MSC-WA-0601	1 mL		100
MS-B-0302W-F	30mg / 2mL / well	Used for clean-up of basic and neutral compounds in plasma and biological samples (For vacuum or positive pressure)	2
MS-A-0302W	30mg / 2mL / well	Used for clean-up of acidic compounds in plasma and biological samples (For vacuum or positive pressure)	2
MS-WA-0302W	30mg / 2mL / well	Used for clean-up of weak acidic compounds in plasma and biological samples (For vacuum or positive pressure)	2

Cleanert® 96-well SPE Plate Series

Sorbent (Polymer series)	10mg / 1mL SPE cartridge 100/PK	30mg / 1mL SPE cartridge 100/PK	60mg / 3mL SPE cartridge 50/PK	30mg / 2mL square 96 well plate 2/PK	50mg / 2mL square 96 well plate 2/PK
Cleanert® PEP	PE0101	PE0301	PE0603	PE0302-W	PE0502-W
Cleanert® PEP-2	PE0101-2	PE0301-2	PE0603-2	PE0302-2W	PE0502-2W
Cleanert® PAX	AX0101	AX0301	AX0603	AX0302-W	AX0502-W
Cleanert® PCX	CX0101	CX0301	CX0603	CX0302-W	CX0502-W
Cleanert® PWAX	WA0101	WA0301	WA0603	WA0302-W	WA0502-W
Cleanert® PWCX	WC0101	WC0301	WC0603	WC0302-W	WC0502-W
Cleanert® PS	PS0101	PS0301	PS0603	PS0302-W	PS0502-W

Sorbent (High pure silica series)	50mg/1mL SPE cartridge 100/PK	100/1 mL SPE cartridge 100/PK	50 mg/2 mL square 96 well plate 2/PK	100 mg/2 mL square 96 well plate 2/PK
Cleanert® AQ C18	S180501-AQ	S181001-AQ	S180502AQ-W	S181002AQ-W
Cleanert® C18 (end capped)	S180501	S181001	S180502-W	S181002-W
Cleanert® C18 (non-end capped)	S180501-N	S181001-N	S180502-NW	S181002-NW
Cleanert® C8	S080501	S081001	S080502-W	S081002-W
Cleanert® SAX	SA0501	SA1001	SA0502-S	SA1002-S
Cleanert® SCX	SC0501	SC1001	SC0502-W	SC1002-W
Cleanert® CN	CN0501	CN1001	CN0502-W	CN1002-W
Cleanert® NH2	NH0501	NH1001	NH0502-W	NH1002-W
Cleanert® PSA	PA0501	PA1001	PA0502-W	PA1002-W
Cleanert® COOH	CH0501	CH1001	CH0502-W	CH1002-W
Cleanert® PRS	PR0501	PR1001	PR0502-W	PR1002-W
Cleanert® Diol	DI0501	DI1001	DI0502-W	DI1002-W
Cleanert® Silica	SI0501	SI1001	SI0502-W	SI1002-W
Cleanert® Phenyl	PH0501	PH1001	PH0502-W	PH1002-W
Cleanert® Amide	HI0501	HI1001	HI0502-W	HI1002-W

Integrated SPE Plate

Product	Specification	Loose cartridge (96/PK)	Assembled plate (1/PK)
Cleanert® PEP-2	30mg / 3mL / well	PE0303-2LDW	PE0303-2DW
	50mg / 3mL / well	PE0503-2LDW	PE0503-2DW
Cleanert® PEP	30mg / 3mL / well	PE0303-LDW	PE0303-DW
	50mg / 3mL / well	PE0503-LDW	PE0503-DW
Cleanert® PAX	30mg / 3mL / well	AX0303-LDW	AX0303-DW
	50mg / 3mL / well	AX0503-LDW	AX0503-DW
Cleanert® PCX	30mg / 3mL / well	CX0303-LDW	CX0303-DW
	50mg / 3mL / well	CX0503-LDW	CX0503-DW
Cleanert® PWAX	30mg / 3mL / well	WA0303-LDW	WA0303-DW
	50mg / 3mL / well	WA0503-LDW	WA0503-DW
Cleanert® PWCX	30mg / 3mL / well	WC0303-LDW	WC0303-DW
	50mg / 3mL / well	WC0503-LDW	WC0503-DW

Product	Specification	Loose cartridge (96/PK)	Assembled plate (1/PK)
Cleanert® AQ C18	50mg / 3 mL / well	S180503AQ-LDW	S180503AQ-DW
	100mg / 3 mL / well	S181003AQ-LDW	S181003AQ-DW
Cleanert® C18 (end capped)	50mg / 3 mL / well	S180503-LDW	S180503-DW
	100mg / 3 mL / well	S181003-LDW	S181003-DW
Cleanert® C18-N (non end capped)	50mg / 3 mL / well	S180503-NLDW	S180503-NDW
	100mg / 3 mL / well	S181003-NLDW	S181003-NDW
Cleanert® C8	50mg / 3 mL / well	S080503-LDW	S080503-DW
	100mg / 3 mL / well	S081003-LDW	S081003-DW
Cleanert® CN	50mg / 3 mL / well	CN0503-LDW	CN0503-DW
	100mg / 3 mL / well	CN1003-LDW	CN1003-DW
Cleanert® NH2	50mg / 3 mL / well	NH0503-LDW	NH0503-DW
	100mg / 3 mL / well	NH1003-LDW	NH1003-DW
Cleanert® PSA	50mg / 3 mL / well	PA0503-LDW	PA0503-DW
	100mg / 3 mL / well	PA1003-LDW	PA1003-DW
Cleanert® SAX	50mg / 3 mL / well	SA0503-LDW	SA0503-DW
	100mg / 3 mL / well	SA1003-LDW	SA1003-DW
Cleanert® COOH	50mg / 3 mL / well	CH0503-LDW	CH0503-DW
	100mg / 3 mL / well	CH1003-LDW	CH1003-DW
Cleanert® PRS	50mg / 3 mL / well	PR0503-LDW	PR0503-DW
	100mg / 3 mL / well	PR1003-LDW	PR1003-DW
Cleanert® SCX	50mg / 3 mL / well	SC0503-LDW	SC0503-DW
	100mg / 3 mL / well	SC1003-LDW	SC1003-DW
Cleanert® Silica	50mg / 3 mL / well	SI0503-LDW	SI0503-DW
	100mg / 3 mL / well	SI1003-LDW	SI1003-DW
Cleanert® Diol	50mg / 3 mL / well	DI0503-LDW	DI0503-LDW
	100mg / 3 mL / well	DI1003-LDW	DI1003-LDW
Cleanert® Hilic	50mg / 3 mL / well	DI0503-LDW	HI0503-LDW
	100mg / 3 mL / well	DI1003-LDW	HI1003-LDW
Cleanert® Phenyl	50mg / 3 mL / well	DI0503-LDW	PH0503-LDW
	100mg / 3 mL / well	DI1003-LDW	PH1003-LDW

Cleanert® SLE (Supported Liquid Extraction)Technology and Sorbents

Material	Description	Specification, Package	Cat. Number
Special treated diatomite SLE -AQ series (Neutral)	Large Particle; Neutral SLE cartridges	200mg / 3mL, 50/Pk	HC2003Q-7
		500mg / 6mL, 30/Pk	HC5006Q-7
		1g / 6mL, 30/Pk	HC0006Q-7
		2g / 12mL, 20/Pk	HC200012Q-7
		4g / 25mL, 15/Pk	HC400025Q-7
		10g / 60mL, 10/Pk	HC1000060-7
		20 g/60 mL, 10/Pk	HC2000060-7
	Large Particle; Neutral SLE well plates	200mg / 2mL / well, 2/Pk	HC2002Q-7W
		300mg / 2mL / well, 2/Pk	HC3002Q-7W
		400mg / 2mL / well, 2/Pk	HC4002Q-7W
	Large Particle; Neutral SLE deep array well plates	500mg / 3mL / well, 1/Pk	HC5003Q-7DW
		600mg / 3mL / well, 1/PK	HC6003Q-7DW
	Small particle; Neutral SLE well plates	200mg / 2mL / well, 2/Pk	HC2002SQ-7W
		300mg / 2mL / well, 2/Pk	HC3002SQ-7W
		400mg / 2mL / well, 2/Pk	HC4002SQ-7W
	Small particle; Neutral SLE deep array well plates	500mg / 3mL / well, 1/Pk	HC5003SQ-7DW
		600mg / 3mL / well, 1/PK	HC6003SQ-7DW
Special treated diatomite SLE-AQ series (Basic)	Small particle; Basic SLE cartridges	200mg / 3mL, 50 /Pk	HC2003Q-9
		500mg / 3mL, 50 /Pk	HC5003Q-9
		500mg / 6mL, 30 /Pk	HC5006Q-9
		1g / 6mL, 30 /Pk	HC0006Q-9
		2g / 12mL, 20 /Pk	HC200012Q-9
		4g / 25mL, 15 /Pk	HC400025Q-9
		10g / 60mL, 10 /Pk	HC1000060-9
		20g / 60mL, 10 /Pk	HC2000060-9
	Large Particle; Basic SLE well plates	200mg / 2mL / well, 2 /Pk	HC2002Q-9W
		300mg / 2mL / well, 2 /Pk	HC3002Q-9W
		400mg / 2mL / well, 2 /Pk	HC4002Q-9W
	Large Particle; Basic SLE deep array well plates	500mg / 3mL / well, 1 /Pk	HC5003Q-9DW
		600mg / 3mL / well, 1 /Pk	HC6003Q-9DW
	Small particle; Basic SLE well plates	200mg / 2mL / well, 2/Pk	HC2002SQ-9W
		300mg / 2mL / well, 2/Pk	HC3002SQ-9W
		400mg / 2mL / well, 2/Pk	HC4002SQ-9W
	Small particle; Basic SLE deep array well plates	500mg / 3mL / well, 1/Pk	HC5003SQ-9DW
		600mg / 3mL / well, 1/PK	HC6003SQ-9DW
Special treated diatomite SLE bulk sorbent	Large Particle; Neutral SLE bulk sorbent	1 kg/Package	HC1001000-7
	Large Particle; Basic SLE bulk sorbent	1 kg/Package	HC1001000-9
	Large Particle; Neutral SLE bulk sorbent	18 kg/Package	HC018-7
	Large Particle; Basic SLE bulk sorbent	18 kg/Package	HC018-9

General Supplies

Cat.No.	Description	Package
AV1130-6	300 µL insert, flat bottom, clear glass, 31x6 mm	100/pk
AV1131-6	250 µL micro-insert, clear glass, with assembled plastic spring, 29x6 mm	100/pk
AV1132-6	300 µL micro-insert, clear glass, 31x6 mm	100/pk
AV1103-P	300 µL PP screw neck Micro-vial, transparent	100/pk
AV1113-P	300 µL PP screw neck Micro-vial, amber	100/pk
AV1100-6	1.5 mL short thread vial, clear glass	100/pk
AV1110-0	1.5 mL short thread vial, amber glass	100/pk
AV1101-6	1.5 mL short thread vial, clear glass, label and filling lines	100/pk
AV1111-0	1.5 mL short thread vial, amber glass, label and filling lines	100/pk
AV2100-A	9 mm screw neck cap, center hole; red silicone/ white PTFE septa	100/pk
AV2200-0	9 mm screw neck cap, center hole; red silicone/ white PTFE septa, slitted	100/pk
AV2300-0	9 mm screw neck cap, closed top; red silicone/ white PTFE septa	100/pk

Sample Preparation Apparatus

Cat.N	Products
VM96	96-Well Vacuum Manifold
SPE-M96	96-Well Positive Pressure SPE Equipment
SPE-M48	MULTI-SPE M48 Solid Phase Extraction Manifold
NV-96G	Nitrogen Evaporator for 96 Well Plates
NV-8G	Nitrogen Evaporator for 8 Postion
NV-15G	Nitrogen Evaporator for 15 Position
NV24A-11	Nitrogen Evaperator for 11 Postion

Columns for LC-MS Analysis

Bonna-Agela Technologies offers a broad line of HPLC columns for Bio-molecules, including reversed phase, normal phase, ion-exchange, HILIC and size exclusion columns. All column's packing materials are made of ultra pure silica, bonded with pure silanes to ensure the surface inertness. They offer the flexibility to meet the needs of bioanalysis.
With the development of UHPLC technology, it became the platform of choice for bioassay. Agela offers a series of sub-2 μm columns to meet the needs of various applications.
Through a core-shell structure, Bonshell columns represent a new level of performance.It presents high efficiency, high resolution, and reduces analysis time separation.

HPLC Column Series

Good polar retention; balanced separation power with lifetime, contamination resistance and consistency

Unisol C18 (2) column is a universal reversed phase column through carefully optimizing the pore size, surface and carbon content adding with Bonna-Agela's innovative surface modification and unique bonding processes. It has excellent balance of hydrophilic, retention, resolution and contamination tolerance. In addition, the Unisol C18 (2) column demonstrates good retention capability for the different polar compounds. Since it is less affected by the pH of mobile phase and buffer concentration, the Unisol C18 (2) column provides excellent reproducibility.
It also provides better peak shape and consistency than PhenomenexPolar RP and YMC AQ; equivalent to Waters T3

Characteristics

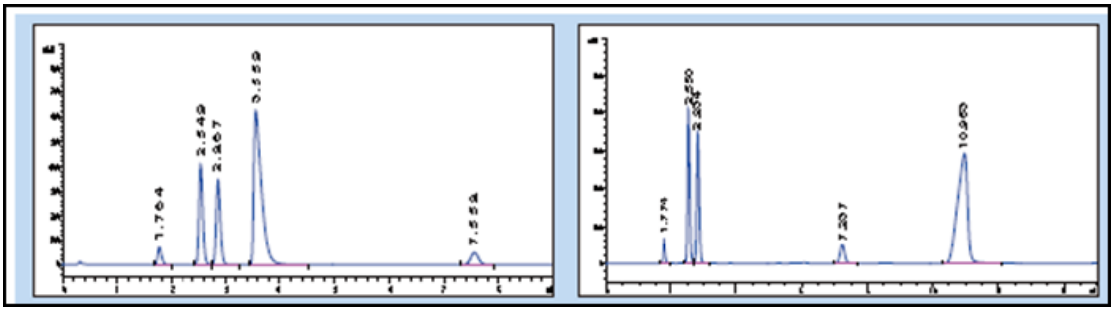
Unisol C18(2): Metal Impurity < 30 ppm; Pore Size: 110 Å; Specific Surface Area: 340 m^2/g;
Available Particle Size: 3 μm, 5 μm or 10 μm; Single end-capped; Carbon Loading:14 %; pH=1.5-9.0

Main Features

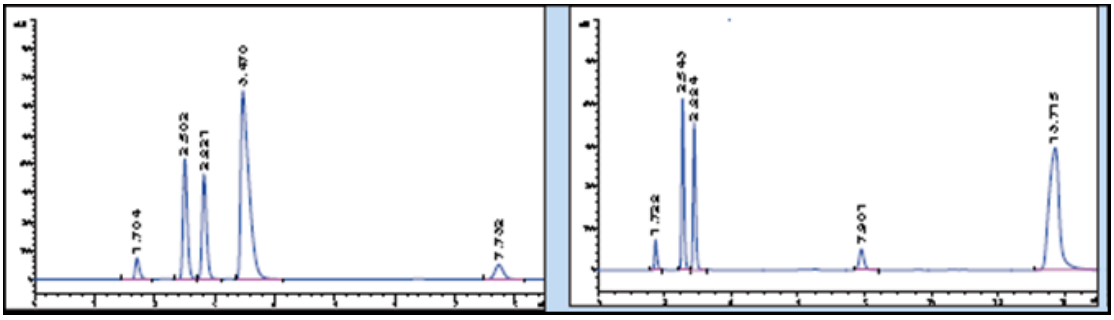
- The density of silicon hydroxyl: 100% water compatible and enhanced retention and separation for polar isomer compounds
- Reduce the quantity of active silicon hydroxyl and blocked metal ions: good peak shape for basic compounds
- Reduce the quantity of ion exchange sites: less affected by the pH mobile phase and buffer concentration.
- Weakens nonspecific adsorption: Good tolerance of biological matrix.

The activity testing of silicon hydroxyl

The Unisol C18 (2) columns provide symmetric peak and good resolution for acidic, neutral and basic compounds at usual pH range. Retention of basic compound is less changed when the pH of mobile phase changes.
Sample: Uracil, Phenol, dimethylphthalate, naphthalene, amitriptyline
Mobile Phase: Methanol: 20 mM potassium phosphate buffer (pH2.6 / pH5.8) =70: 30
Flow Rate: 1.00 mL/min
Temperature: 30℃
Detector: UV 254 nm



Unisol C18(2), 5 μm, 4.6×250 mm
pH=2.6 Rt(amitriptyline)=3.559 pH=5.8 Rt (amitriptyline)=10.963



Waters Atlantis T3, 5 μm, 4.6×250 mm
pH=2.6 Rt(amitriptyline)=3.470 pH=5.8 Rt(amitriptyline)=13.715

Venusil® ASB Series Column

Extremely low bleed and high sensitivity; may retain very polar analytes

The Venusil® ASB series columns are specially designed for the separation of polar compounds from low (extremely stable at pH=0.8) to medium pH. The stationary phase is bonded with unique bulky silanes that sterically protects the siloxane bond. We offer a line of bonding chemistry of C1, C3, C8, C18 and Phenyl groups presenting a broad selection of different polarity for various applications. These columns can be used to replace Zorbax SB series.

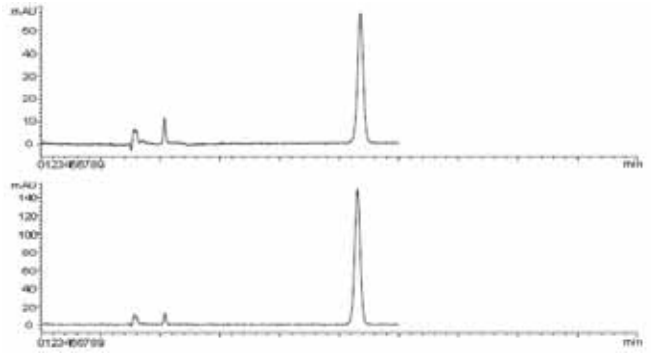
Main features

- Low pH stability: great stability even at pH=0.8
- Peak shape and efficiency: Excellent peak symmetry for basic compounds comparing to other brand polar phase columns
- Polar C18 phase: Very strong separation power for polar compounds
- 100% aqueous compatible: Much better peak shape, retention and efficiency
- Five different bonded phases provide broad selectivity

Venusil® ASB column

Phase	Particle size	Pore size	Carbon loading	Surface Area	End-capped style	pH range
C18	3,5,10 μm	150 Å	12 %	200 m^2/g	No	0.8-7.5
C8	3,5,10 μm	150 Å	7 %	200 m^2/g	No	1.0-7.5
C3	5 μm	150 Å	4 %	200 m^2/g	No	1.0-7.5
C1	5 μm	150 Å	2 %	200 m^2/g	No	1.0-7.5
phenyl	5 μm	150 Å	6 %	200 m^2/g	No	1.0-7.5
C18 (T)	5 μm	300 Å	5 %	80 m^2/g	No	0.8-7.5
C8 (T)	5 μm	300 Å	3 %	80 m^2/g	No	1.0-7.5

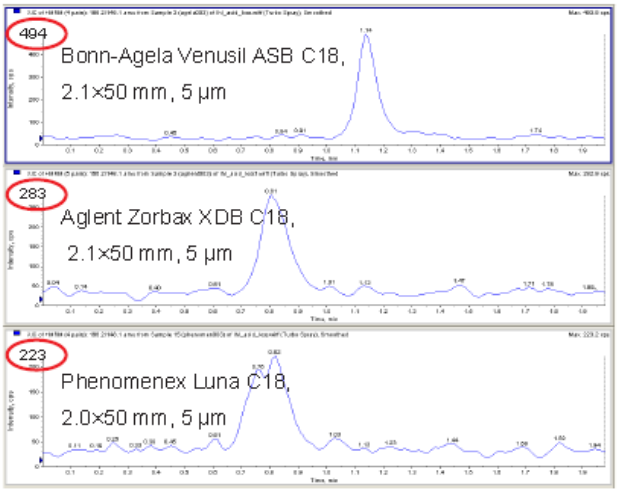
The Stability in the Low pH



Column: Venusil® ASB C18, 4.6×150 mm, 5 μm
Sample: Naphthol
Aging: 40℃ , TFA in 80 % methanol (pH=1.0), 400 hours
Mobile Phase: TFA in 80 % methanol (pH=1.5)
Flow Rate: 1 mL/min
Injection: 5 μL
Temperature: 30℃

Beffer LC-MS Sensitivity

2-3 times higher than many popular brands (low bleeding, inert surface and high efficiency)



Durashell C18 (2) Column

Bonna-Agela Durashell column has a wide pH range from 1.5 to 12.0. Compared with the current products in the market that also can be used at high pH, Durashell employs a hybrid technology on the stationary phase surface, followed by molecular modifications.

The patented technology produces a strong hydrophobic protection layer over the silica surface, allowing the columns to be used at extremely high and low pH. It also reduces excessive hydrophobic interactions between the stationary phase and the analytes, and yet maintains great interfacial kinetics for high efficiency. DurashellC18(2) column has excellent peak symmetry for basic compounds by unique end-capping technology. These columns can be used to replace Phenomenex Gemini or Waters X-bridge.

Characteristics

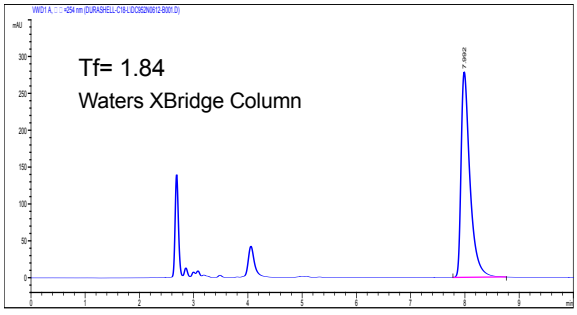
Metal Impurity < 30 ppm; Pore Size: 150 Å; Specific Surface Area: 200 m^2/g;
Available Particle Size: 3 μm, 5 μm and 10 μm.

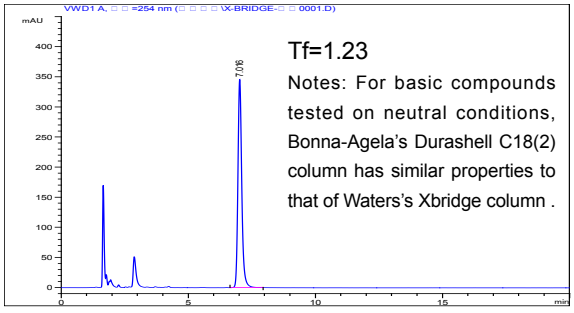
Main features

- Wide pH range (1.5-12.0)
- Minimal silanol activity
- Better efficiency than many other high pH compatible columns
- High loading capacity for basic compounds for preparative applications

Test Results

DurashellC18(2) column





Sample: Amitriptyline
Column: Durashell C18 (2), 4.6x250 mm, 5 μm, 150 Å
Waters XBridge, 4.6x150 mm, 5 μm, 130 Å
Mobile Phase: 80% Methanol, 20 % 5mM KH_2PO_4 (pH 7)
Flow Rate: 1.2 mL/min
Temperature: 25 °C

Durashell HILIC Column

Durashell HILIC stationary phase is made from the hybrid spherical silica particles bonded with a neutral hydrophilic amide group. It can be used for normal phase, reversed phase, or hydrophilic interaction HPLC. Compared with traditional silica and NH_2 columns, the Durashell HILIC columns have better reproducibility and column lifetime. In addition, they have longer lifetime than that of currently marketed HILIC column. It is especially useful for the separation of strong hydrophilic compounds, whether they are acidic, basic or neutral.

Characteristics

Metal Impurity < 30 ppm; No End-capped, pH=1.5-12.0. Pore Size 100 Å; Specific Surface Area: 380 m^2/g;
Carbon Loading 10 %; Available Particle Size: 3 μm, 5 μm and 10 μm.

Main features

- Lower bleed, and longer lifetime than other HILIC columns.
- Strong retention of polar compounds in HILIC mode.
- Unique selectivity and complementary to conventional reversed phase and normal phase.
- pH range (1.5-12.0).

Bonshell Column

A better choice to speed up your HPLC analysis

Bonshell columns represent a new level of performance through a core-shell structure. They are packed with uniform particles, and the structure of particles is 1.7 μm of solid core coated with 0.5 μm of porous layer. It is the best choice if you want to speed up your HPLC analysis without an expensive UHPLC instrument.

Characteristics

Phase	Particle size	Pore size	Carbon loading	Surface Area	End-capped style	pH range
ASB C18	2.7 μm	90 Å	7 %	150 m^2/g	No	1.0-7.5
C18	2.7 μm	90 Å	10 %	150 m^2/g	Double	1.5-9.0

Main features

- Core-shell technology.
- Stable at operating pressures up to 600 bar (9000 psi).
- Conventional column, high column efficiency.

Bonshell ASB C18 Column

A better choice to speed up your HPLC analysis

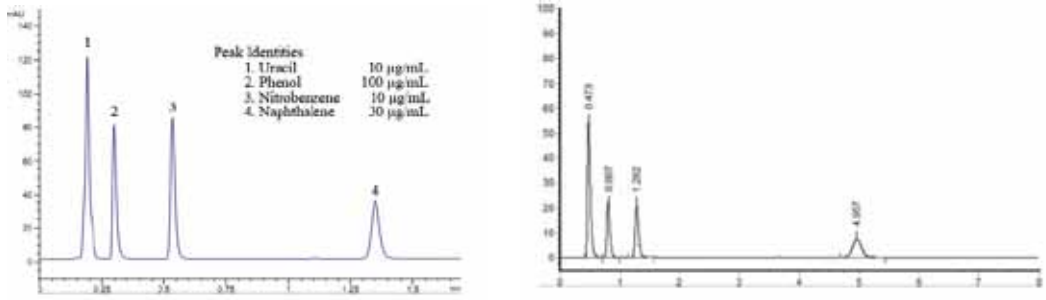
This C18 column has protective butyl side chains to enhance peak shape and separation of basic compounds under neutral and acidic conditions.

Main features

- Core-shell technology
- Stable at operating pressures up to 600 bar (9000 psi)
- Low pH stability: great stability even at pH=0.8
- Very strong separation power for polar compounds
- Compatibility with 100 % aqueous mobile phase

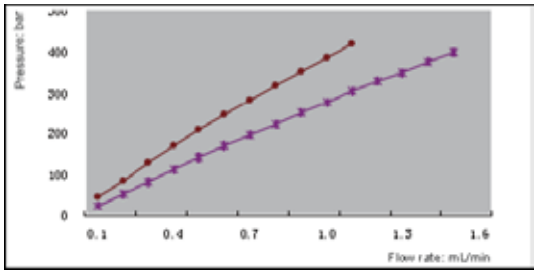
Extremely improve the column efficiency, analysis speed, resolution and sensitivity than columns packed with 3 μm and 5 μm particles

Generating much lower backpressure at optimal linear velocities of mobile phase, you can now achieve 2-3x's the column efficiencies of traditional fully porous 3 μm and 5 μm columns on any LC instrument.
2.7 μm Bonshell ASB C18



Column: Venusil® ASB C18, 3 μm, 2.1×50 mm; Mobile phase: 50 % water and 50 % ACN;
Flow rate: 0.5 mL/min; Temperature: 30 ℃ ; Detection: UV 254 nm

Lower pressure



Column: Venusil® ASB C18, 3 μm, 2.1× 50 mm
Mobile phase: ACN : H_2O=70:30, 25 ℃
Red curve: sub-2 μm column
Rurpe curve: Bonshell
The column pressure lowers to 60% compared to that of UHPLC in the same column efficiency

UPLC Column Series

The chromatography separation technology has been revolutionized by Ultra-Performance Liquid Chromatography (UHPLC) System. Sub 2 μm particles make UPLC analysis quicker and more sensitive than the traditional liquid chromatography.

Bonna-Aglea UHPLC columns are packed with 1.9 μm particles based on ultra pure silica prepared by aggregation of silica sols. This kind of silica has narrow size distribution and no dead pores whose pore size is smaller than 2 nm.

Bonna-Agela UHPLC family includes UHP AQ C18; UHP ASB C18, UHP Innovol C18 and UHP HILIC.

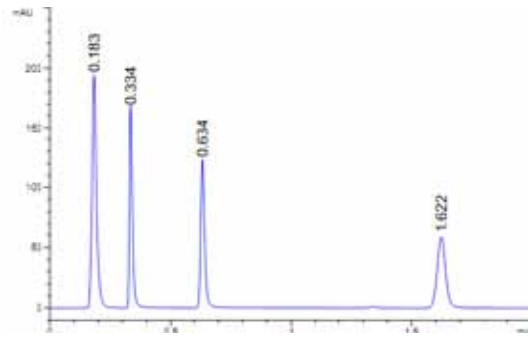
UHP AQ C18 is based on new spherical silica with high purity, with double layer surface modification technology, which reduces the interactions between polar analytes and silica surface significantly and subsequently symmetry for very basic compounds is maximized. UHP ASB C18 is bonded with unique bulky silanes that sterically protect the siloxane bond. UHP Innoval C18 is end-capped twice to ensure an inert stationary phase.

The UHPLC material is packed in newly designed column housings with extremely low void volume, which tolerate back-pressure up to nearly 1000 bar or 15000 psi.

UHP AQ C18

Characteristics

Metal Impurity < 30 ppm; Pore Size: 100 Å; Specific Surface Area: 240 m^2/g; Available Particle Size: 1.9 μm.
Carbon Loading: 8 %; Single end-capped; pH=1.5-9.0



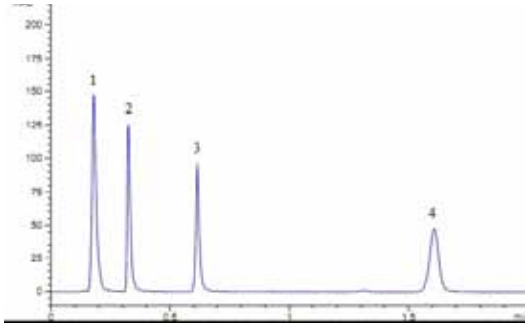
Column: UHP AQ C18, 2.1×50 mm, 1.9 μm
Sample: Uracil (1) Phenol (2) Nitrobenzene (3) and Naphthalene (4) in mobile phase; 1.0 μL
Mobile phase: 50 % Acetonitrile / 50 % Water
Flow Rate: 0.5 mL/min
Pressure: 337 bar
Temperature: 30℃
Detector: UV 254 nm

UHP ASB C18

Characteristics

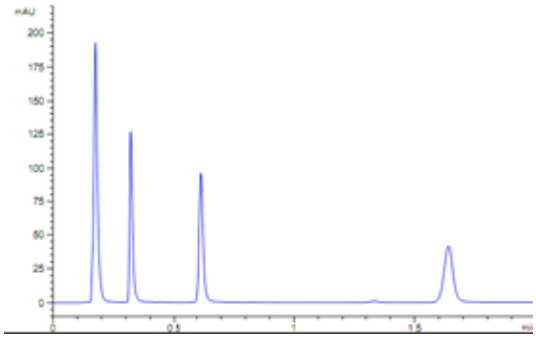
Metal Impurity < 30 ppm; Pore Size: 100 Å; Specific Surface Area 240 m^2/g; Available Particle Size: 1.9 μm.
Carbon Loading: 8%; No end-capped; pH=0.8-7.5

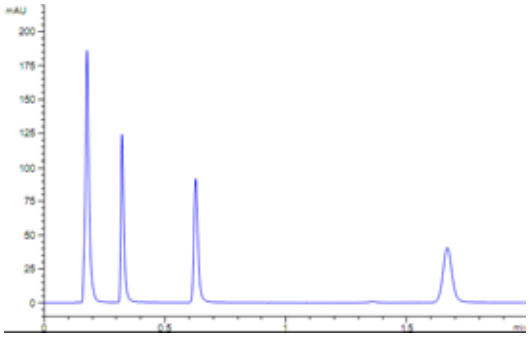
Balanced Retention for Hydrophilic and Hydrophobic Compounds



Column: UHP ASB C18, 2.1×50 mm, 1.9 μm
Sample: Uracil (1) Phenol (2) Nitrobenzene (3)
and Naphthalene (4) in mobile phase; 2 μL
Mobile Phase: 50% Water; 50% Acetonitrile
Flow Rate: 0.5 mL/min
Detector: UV 254 nm
Temperature: 30℃

Pressure test



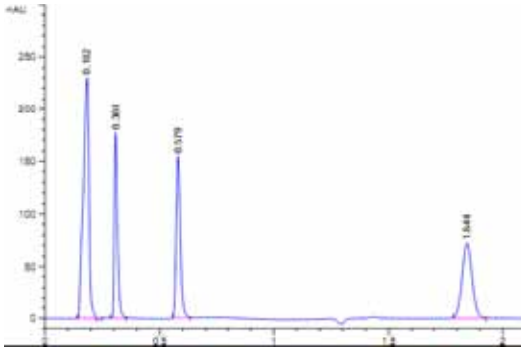


Column: UHP ASB C18, 2.1×50 mm, 1.9 μm
Sample: Uracil (1) Phenol (2) Nitrobenzene (3) Naphthalene (4) in mobile phase
Mobile phase: 50 % Acetonitrile / 50 % Water
Flow Rate: 0.5 mL/min
Temperature: 30℃
Detector: UV 254 nm

UHP Innoval C18

Characteristics

Pore Size: 100 Å; Specific Surface Area: 240 m^2/g; Available Particle Size: 1.9 μm. Carbon Loading:14 %;
Double end-capped; pH=1.5-9.0



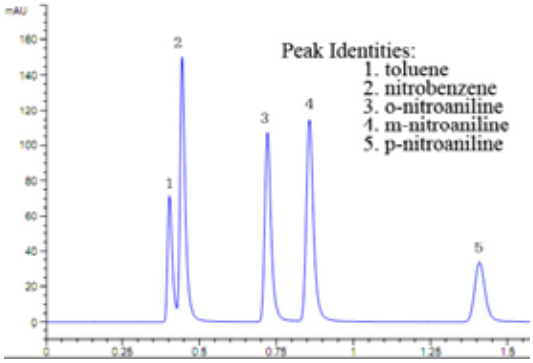
Column: UHP Innoval C18, 2.1×50 mm, 1.9 μm
Sample: Uracil (1) Phenol (2) Nitrobenzene (3) and Naphthalene (4)
in mobile phase; 1 μL .
Mobile phase: 50 % Water, 50 % ACN
Flow Rate: 0.5 mL/min
Pressure: 287 bar
Temperature: 30℃
Detector: UV 254 nm

UHP HILIC

The proprietary nano-surface treatment and bonding process of the packing materials leading to unique performance of the UHP HILIC columns.

Characteristics

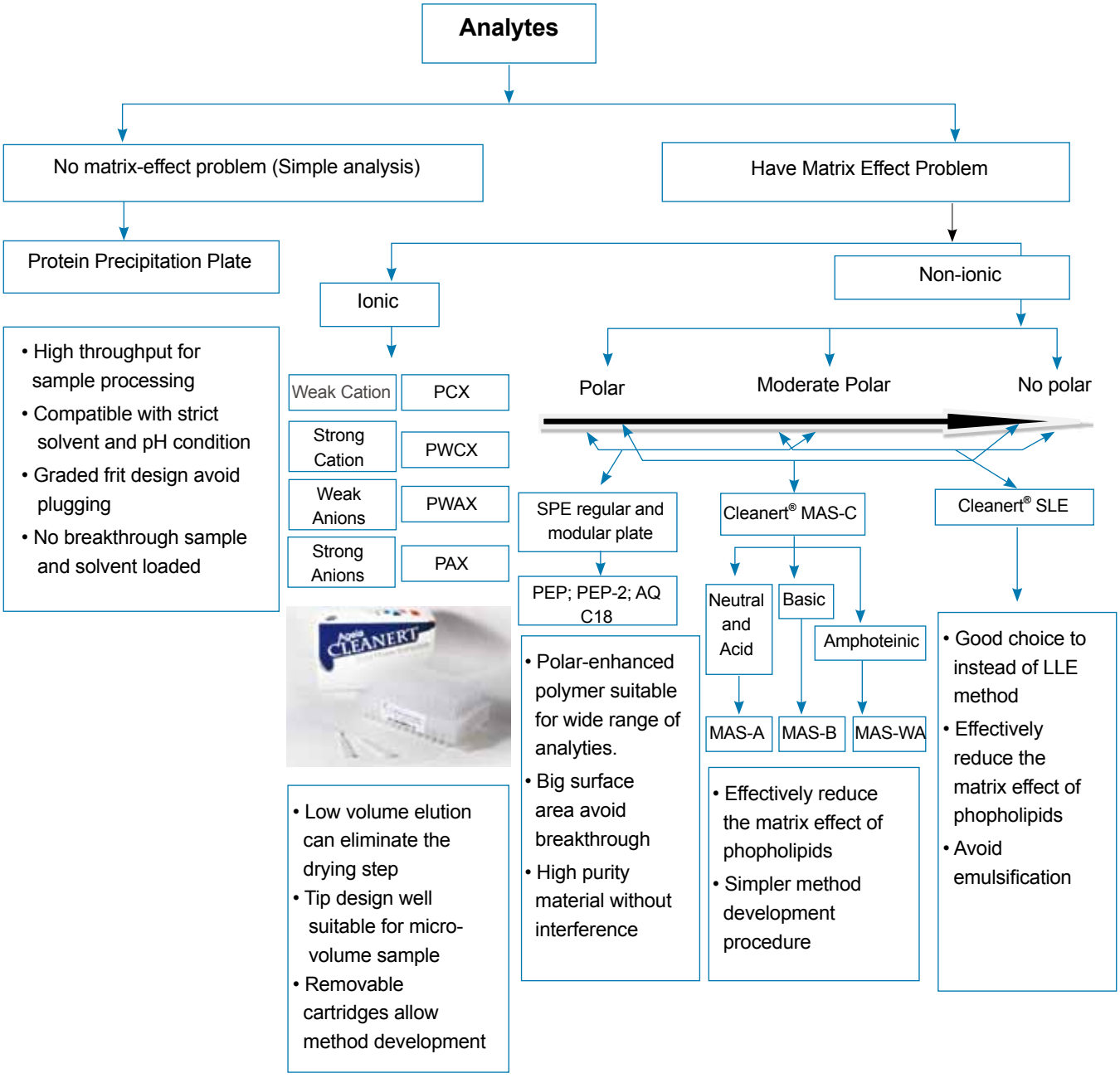
Metal Impurity < 30 ppm; Pore Size: 100 Å; Specific Surface Area: 240 m^2/g; Available Particle Size: 1.9 μm.
Carbon Loading: 3 %; No end-capped; pH=2.0-8.0



Column: UHP HILIC, 2.1×50 mm, 1.9 μm
Mobile phase: 98.8 % chlorobutane; 1 % methanol; 0.2 % water
Flow rate: 0.3 mL/min
Temperature: 30℃
Detector: UV 254 nm

Method Development Guide for Bio-sample Preparation

Product Selection Guide (By Analytes Character)



1) Experiment Requirement

Simple analysis, such as single analyte analysis, detection limit ＞ 1ppb, or analytes without of matrix effect problem.

Suggested sample preparation method—Protein precipitation plate

Why choose PPT plate ?

- Increase method stability
- Avoid contamination of analysis instrument
- Enhance HPLC column life time

2) Experiment Request

High polar compound analysis is always a challenge, because the analytes cannot be adsorb in the SPE material surface. Due to its chemical property, the retention time on the HPLC column is very short, it cannot be separated with the polar matrix effectively, just as the picture shown below, matrix interference will cause inconsistent results.

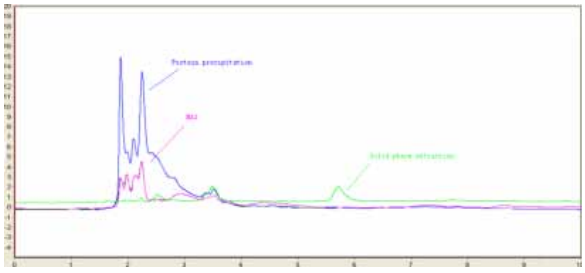
Suggested sample preparation products—Cleanert® MAS-C or Cleanert® OMM SPE Products

Why choose MAS-C plate?

- Different from traditional SPE method. MAS-C series choose special material to adsorb matrix instead of analytes. Since it does not need to select SPE plate, it make the method development easier.
- Fuctionalization material can effectively reduce the matrix effect of phospholipids, enhancing analytes response. Base on the picture below, it show the chromatogram scanned under UV254nm, the weakly retained matrix reduced by MAS method effectively.

OMM SPE Products (Optimized Molecular Modification)

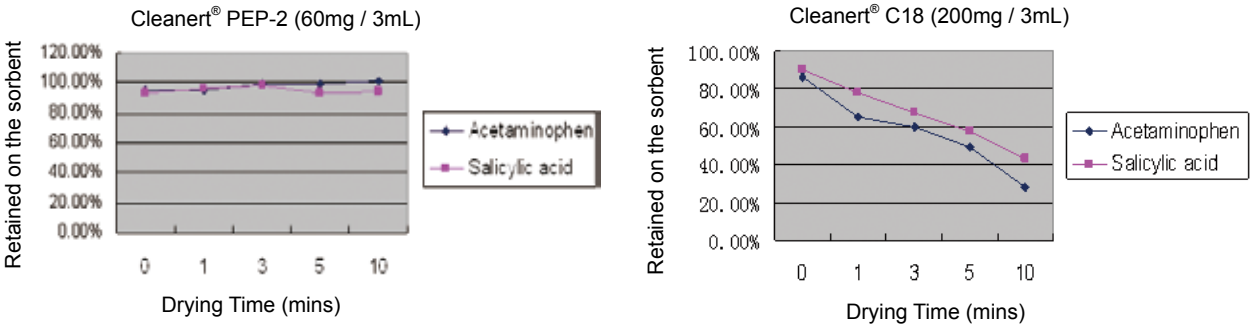
— PEP and PEP-2, PAX, PCX, PWAX, PWCX and PS are all based on polystyrene/divinylbenzene while each phase has different functionality and unique selectivity.



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.

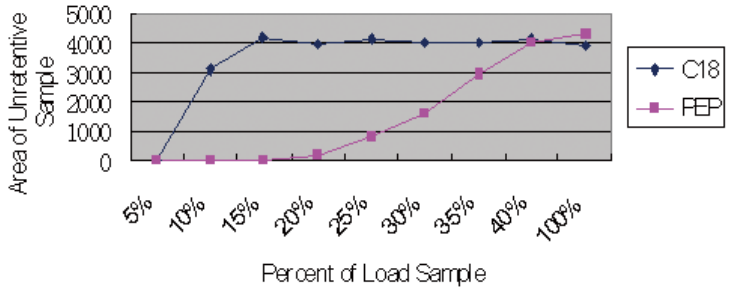
Effect of Drying on Recovery - PEP-2 Versus C18 Sorbents



High Capacity

The Cleanert® PEP series sorbents have mach 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)



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.

	PEP	PEP-2	Brand P	Brand W
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%

The recoveries of three compounds on different SPE materials.

3) Experiment Requirement—Multiple Analytes

Applications involved multiple analytes need the SPE material have a good selectivity for analytes with wide range of polarity.
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, this material can retain most of neutral and ionizeble compounds in liquid samples.

Suggestion Processing Method

	PEP	PEP-2	Brand P	Brand W
Caffeine	98.58%	100.37%	100.12%	103.53%
Metoporolol	80.11%	88.25%	91.23%	90.05%
Salicylic acid	21.36%	109.73%	8.03%	18.79%

4) Experiment Request

Small sample loading volume, low concentration detection.

Suggested sample preparation method-Cleanert® Micro plate

Why choose Micro plate?

- Internally tapered well suitable for micro-volume sample
- High sorbent bed avoids breakthrough
- Low-elution volume can save time of concentration
- Removable cartridges allow flexible combination
- Different sorbent meet the requirement of method development

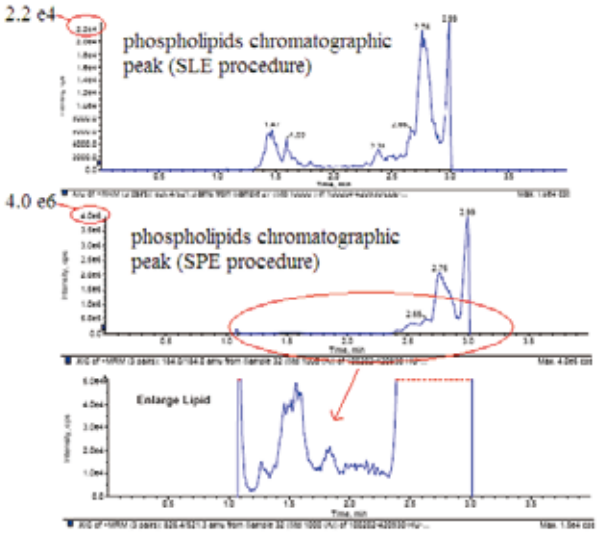
5) Experiment Request

Matrix effect problem often affect the non polar analyte analysis in biological sample, such as phospholipids can make strong interference in LC-MS/MS analysis. Liquid-liquid extraction is often used for clean-up, but LLE method has its limits, such as time costing, large volume solvent usage, also it could not be run automatically by instrument.

Suggested sample preparation method—Cleanert® SLE

Why choose SLE plate ?

- SLE using diatomite earth as the supporting medium, and makes it easy to transfer LLE method to the high-throughput 96 well plate.
- Effectively reduce the matrix effect of phospholipids, enhance analytes response and stability of recovery.
- Avoid emulsification, and easy to operation



Base on the chromatography of phospholipids in plasma treated by three kinds of method. We can find that SLE method got a 100 times response decrease.

- Bonna-Agela develop a method which can remove the matrix effect of phospholipids, and compared them with general method. Result shows that using specific method can enhance the analytes response effectively, besides achieving better stability method.

Protein precipitation

- 50 μL plasma sample was added to each well;
- Added 250 μL ACN-Methanol (9:1)，standed for 3 min;
- Vacuum, dried by N_2.

SLE method:

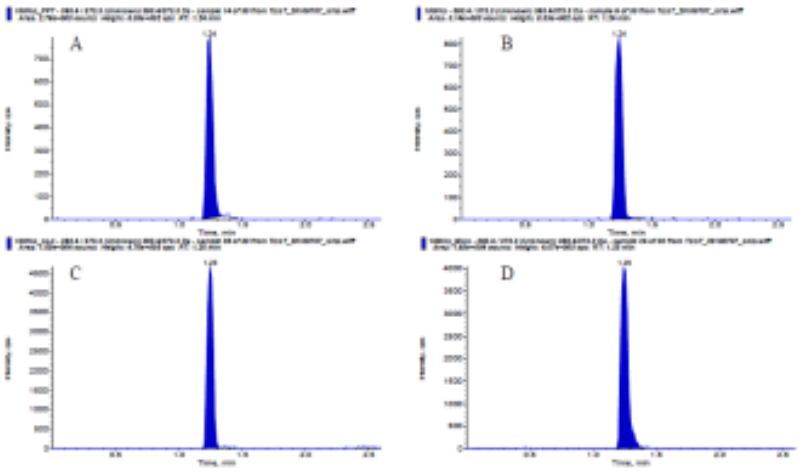
- 50 μL plasma diluted by 50 μL water；
- Load sample solution, stands for 10 min；
- 1.5 mL MTBE elution in 2 times.

SPE method:

- Activated HLB column with 1 mL MeOH, then 1 mL Water
- loaded 50 μL sample
- Washed columns with Water-Methanol (8:2)
- Elute sample with ACN-Methanol (9:1)

MAS-C method:

- 50 μL sample in each well；
- Add 600 μL ACN:Methanol (9：1),stands for 3 min；
- Vacuum the plate，dry by N_2.



Concentration	Response of Dexamethasone in Human Plasma			
	A：Protein Precipitation	B：SPE method	C：Bonna-Agela SLE plate	D：Bonna-Agela MAS plate
10 ng/mL	2.38E+02	2.91E+02	1.32E+03	1.46E+03
100 ng/mL	2.76E+03	3.14E+03	1.50E+04	1.63E+04

6) Method Transfer From LLE to SLE Method

Principle of SLE: Diatomaceous earth packed in the Cleanert® SLE is a kind of porous material with strong absorption ability for aqueous phase, just like the sketch map show.
Base on this principle, SLE just give a solid support medium for LLE process, so that method transfer became more easily.

For Example

Method 1----liquid/liquid extraction	Method 2----SLE(Solid-supported liquid-liquid extraction)
Choose a 2 mL centrifuge tube	Note: SLE Capability of water absorption: 0.8-1 mL/g. Total liquid sample should be 200 µL, so that we can choose a 300 mg/well plate
1. Add 1 mL Ethyl acetate into 100 µL plasma.	1. Add 100 µL diluted with 100 µL buffer; Note: Dilution will decrease the viscosity of sample, so that is helpful for sample diffusion; Adjust the pH of the sample solution can restrain ionization, and make extraction more efficiently. 1M ammonium buffer (pH 9-10) are recommended for basic analytes and 1M phosphate buffers (pH 2-3) are recommended for acidic analytes.
2. Vortex for 5 min	2. Standing for 10 min. Note: Give enough time for sample adsorption into the diatomite earth micro-pore.
3. Centrifuge at 4000rpm for 10 min	3. Elute each well with totally 1.5 mL Ethyl acetate by twice, and pump the well with vacuum for 2minutes. Note: Ratio of elution solvent and sample should be more than 3:1; Elution with 2-3 times aliquots instead of one may improve extraction efficiency and recovery. MTBE is widely used as elution solvent, and ethyl acetate, DCM and mixed solvents are also good choices for elution solvent. After solvent run off, apply vacuum can collect all the elution solvent from the loose.
4. Collect the supernatant and evaporate it at 45℃ by nitrogen blowing.	4. Collect the eluant and evaporate it at 45℃ by nitrogen blowing. Note: eluant collection can be achieved by 96well plate vacuum manifold, positive pressure manifold; concentrator for 96 well plate can be used for evaporate.
5. Reconstitute it with 200 µL Methanol.	5. Reconstitute it with 200 µL methonal.

7) Experiment Requirement

Biological sample analysis usually uses LC-MS/MS which have high sensitivity. Interference from SPE material and plastic material sometimes will occur to extremely low level analysis.

Most of the SPE material use silica or polymer base, analytically pure solvent and raw material is usually used in synthesis process. Soluble substance from elution process will affect the PPT level analysis.

Bonna-Agela provides customized trace level interference SPE material for suckion kind of applications. Chromatographically pure level solvent is used, and by additional washing process, specific interference can be decreased by controllable level.

8) Experiment Requirement

Improve throughput is an important request for today's method development. Clinical laboratory and DMPK researchers have thousands of sample that have to been finished in one day, giving it big challenge to operator. Save seconds by optimize a method is also very meaningful in saving hours in one day. Agela give some convenient methods for saving valuable time for you.

Micro plate vs regular SPE plate (Reduce 30 min per sample)

Modular Micro Plate	Regular SPE plate
 20min	 50min
200 µL methanol and 200 µL water Diluted biological sample 50 µL 200 µL Water (or 5% organic solvent) Dry the cartridge by vacuum 50 µL MeOH or ACN Inject directly	1 mL MeOH or ACN; 1 mL Water Diluted biological sample 100 µL 1 mL Water (or 5% organic solvent) Dry the cartridge by vacuum 500 µL MeOH or ACN Concentration and reconstitution

SLE vs LLE (Reduce 20 min per sample)

SLE Plate	LLE plate
 20min Add 100 µL diluted with 100 µL buffer， and load the sample to plate; Standing for 5 min Elute each well with totally 1.5 mL Ethyl acetate by twice, and pump the well with vacuum for 2 min. Collect the eluant and evaporate it at 45℃ by nitrogen blowing. Reconstitute it with 200 µL methanol	 40min Add 100 µL diluted with 100 µL buffer; Add 1mL Ethyl acetate into 200 µL diluted plasma Vortex for 5 min. Centrifuge at 4000 rpm for 10 min. Collect the solvent layer and evaporate it at 45℃ by nitrogen blowing. Reconstitute it with 200 µL methanol

PPT Plate vs centrifuge (Reduce 30 min)

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

PEP-2 vs RP Polymer -- No need for sampel pH adjustment

	PEP-2	Hydrophilic-lipophilic balance RP Polymer(PEP or HLB)
Condition	MeOH or ACN; Water	MeOH or ACN; Water
Sample Loading	Diluted biological sample, no	Diluted biological sample;
Wash	need for pH adjustment	pH adjustment by buffer;
	Water (or 5% organic solvent)	Water (or 5% organic solvent)
	Dry the cartridge by vacuum	Dry the cartridge by vacuum
Elution	MeOH or ACN	MeOH or ACN

9) Experiment Requirement --Low Detection Limit

Even if high sensitive instrument is not available, Lower detection limit still can be achieved by enhancing sample clean up to get a lower baseline.

Another choice is using larger sample loading volume followed by further concentration.

Through more effective sample preparation method to get a lower base line is another choice to get extremely low quantitative limit. Double layer cartridge clean up is effective in some critical compounds analysis. Extremely hydrophobic analytes, such as fatty acid, Vitamin D, their polar is too similar as the endogenous matrix in plasma, so as to very easy to have matrix effect problem.

SLE can extract them out, but a further silica plate tandem with SLE plate is helpful to separate the matrix with the analytes base on their difference retention force on the silica surface.

MAS-M plate which is base on mix-phase bonding material can solve the matrix interference problem for fatty acid analysis in plasma. Reverse phase combined with ion exchange chemical group can retain the non polar analytes with matrix on the surface. Water and neutral organic solution can get rid of the matrix effectively without any loss of analytes. Adjusting the elution solvent pH can finally elute out the fatty acid

10) Solution For Flow Issues

Clogging causes the operators a lot of problems, especially for sticky samples, such as whole blood, plasma and horse urine. For 96 well plate operating, even when one well is clogged, it will affect the whole experiment, and sometimes it is irreparable when sample is limited.

Bonna-Agela provides Plus series for most of SPE products, a pre-filter frit is added to intercept protein to avoid, avoid clogging effectively.

Polymer, C18 SPE plate also filtration plate and PPT plate also have a plus series for sticky sample.

For sticky sample, dilution by water or buffer is also necessary; 2 times dilution is suggested for whole blood sample, and 1 time dilution for plasma and urine samples.

For protein precipitation plate or filtration plate, 0.04Mpa vacuum is suggested to provide for sample operation, while 0.08 Mpa vacuum for most of the SPE plate.

11) Protein Binding Issues

Using H_3PO_4 buffer is helpful to break the binding between analytes and protein in sample.

12) Unstable Analytes

Bonna-Agela provide Biological Cooler instrument, which can be used instead of ice-bath to make sure the temperature sensitive analytes stability.

Applications

Hydrochlorothiazide in Plasma — PPT Method

EAB10002

Experimental Details

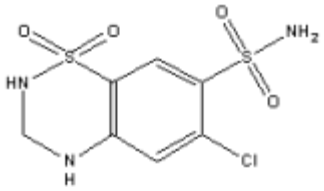


Figure 1 Chemical structure of Hydrochlorothiazide

Sample Preparation

(1) Put Cleanert® PPT and 96-well plate onto the SPE manifold
(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 N2, then resolve with 200 μL mobile phase.

Instrumentation

Instrumentation: LC-MS/MS, API 4000
Column: Venusil® ASB C18, 2.1×150 mm, 5 μm
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

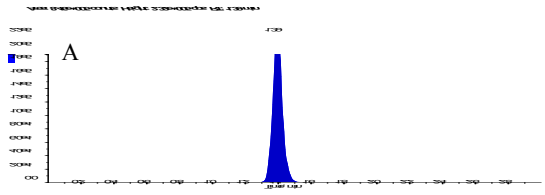


Figure 2 Chromatogram of Hydrochlorothiazide standard solution

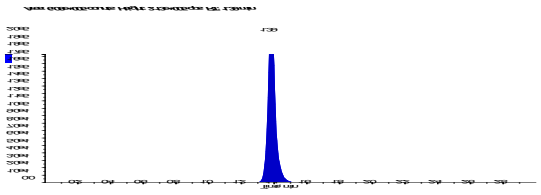


Figure 3 Chromatogram of plasma sample spiked with Hydrochlorothiazide

Table 2 Recovery data of spiked sample

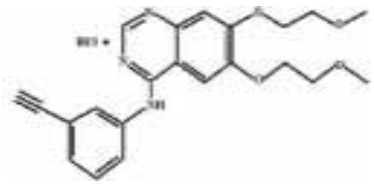
Concentration (ng/mL)	Recovery (%)						Average recovery (%)	RSD (%)
	1	2	3	4	5	6		
20	79.4	75.8	76.5	74.9	75.1	78.4	76.7	2.39

Ordering Information

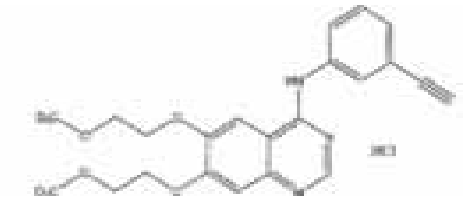
Products	Specification	Cat.No
Cleanert® PPT	2 mL/well	96CD2025-Q
Venusil® ASB C18	2.1×150 mm, 5 μm, 150 Å	VS951502-0
96-well collection plate	2.2 mL Squaral well	96SP2036-2
96-well vacuum manifold	adapt to 96 well plate	VM96
NV-96G for 96 Well Plates	adapt to 96 well plate	NV96-G
Acetonitrile	HPLC, 4 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, 31x6 mm	AV1132-6

Erlotinib Hydrochloride in Plasma — SLE

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® Silica, 3×50 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.

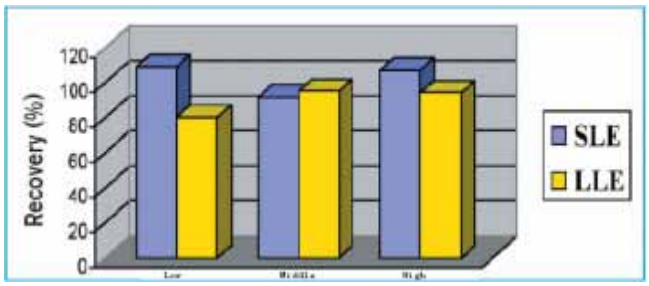


Figure 2 The comparison of recovery between SLE and LLE

(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.

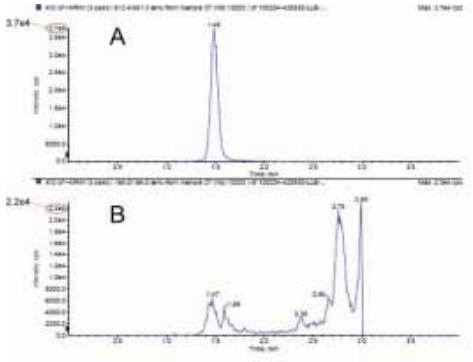


Figure 3 Peak of drug (A) and phospholipids (B) by SLE method

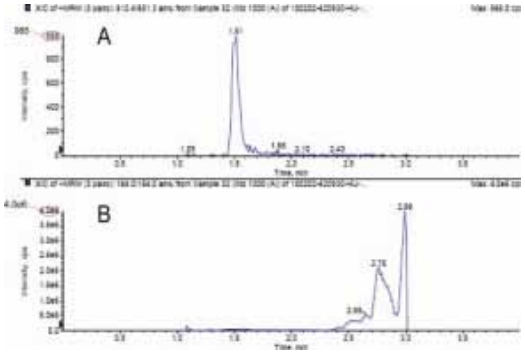


Figure 4 Peak of drug (A) and phospholipids (B) by SPE method

Ordering Information

Products	Specification	Cat.No
Cleanert® SLE plate	200 mg/well	HC2002Q-9W
Venusil® Silica	3×50 mm, 3 μm	VSi930503-0
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
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 — SLE method

EAB10004

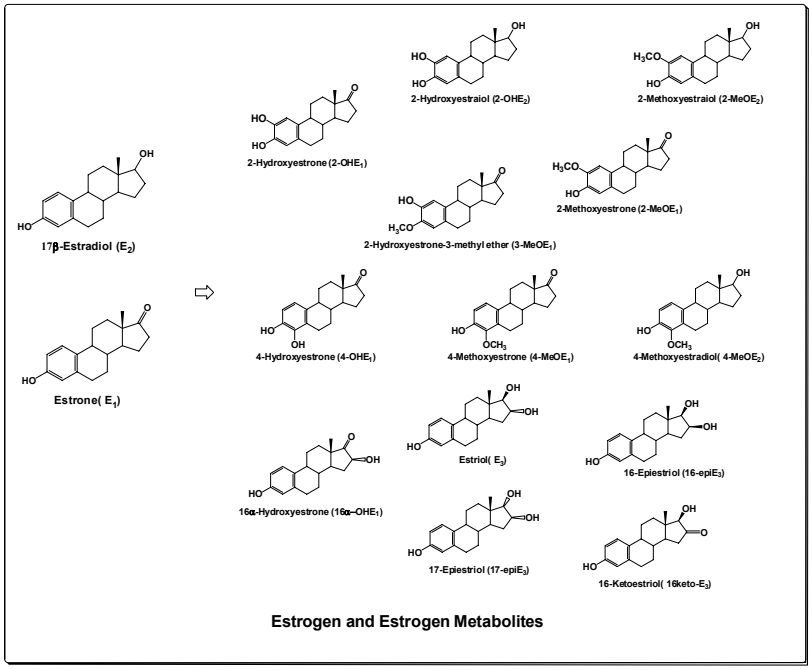
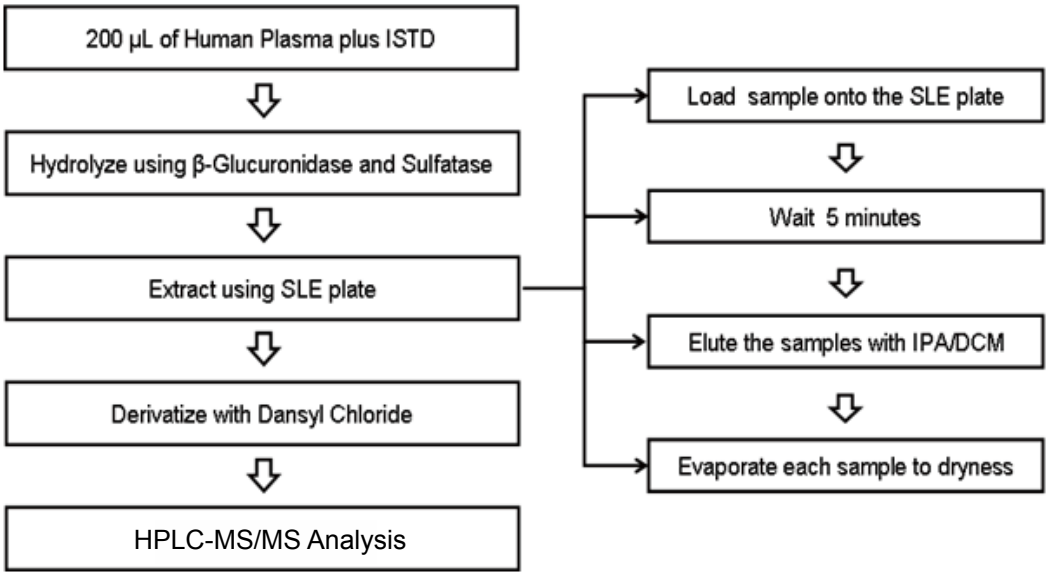


Figure 1 Chemical structure of Estrogens and Estrogens Metabolites

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

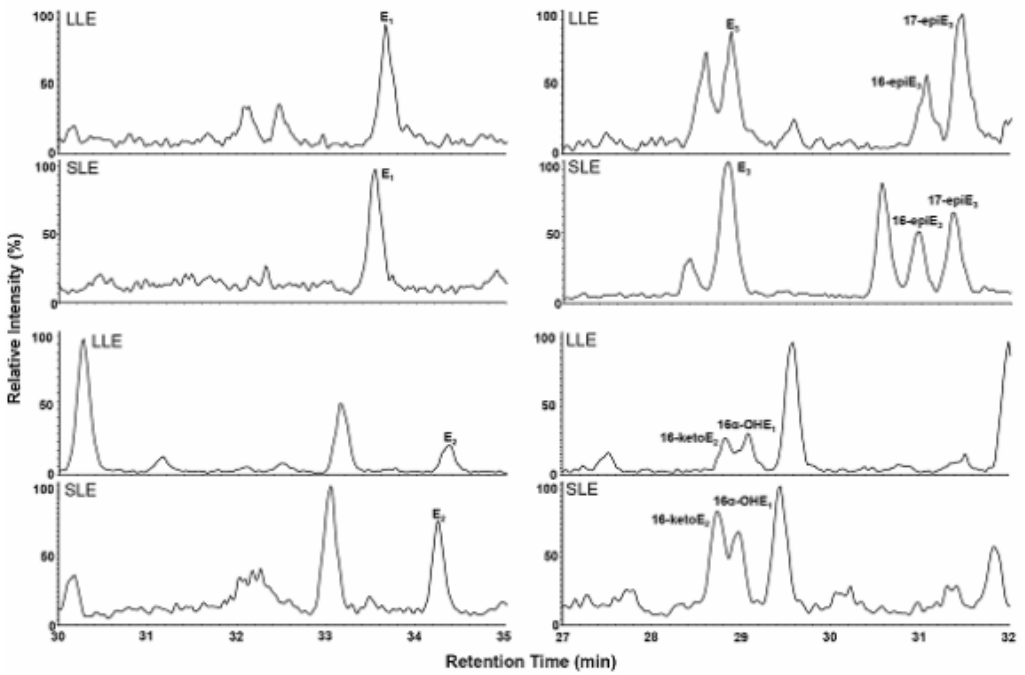


Figure 2 LLE and SLE method comparison at 10 pg/mL (LLOQ)

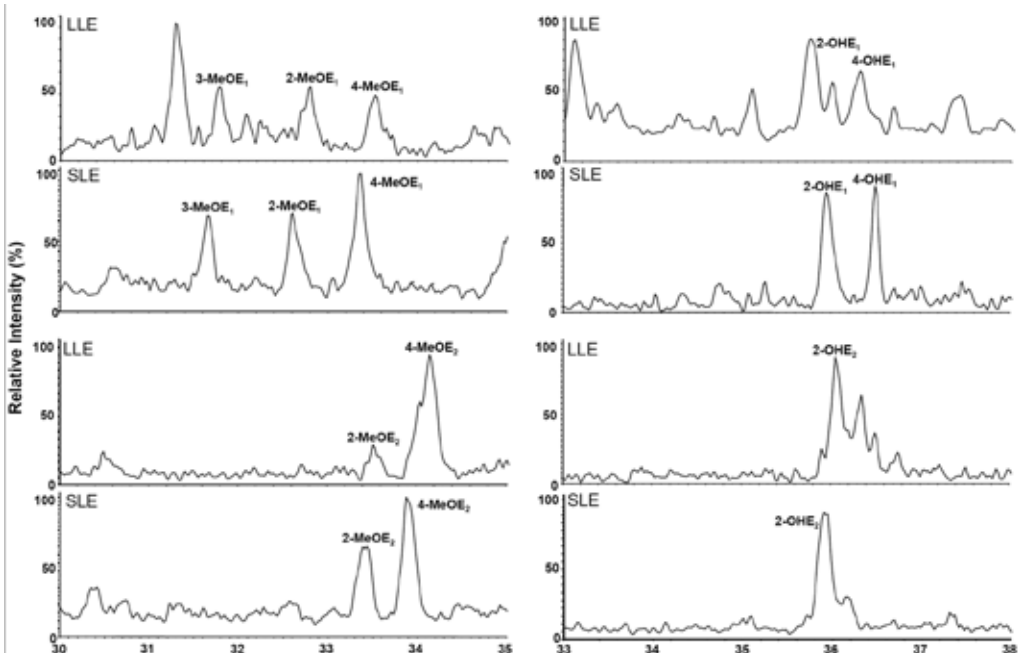


Figure 3 LLE and SLE method comparison at 10 pg/mL (LLOQ).

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	HC3002Q-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 — SLE Method

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.

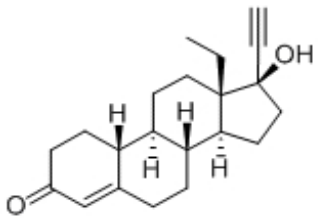


Figure 1 Chemical structure of levonorgestrel

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

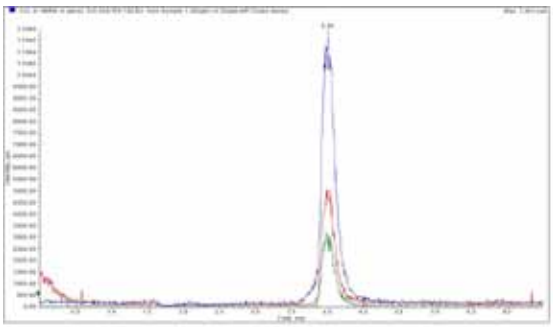


Figure 2 Chromatogram of 20 ng/mL lenovorgestrel standard solution

Comparison of Phospholipids elimination between SLE and LLE

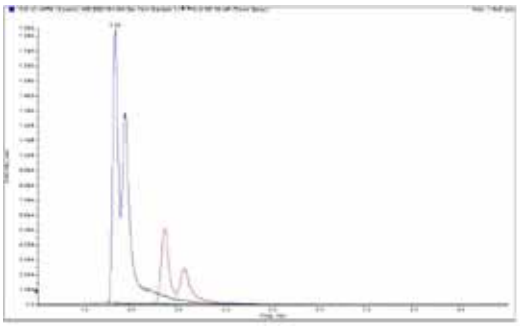


Figure 3 Chromatogram of phospholipids in the sample treated by LLE

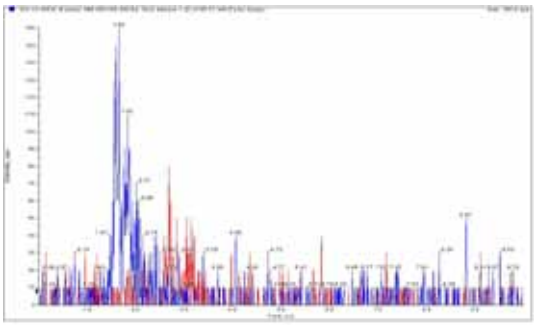


Figure 4 Chromatogram of phospholipids in the sample treated by SLE

(2) Recovery

Table 2 Recoveries of levonorgestrel treated by SLE

Analyte	Concentration (ng/mL)	Sample - % 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	500mg / 3mL / well	HC5003Q-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
Acetonitrile	HPLC, 4 L	AH015-4
Methanol	HPLC, 4 L	AH-230-4
Hexane	HPLC, 4 L	AH216-4
Acetic Ether	HPLC, 4 L	AH100-4
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

Analysis of Telmisartan in Plasma by SLE Pre-treatment and LC-MS/MS

RAB10001

Experimental Details

Sample Preparation

The experiment employed Cleanert® SLE-S 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-S 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.

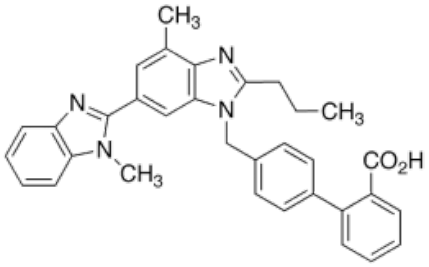


Figure 1 Chemical Structure of Telmisartan

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

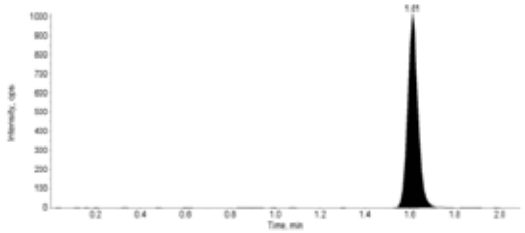


Figure 2 Lower limit of quantization human EDTA plasma sample (2 ng/mL)

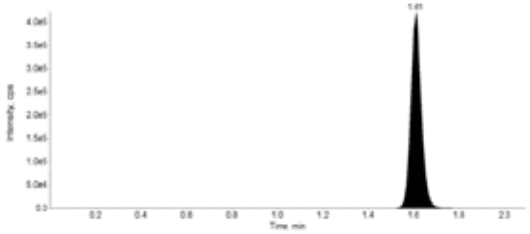


Figure 3 Higher limit of quantitation human EDTA plasma sample (1000 ng/mL)

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	400mg/2mL/well	HC4002Q-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

EAB10005

Experimental Details

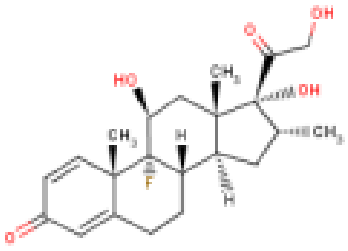


Figure 1 Chemical structure of dexamethasone

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.

(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

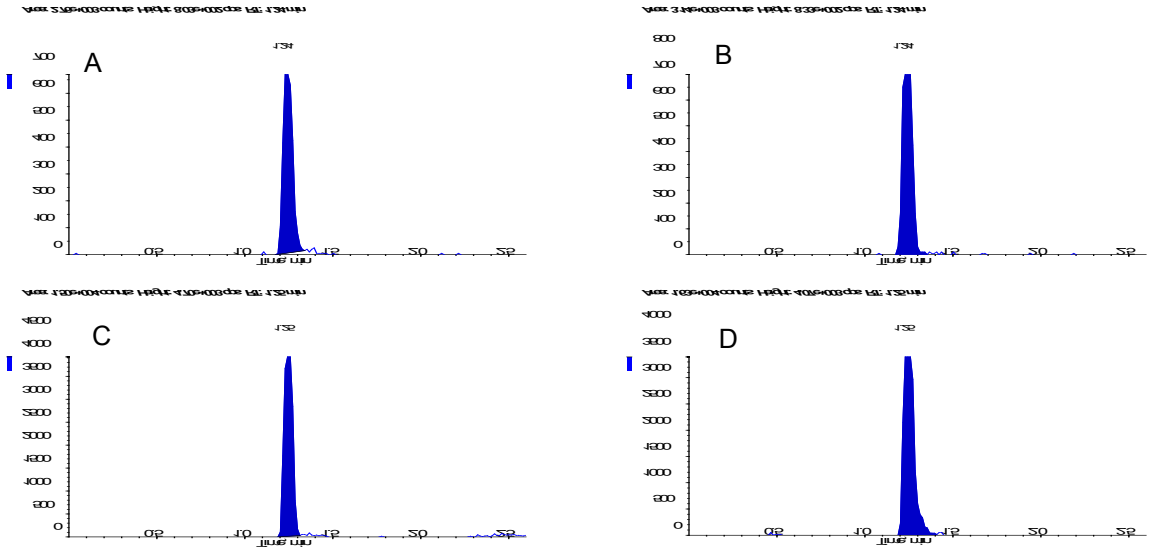


Figure 2 The MRM spectra of sample processed by traditional PPT method (A), Cleanert PPT(B), SLE (C) and MAS (D)

(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

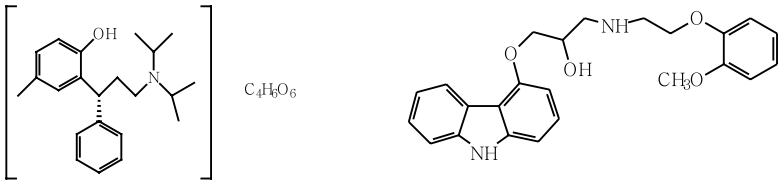
SLE and MAS method perform excellent in phospholipids and protein removing, which raise the signal by 5 times.

Ordering Information

Products	Specification	Cat.No
Cleanert® PPT	2mL / well	96CD2025-Q
Cleanert® SLE plate	200mg / 2mL / well	HC2002Q-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 — MAS Method

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

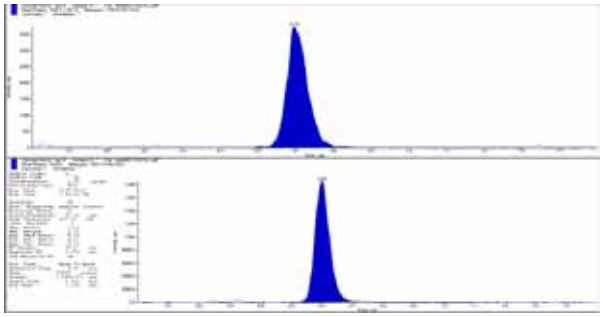


Figure 2 MRM gram for internal standard of tolterodine (A) and carvedilol (B)

(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
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

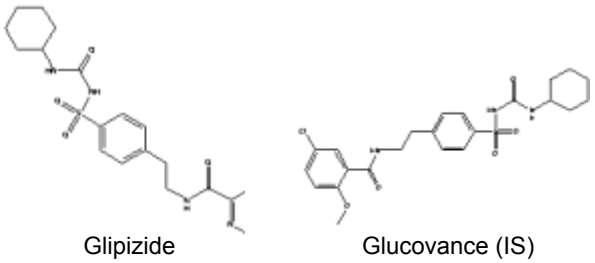
Glipizidein Plasma — MAS Method

RAB10002

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



Glipizide Glucovance (IS)

Figure 1 Chemical structure of glipizidein and glucovance

Instrumentation

Instrumentation: LC-MS/MS, API 5000
Column: Venusil® AQ C18, 2.1×150 mm, 5 µm
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

Compounds	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
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

Comparison of MAS and PPT Method in Propranolol Detection in Plasmas Sample

EAB10007

Experimental Details

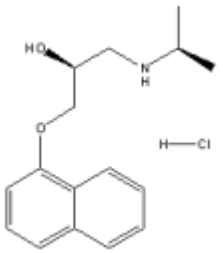


Figure 1 Structure of propranolol

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.

(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

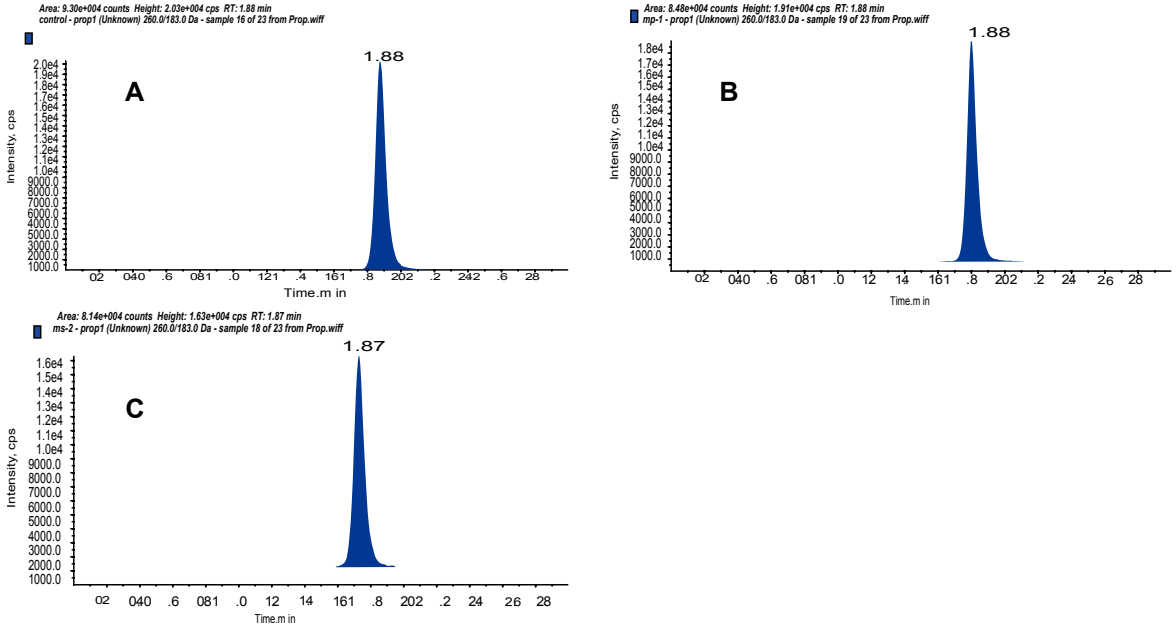


Figure 2 MRM Spectra of Propranolol Standard Solution (A) and Spiked Standard Processed by MAS(B) and PPT(C)

(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
Acetonitrile	HPLC, 4L	AH015-4
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

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.

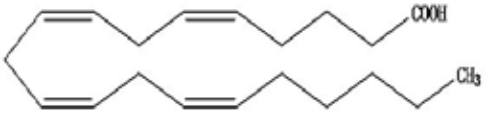


Figure 1 Chemical structure of arachidonic acid

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) 14.83%
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%

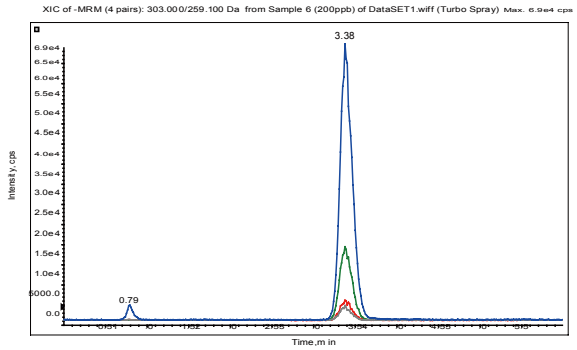


Figure 2 Chromatogram of 1ppm AA standard solution

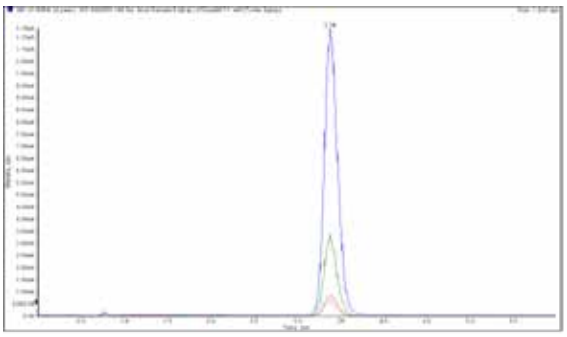


Figure 3 Chromatogram of plasma sample

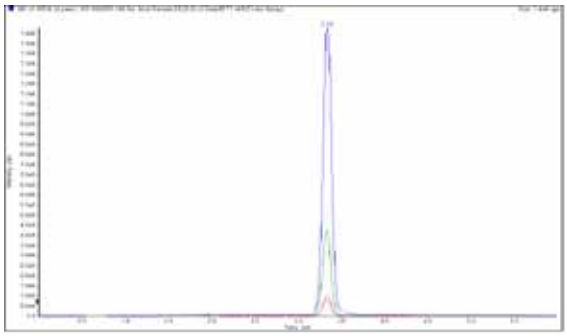


Figure 4 Chromatogram of plasma sample spiked with 1ppm AA standard (Protein precipitation plate)

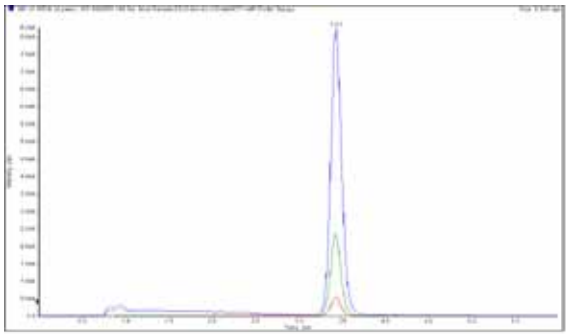


Figure 5 Chromatogram of plasma sample spiked with 1ppm AA standard (Brand W plate)

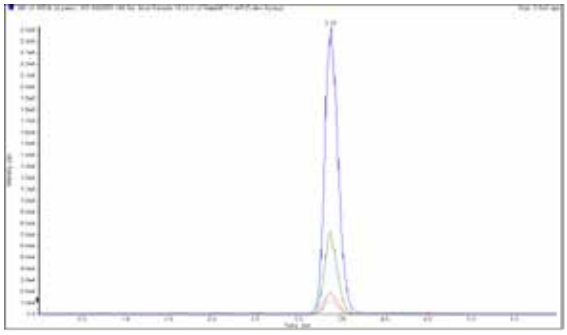


Figure 6 Chromatogram of plasma sample spiked with 1ppm AA standard (Cleanert® MAS-M plate)

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

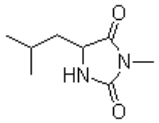
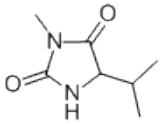
Products	Specification	Cat.No
Cleanert® PPT plate	2mL / well	96CD2025-Q
Cleanert® MAS-M plate	50mg / 2mL / well	MS-M-0502W
Venusil® ASB C18	2.1 mm×150 mm, 3 μm, 150 Å	VS931502-0
96-well collection plate	2.2 mL Squaral well	96SP2036-2
96-well vacuum manifold	adapt to 96well plate	VM96
Acetonitrile	HPLC, 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
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

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.

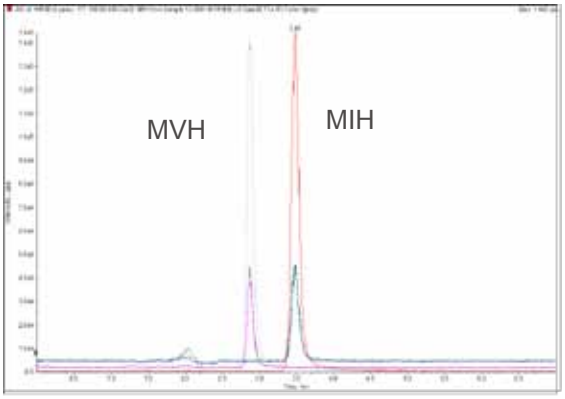


Figure 1 Chromatogram of MVH and MIH (250ng/mL)

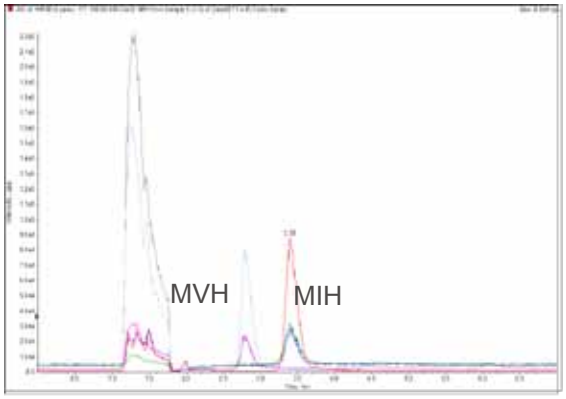


Figure 2 Chromatogram of hemoglobin spiked with 50mg/kg MVH

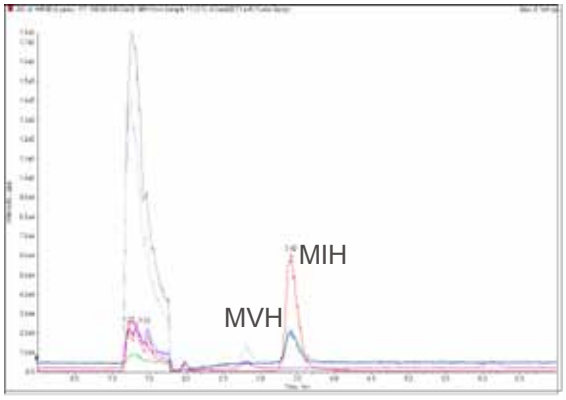


Figure 3 Chromatogram of actual hemoglobin

Table 4 Recoveries and Precisions

Compound	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	30mg/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
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
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

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.

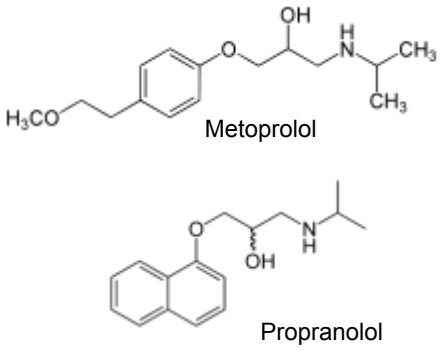


Figure 1 Structures of Metoprolol and Propranolol

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

Analytes	Q1	Q3
Metoprolol	268.3	191.0
Propranolol	260.1	157.1
	260.1	183.1

Result

(1) Chromatogram

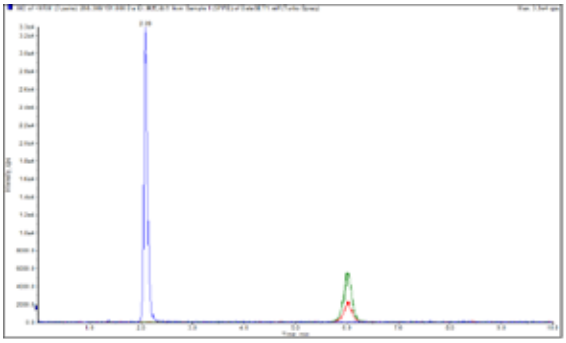


Figure 2 Chromatogram of 5ppb Metoprolol and Propranolol

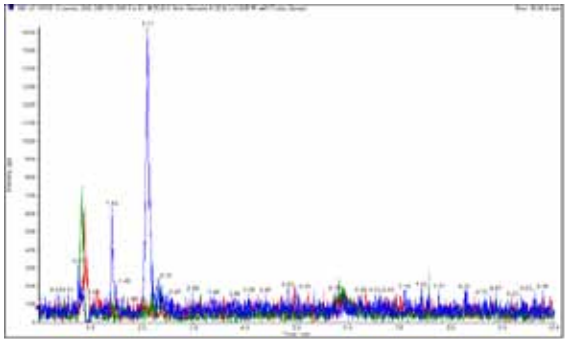


Figure 3 Chromatogram of blank sample

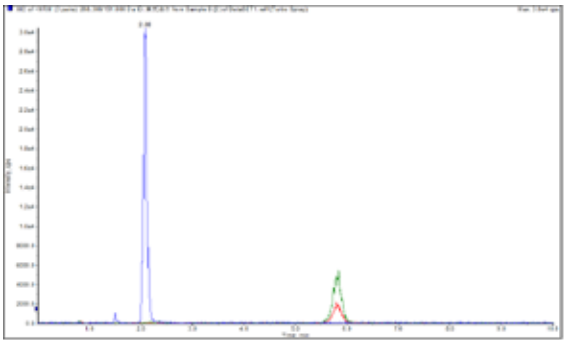


Figure 4 Chromatogram of spiked plasma sample with 5ppb Metoprolol and Propranolol

(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)	Sample - % 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
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,31x6mm	AV1132-6

Extraction of Indomethacin and Ibuprofen from Small Volume Biological Fluid Samples Using a New Versatile µElution SPE 96-well Plate Format

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.

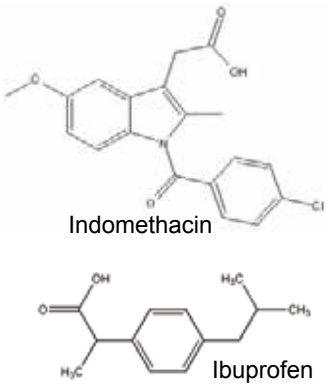


Figure 1 Chemical structure of Indomethacin and Ibuprofen

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

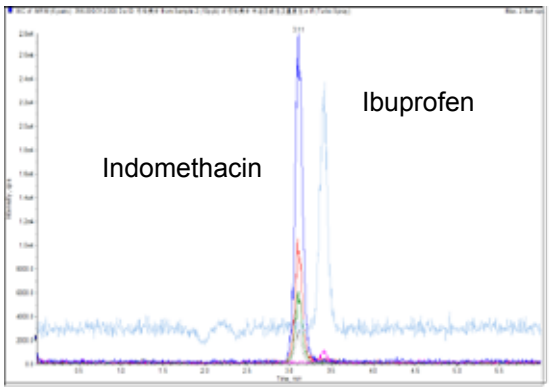


Figure 1 Chromatogram of spiked plasma sample with 10 ng/mL Indomethacin and Ibuprofen

(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-1	Sample - % 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

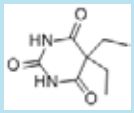
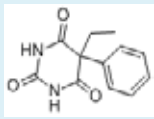
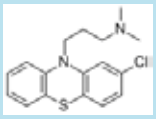
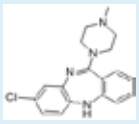
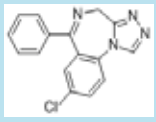
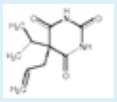
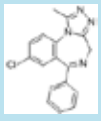
Products	Specification	Cat.No
Cleanert® PEP Micro Plate	5mg/well	PE00501-MW
Venusil® XBP C18	2.1 mm×150 mm, 5 μm, 120 Å	VX951502-A
96-well collection plate	2.2 mL Squaral well	96SP2036-2
96-well vacuum manifold	adapt to 96well plate	VM96
Acetonitrile	HPLC, 4 L	AH015-4
1.5 mL vials	1.5 mL short thread 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
Filtration membrane(Nylon)	φ13; 0.22 μm	AS021320-T
Disposable Syringe	2 mL, needless	ZSQ-2ML

Hypnotic Drugs Detection in Blood Sample

EAB10008

This experiment used Qdaura® SPE-40 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	Structure	Formula	Molecular Weight	CAS Number
Barbital		$C_8H_{12}N_2O_3$	184.19	57-44-3
Phenobarbital		$C_{12}H_{12}N_2O_3$	232.24	50-06-6
Chlorpromazine		$C_{17}H_{19}ClN_2S$	318.86	50-53-3
Clozapine		$C_{18}H_{19}ClN_4$	326.82	5786-21-0
Estazolam		$C_{16}H_{11}ClN_4$	294.74	29975-16-4
Aprobarbital		$C_{10}H_{14}N_2O_3$	210.23	77-02-1
Alprazolam		$C_{17}H_{13}ClN_4$	308.76	28981-97-7

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® SPE-40 (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

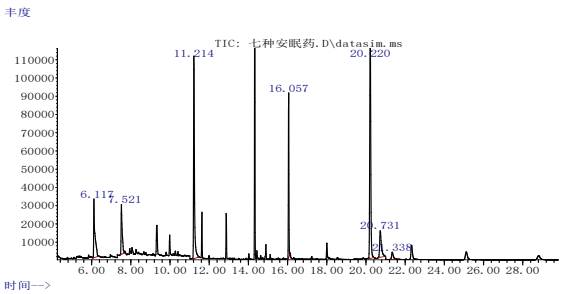


Figure 1 Chromatogram of hypnotic drugs standard solution

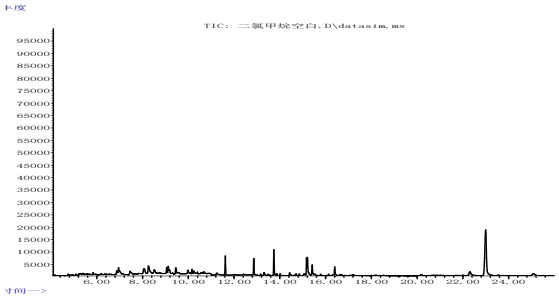


Figure 2 Chromatogram of blood sample

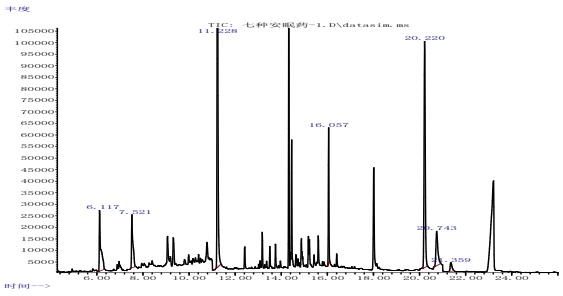


Figure 3 Chromatogram of hypnotic drugs spiked to blood sample

(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® SPE-40 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.

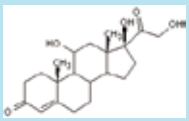
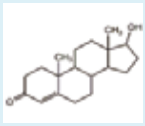
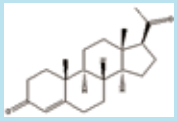
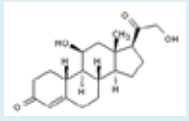
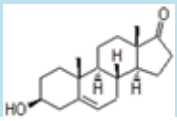
Ordering Information

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
Methanol	HPLC, 4 L	AH-230-4
dichloromethane	HPLC, 4 L	AH300-4
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
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

RAB10007

Table 1 Information of the analytes

Compounds	Chemical Structure	CAS Number
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	t_R/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

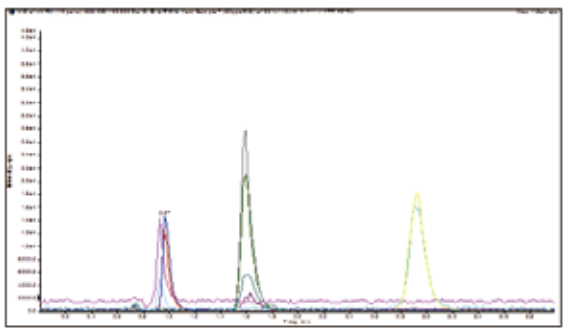


Figure 1 Chromatogram of 30 ppb Steroid hormones standard solution

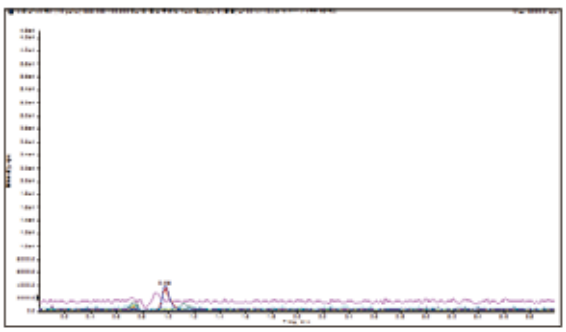


Figure 2 Chromatogram of serum sample

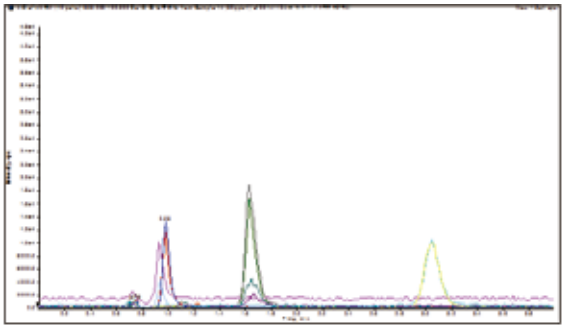


Figure 3 Chromatogram of Serum sample spiked with 30 ppb Steroid hormones standard solution

(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%

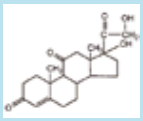
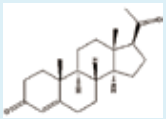
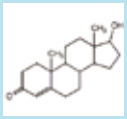
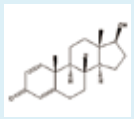
Ordering Information

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
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, 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

RAB10008

Table 1 Information of the analytes

Compounds	Chemical Structure	CAS Number
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 (200mg / 3mL) 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	t_R/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	2.62	405.3	359
		405.3	328.8
		405.3	300.9

Result

(1) Chromatogram

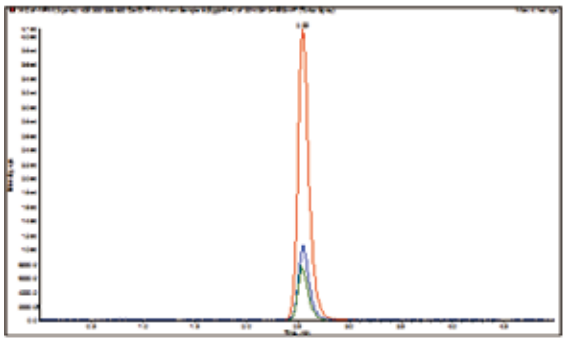


Figure 1 Chromatogram of Cortisone (5 ng/mL)

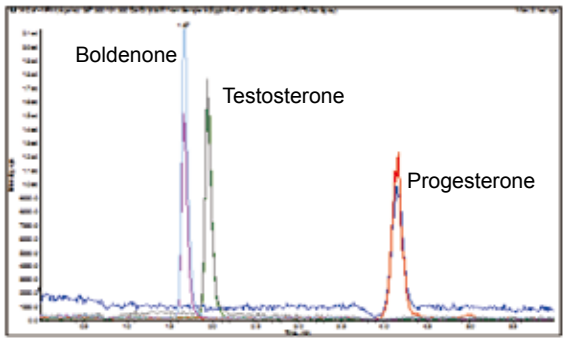


Figure 2 Chromatogram of boldenone, testosterone and progesterone (5 ng/mL)

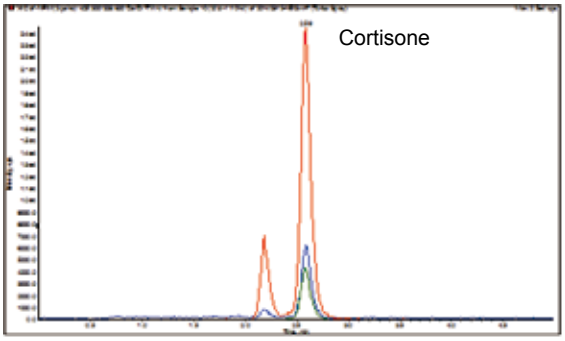


Figure 3 Chromatogram of actual serum sample (negative mode)

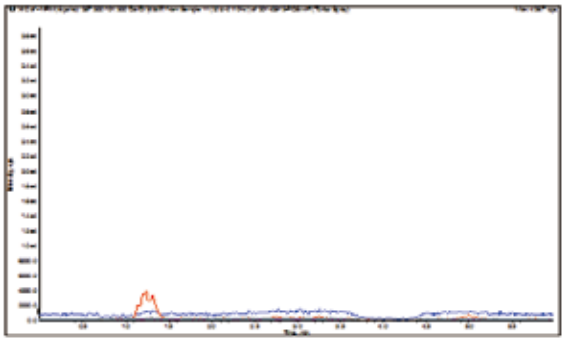


Figure 4 Chromatogram of actual serum sample (positive mode)

(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

Compounds	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	200mg / 3mL / well, PH=9	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
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, 31×6 mm	AV1132-6
Filtration membrane(PTFE)	φ13; 0.22 μm	AS041320-T
Disposable Syringe	2 mL,needless	ZSQ-2ML

Cleanert® MAS-M for the Extraction of Arachidonic Acid from Faeces Sample

RAB10009

Experimental Details

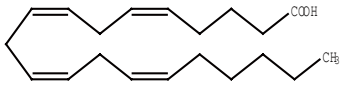


Figure 1 Chemical structure of arachidonic acid

Standard solution

Arachidonic acid (AA) was chose to represent fatty acid.
Stock solution was prepared by dissolved 10 mg of AA in 100 mL of methanol. The stock solution was diluted with a mixture of acetonitrile:water (70:30,v/v) to obtain work solution.

Sample pre-treament

50mg of faeces sample was added to centrifuge tube, then mixed by 0.5 mL 1% formic acid in water and vortexed for 2 min, added 1.5 mL methanol into the tube, ultrasonic for 10min, and centrifuged under 6000r/min for 5 min. The supernatant was collected into a clean vial. Added 1.5 mL methanol into the tube and repeat the extraction process. Combined supernatants and filtrated by PTFE filter (0.22 μm), the filtrate was collected and then mixed with 0.6mL ammonium hydroxide.

Sample Preparation

The pre-treatment sample was loaded into each well of Cleanert® MAS-M plate which was conditioned with 600 μL methanol and 600 μL water sequentially. The plate was washed with 600 μL of water followed by 600 μL of methanol. The target compounds were eluted with 600 μL of 3% formic acid in acetonitrile. The eluates were concentrated at 45℃ under a gentle stream of nitrogen to dryness. The residues were reconstituted with 100 μL of acetonitrile:water (70:30,v/v) separately for further analyses.

Instrumentation

Instrumentation: LC-MS/MS, API 4000+
HPLC Column: Venusil® ASB C18, 2.1×50 mm, 3 μm,150 Å
Mobile Phase: Acetonitrile:Water = 70:30(v/v)
Flow rate: 0.2 mL/min
Injection: 5 μL
LC Instrument: Shimadzu LC-20A
Ion source: ESI - Negative
Scan mode: MRM

Table 1 MS/MS transitions and Retention time of AA

Analyte	t_R/min	Q1	Q3
AA	3.3	303.0	259.1
			230.9
			205.1
			177.1

Result

(1) Chromatogram

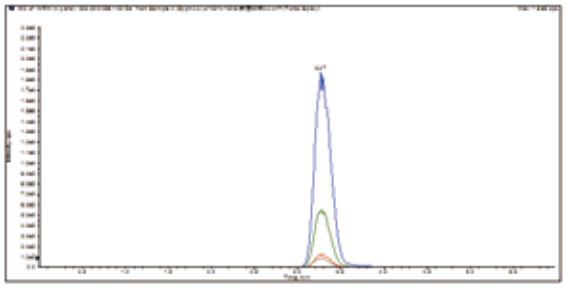


Figure 1 Chromatogram of 5 μg/mL AA standard

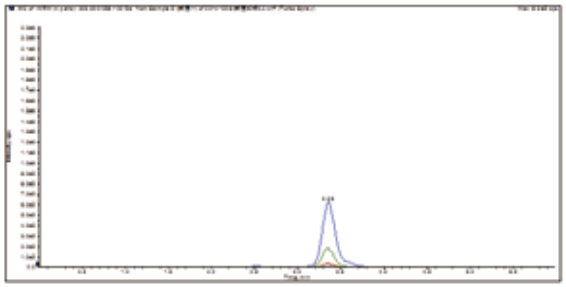


Figure 2 Chromatogram of AA in faeces sample

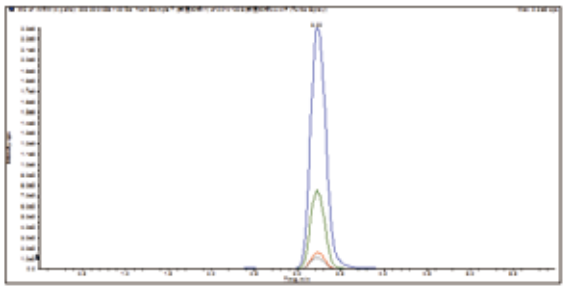


Figure 3 Chromatogram of faeces sample spiked with 5 μg/mL AA standard

(2) Recovery data

There was approximately 3 μg/g of AA in the mouse faeces in the experiment. Background should be subtracted from the response of AA in spiked samples to calculate the recovery data.

Table 3 Recovery data

Spiked concentration	AA
10μg/g	88.9%

Ordering Information

Products	Specification	Cat.No
Cleanert® MAS-M plate	50mg / 2mL / well	MS-M-0502W
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
Acetonitrile	HPLC, 4 L	AH015-4
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Filtration membrane(PTFE)	φ13; 0.22 μm	AS041320-T
Disposable Syringe	2 mL,needless	ZSQ-2ML

Appendix

Cross Reference

Agela	Waters	Biotage	Aglient	Supelco	Phenomenex
Cleanert® Protein Precipitation Plate	Sirocco™ Protein Precipitation Plate	ISOLUTE® PPT+	Captiva ND lipids Protein Precipitation Plate	96-Well Protein Precipitation Filter Plate	STRATA IMPACT Plate
Cleanert® FAST series	None	MULTI 96 filters	Captiva series	None	None
Cleanert® SLE series	None	IsoluTE® SLE+	ChemElut series	None	None
Cleanert® Polymer 96 wellplate	Oasis 96-well Plate	EVOLUTE series	Bond Elute Plexa series	Discovery® SPE 96-Well Plates	Strata series
Cleanert® micro 96 wellplate	96-well µElution Plate	None	None	None	None
Cleanert® MAS-C 96 wellplate	Ostro Sample Preparation Products	None	None	HybridSPE Phospholipid	Iphree Phospholipid Removal Plates

Major HPLC Columns for LC/MS Applications, Unique Features, Benefits and Advantages

Bonna-Agela Products	Features	Benefits	Advantages	Comparison
ASB C18, C8, Ph, C4, C1 unique	Combine bulky protection on silane and special treatment for low acidity silanol; non-end cap	Extremely low bleed and high sensitivity, more significant on short chain; may retain very polar analytes	In formic acid buffered mobile phase, clearly better peak shape and consistency compared to Zorbio-analysisx SB	Zobio-analysisx SB family
Unisol C18 (2)	mid-polar; medium surface area, special surface treatment and special end cap	Good polar retention; bio-analysislanced separation power with lifetime, contamination resistance and consistency	Better peak shape and consistency than Phenomenex Polar RP and YMC AQ; similar to Waters T3	Phenomenex Polar RP; YMC-AQ; Waters Atlantis T3
Durashell C18 (2)	Less polar, hybrid surface	Broad pH tolerance; very inert surface; contamination tolerance, long lifetime	Similar to or better than Phenomenex Gemini for some applications	Phenomenex Gemini; Waters X-bridge
Durashell AQ C18 unique	Special end cap to create AQ C18 on hybrid surface	Enhanced polar retention and long lifetime at broad pH	Better lifetime than other AQ columns at pH>7	Phenomenex Polar RP, Waters Atlantis T3
Durashell Amide and amine unique	First HILIC columns on hybrid surface	low bleed , better consistency and long lifetime	Lower bleed, and longer lifetime than other HILIC columns	Compared to most of HILIC columns
RAM RP unique	Co-phases of C18 and PEG	Unique separation pattern; contamination resistant	Better separation of analytes from bio-matrix	Regular C18, AQ C18

Some Unique Columns for Pharmaceutical QA/QC and Preparative Applications

Bonna-Agela Products	Features	Benefits	Advantages	Applications
Mixed C18/ amide unique	Combination of RP and HILIC	Unique and predictable selectivity; broad range of polar and non-polar in one sample	Easier and predictable separation improvement; analyse non-polar compounds and lower metabolites in one run	Complex mixture sample, impurity profiling; metabolite studies
Venusil® AQ-C18 unique	Large surface, large number of low acidity silianol residue	Extremely powerful separation of steric isomers	Dual mechanism and better steric recognition	EP method for prednisolone, other steroid isomers;
Durashell AM Unique	Unique bonding and end caping on hybrid surface	Better separation, narrow and symmetric peak when overload	Better impurity detection limit; better lifetime at pH>7	Many antibiotics; complex samples low level impurity profiling
Mix RP unique	Mixed bonding of long and short chain silane	Better selectivity by shape; higher load ability	Better separation and purification of peptide	Organic isomer purification

SPE Media and Other Material Products for LC-MS Application in Bio-analysis and Clinical Diagnostics

Bonna-Agela Products	Features	Benefits	Advantages	Applications
PEP-2 unique	Combination of amide and urea	No need to adjust pH of sample and rinsing solvents	Simpler and more consistent than Waters Oasis HLB	Biological sample clean up
PP/Phosphlipid remover unique	Only polymer bio-analysissed on the market	More consistent, no worries on irreversible adsorption	More consistent and predictable than zirconia	Protein precipitation combined with lipid removal
Hallow fiber Very unique	Hallow fiber in spin tube and 96 well-plate format	Purely remove interference by molecule size; good for very polar and neutral, multi-analytes in one run	Cleaner than protein precipitation; consistent and convenient;	Remove protein and other large molecule from biological samples
AQ-C18 unique	Narrow pore size and pore distribution; silica bio-analysisse AQ-C18	Less dryness effects; low bleed/less interference on trace analysis; cleaner	Cleaner sample than polymer bio-analysisse at trace level	Any bio-analysis for low to mid-polar analytes
PEP/PCX/PWCX/ PAX/PWAX	Equivalent to Waters Oasis family	Using special top frits to avoid clogging by nano particles	Tightly control on pore size to get cleaner sample	Biological sample clean up
SLE unique	Equivalent to Biotage , with hydrophilic top frits	More convenient with more choices; cleaner than Liquid-Liquid Extraction	More choices on pH, size better flow characteristic than	Replace L/ L extraction to increase productivity and reduce phospherlipid
SLE highly inert unique	Specially deactivated	Reduce the chelating effect; improve recovery of multifunctional analytes	Biotage Better recovery of multi-hydroxy, multi-amide	Bio-analysis of multi-hydroxycontaining analytes

Bonna-Agela Products	Features	Benefits	Advantages	Applications
Modular Micro plates unique	Equivalent to Waters u-elution plate; with modular design	More convenient; No need of dry-down compared to regular SPE	More convenient than Waters for method development and correct failed wells	Biological sample clean-up; low volume sample or to avoid dry-down step
Modular Deep plate unique	3.3 mL volume per well; modular design	For large volume size (needed for very dilute sample)	70% larger than regular plate; more convenient as modular Can use either	Large volume biological sample cleanup and enrichment
PPT plates	Equivalent to Agilent PPT plates	Low cost, fast and simple	vacuum, centrifuge or positive pressure; can hold ACN	Remove protein from biological sample

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