Agela Technologies

Bonna-Agela

Sample Preparation Products

Official Website

Best Value
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Innovation to Benefit Customers

Sample Preparation

Better Solutions for Chromatography

Chemical Analysis

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Bonna-Agela Technologies — A Global Supplier for Chromatography Solutions

Bonna-Agela, is dedicated to improve the future in Chromatography Science & to assist scientists worldwide in achieving their scientific goals by using chromatography products from Bonna-Aegla used in scientific discovery and analysis. Benefiting from years of research and product development, we are industry specialist in silica and polymer bonding technologies by which we produce solution in field of Sample Preparation, HPLC Columns and Preparative Purifications. We also cater Instrumentation for Sample Preparation and Purification for scientists interested in Atomization of their analysis processes.

Our core objective is to provide total solutions to Pharmaceuticals, Bioanalysis, Food Safety, Environmental & Clinical applications and scientist involved in these fields.

With our customers and industry, we earned reputation for innovative expertise, uncompromised quality maintain system, and comprehensive solution tailored to customer requirements from a decade. The very result of this is we have developed trust and faith among our customers and we are progressive towards developing new solutions for our customers.

Our main objective is to give our customers utmost importance and quick response.

How to Place Orders

USA office is open from 9:00 am to 5:00 pm Pacific Standard Time, Monday through Friday.
To place an order or receive a quote, you may choose from the following contacts:

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Sample Preparation Products

Introduction

Solid phase extraction (SPE) is one of the most common techniques in sample preparation. It is replacing the conventional time-consuming liquid-liquid extraction (LLE) method. The process of SPE is a miniature version of High Pressure Liquid Chromatography. SPE, utilizing the same type of stationary phases as used in liquid chromatography columns, could be practiced in different mechanisms including normal phase, reversed phase, ion-exchange, affinity chromatography and mixed-mode in the fields of agricultural and food, bioanalytical, environmental and forensic applications.

Cleanert® SPE Cartridges, Well Plates and Media

Filtration Plate

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

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

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

Common Filters

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

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

MAS (Multi-function Impurity Adsorption SPE)

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

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

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

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

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

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

Traditional line:

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

Bonded Silica SPE Cartridges

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

Traditional line:

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

Unique: AQ-C18, HILIC (Amide)

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

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

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

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

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

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

SPE Product

Bonded Silica Gel	Polymer	Non-silica Adsorptive Material	Mixed Material	Specialized SPE Cartridge	Clean-up Cartridges for IC	Cleanert® 96 Well Plate Series
AQ C18,C18, C18-N,C8, NH$_2$,COOH, Silica,PSA, PRS,SCX, SAX,Diol	PEP,PEP-2, HXN,PS,PAX, PCX,PWAX, PWCX	Florisil, PestiCarb, Alumina-N, Alumina-A, Alumina-B	C8/SCX, PestiCarb/NH$_2$, PestiCarb/PSA, SAX/PSA	SUL-5,HXN, TPT,SLE, DNPH-Silica, EPH,MCPD, Bap,PAE, DEHP,LDC	IC-RP,IC-P, IC-H,IC-Na, IC-Ag,IC-Ba, IC-A,IC-M, IC-Ag/H, IC-Ag/Na, IC-BaAgH, IC-BaAgNa	Protein Precipitation Plate, Filtration Plate, Micro Plate, Deep well detachable Plate, MAS Plate (phospholipid removal)

Product Format Classification

1. SPE Cartridges

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


SPE cartridge

2. 96 Well Plates

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


96 well plate 2 mL


96-well plate 1 mL

3. Micro Plate Series

Micro plate allows a small amount of sample loading and elution.
It was constituted by base plate and individual coose cartridges, and they allow different SPE materials in one plate, which are suitable for method development.

Cleanert® micro plate

4. Deep Well Detachable Plate

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

Deep well detachable plate

5. Clean-up Cartridges for Ion-chromatography Application

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

Cleanert® IC cartridges

6. MAS-QuEChERS

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

Cleanert® MAS -QuEChERS

7. Bulk Media

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

Bulk media

8. Cartridge Format

General cartridge

Flangeless cartridge

LDC cartridge

LRC cartridge

Glass cartridge

Featured Products

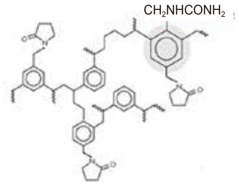
OMM Technology (Optimized Molecular Modification) for SPE

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

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

Cleanert® PEP-2

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



Particle Characteristics

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

Suggestion Processing Method

Neutral and Basic Compounds (metoprolol)

Condition: MeOH or ACN; Water

Sample Loading: Diluted biological sample

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

Elution: MeOH or ACN

Acidic Compounds (salicylic acid)

Condition: MeOH or ACN; Water

Sample Loading: Diluted biological sample

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

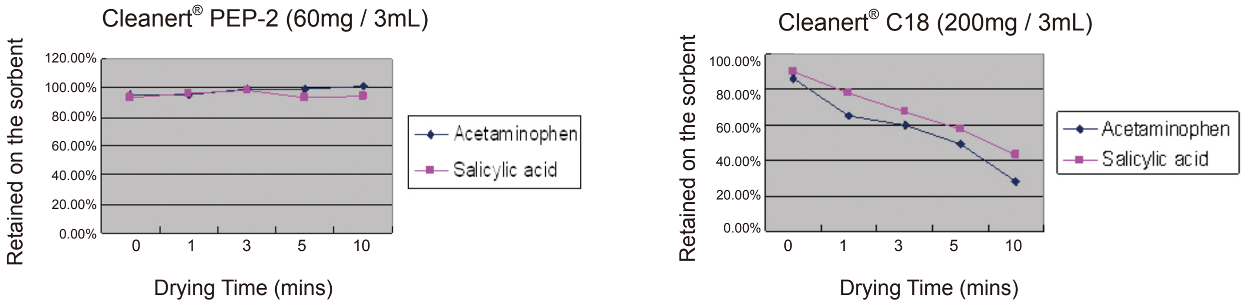
MeOH or ACN (For PEP-2, add some acid in the solvent will improve the recoveries for acid compounds.)

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

Good Water-Wettability

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

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



High Retention for Polar Compounds and Acid Compounds

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

The recoveries of three compounds on different SPE materials

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

* No pH adjustment for sample loading solution

Ordering Information

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

Cleanert® PEP

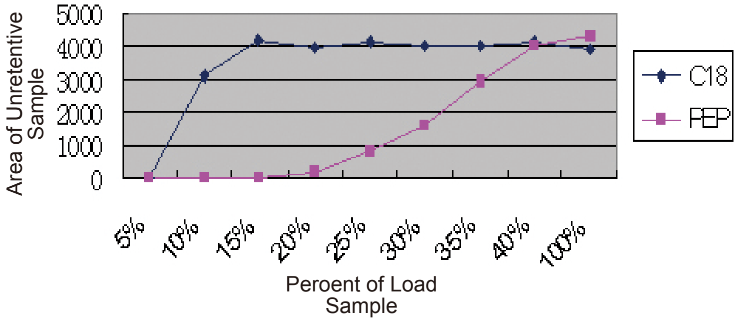
Cleanert® PEP is made of polydivinylbenzene on which the surface is functionalized with vinyl pyrrolidone. The material has a balanced hydrophilic and hydrophobic property and can be used in the entire pH range of 1-14.

PEP can be used to extract a variety of polar and non-polar compounds. Some highly hydrophilic compounds which have little retention on C18 columns, such as chlorinated phenols, phosphate esters and drug metabolites, can be effectively retained on PEP.

High Capacity

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

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

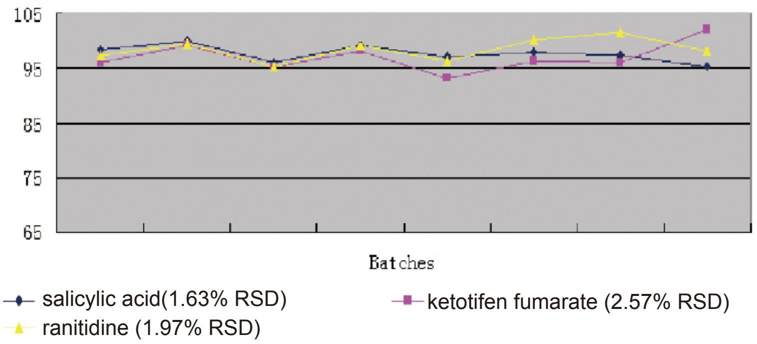


Excellent Batch-to-Batch Reproducibility

The OMM technology offers consistent surface modification, resulting in excellent batch-to-batch reproducibility.

Multiple batches of Cleanert® PEP series have been successfully used for various compounds with consistent results.

Batch to batch reproducibility of PEP

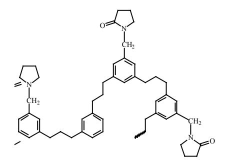


Particle Characteristics

Functionalized polymer sorbents; Average particle size: 40-60 μm.

Average pore size: 70 Å; Specific surface area: 600 m^2/g.

Ordering Information

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

Cleanert® PAX (RP/Strong Anion Exchange)

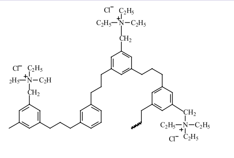
Description

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

Particle Characteristics

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

Ordering Information

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

Cleanert® PWAX (RP/Weak Anion Exchange)

Description

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

Particle Characteristics

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

Suggested processing Method for PAX and PWAX

Condition with Methanol and then Water

Sample loading (adjust pH with acid solution)

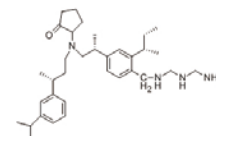
Wash with 2% $NH_3 \cdot H_2O$ in water

Wash with methanol

Elution with 2% formic acid in methanol

Evaporation and reconstitution

Ordering Information

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

Cleanert® PCX (RP/Strong Cation Exchange)

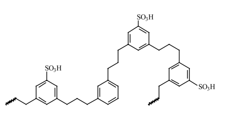
Description

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

Particle Characteristics

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

Ordering Information

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

Cleanert® PWCX (RP/Weak Cation Exchange)

Description

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

Particle Characteristics

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

Suggested processing Method for PCX and PWCX

Condition with Methanol and then Water

Sample loading (adjust pH with basic solution)

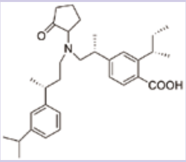
Wash with 2% formic acid in water

Wash with methanol

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

Evaporation and reconstitution

Ordering Information

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

Cleanert® PS

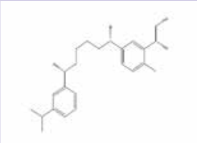
Description

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

Particle Characteristics

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

Ordering Information

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

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

Description

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

Particle Characteristics

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

Ordering Information

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

Cleanert® Micro Plate

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

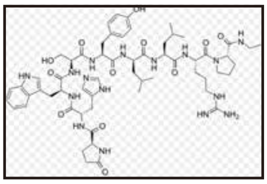
The micro plate optimizes the configuration of the sorbents (PEP-2, PEP, PCX, PAX, PWCX, and PWAX), packed with 30 μm particle size sorbent to enhance the efficiency of column.

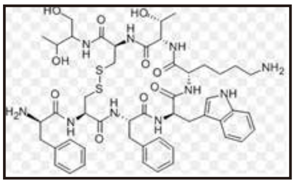
Cleanert® micro plate allows low-elution volume with sensitive, robust, and reproducible results without time-consuming evaporation and reconstitution step.

Innovative Features

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

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


Leuprolide


Octreotide

Consumables

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

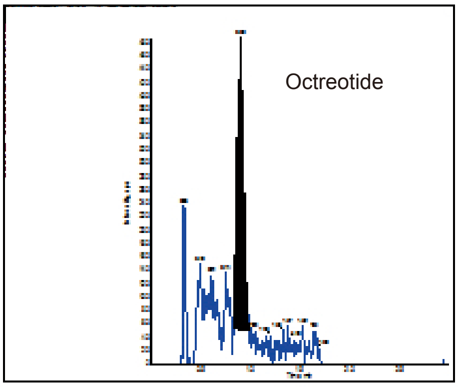
SPE processing method

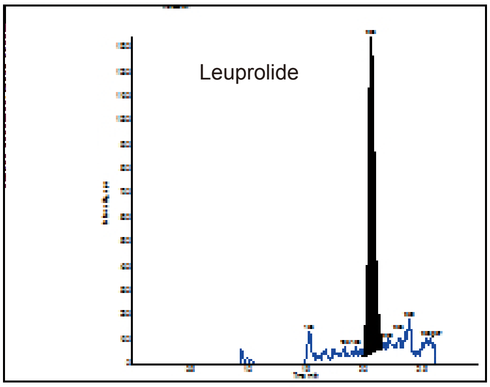
Condition	Condition the plate with 0.4 mL of 5% HCOOH in methanol ; 0.4 mL of methanol; 0.4 mL of water one by one (apply vacuum to each step).
Sample loading	Combine 100 µL of serum sample, 30 µL of IS-D , 200 µL of 100 mM ammonium acetate buffer together. And transfer the mixture to the preconditioned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

Recoveries of Leuprolide and Octreolide

Recovery		Leuprolide Peak Area				Octreotide Peak Area			
		QCL		QCH		QCL		QCH	
Bonna-Agela PWCX micro plate	Mean	2240	4339	310486	703048	4085	5420	378178	593745
	SD	313	230	19215	4678	245	535	4342	3294
	%CV	13.95	5.30	6.19	0.67	11.75	10.67	1.56	0.63
	n	3	2	3	2	3	2	3	2
	Recovery %	51.61		44.16		72.53		63.11	

Chromatograms of serum samples spiked with Octreotide and Leuprolide





Detection of Metoprolol and Propranolol in Plasma using Micro Plate

Sample pretreatment

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

Condition: 200 μL of methanol and 200 μL of water

Sample Loading: 100 μL of plasma

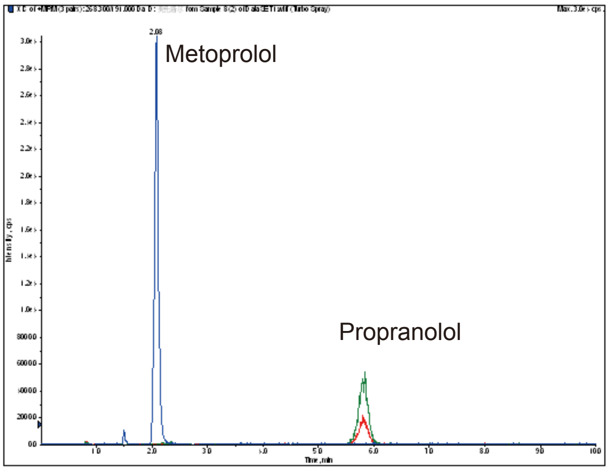
Wash: 200 μL of 5% methanol water solution

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

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

HPLC condition

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



Chromatogram of spiked plasma sample with 5 ppb metoprolol and propranolol

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

Ordering information

Product	Specification	Loose cartridge (100/PK)	Assembled plate (1/PK)
Cleanert® PEP-2	5mg / 1mL / well	PE00501-2LMW	PE00501-2MW
	10mg / 1mL / well	PE0101-2LMW	PE0101-2MW
Cleanert® PEP	5mg / 1mL / well	PE00501-LMW	PE00501-MW
	10mg / 1mL / well	PE0101-LMW	PE0101-MW
Cleanert® PAX	5mg / 1mL / well	AX00501-LMW	AX00501-MW
	10mg / 1mL / well	AX0101-LMW	AX0101-MW
Cleanert® PCX	5mg / 1mL / well	CX00501-LMW	CX00501-MW
	10mg / 1mL / well	CX0101-LMW	CX0101-MW
Cleanert® PWAX	5mg / 1mL / well	WA00501-LMW	WA00501-MW
	10mg / 1mL / well	WA0101-LMW	WA0101-MW
Cleanert® PWCX	5mg / 1mL / well	WC00501-LMW	WC00501-MW
	10mg / 1mL / well	WC0101-LMW	WC0101-MW
Base plate	/	/	96WKB
Removing Tool	/	/	RM01

Cleanert® PPT Protein Precipitation Plate

Protein precipitation is often used as a fast sample preparation method in bioanaylsis. Protein denaturation with acetonitrile in a centrifuge tube is the most used method which is laborious, time consuming, and also not compatible to automatic instruments. Cleanert® PPT plates were designed for high throughput and automated protein precipitation processing.

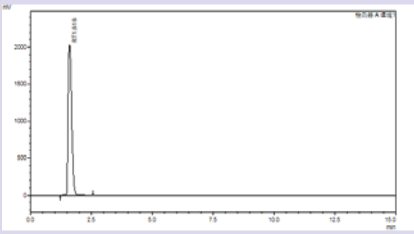
Bonna-Agela use high pure material and extractable residue were controlled strictly for every lot. 1mL and 2mL 96 well plates for sample loading are optional.

1 mL round well plate format

2 mL square well plate format

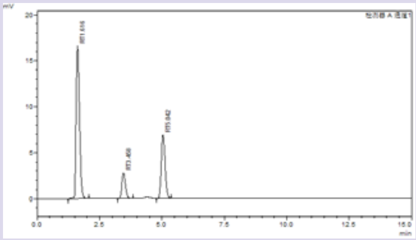
Advantages

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



Background Testing

No peak area is > 1% of uracil



Recovery Testing

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

PPT Plate vs centrifuge

PPT Plate for 96 samples

15 mins

Load 600 μL of ACN and 200 μL of plasma into PPP plate
Mix well
Collect the eluant by vacuum

Centrifuge for one sample

45 mins

Add 1 mL of ACN and 200 μL of Plasma into centrifuge tube
Vertex for 10 mins
Centrifuge for 10 mins
Transfer the supernatant

Typical processing method

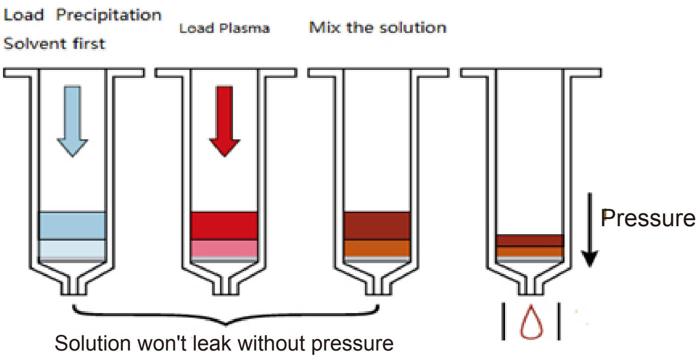
Place the Cleanert® PPT plate onto a vacuum manifold or positive pressure device;

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

Add 20-300 μL of plasma into each tube;

Mix the solvent and sample together by vortex or pipette;

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



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

Ordering Information

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

Cleanert® FAST—Filtration Plate Series

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

▲ Membranes: Glass fiber, PTFE, PP, Cellulose Acetate;

▲ Pore size: 0.22 μm, 0.45 μm, 1 μm, 5 μm are optional;

▲ 1 mL round well and 2 mL square well 96 well plate were available for filtration series.

1 mL Filtration plate-round well

2 mL Filtration plate- square well

Ordering Information

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

Cleanert® MAS (Multi-function Impurity Adsorption SPE)

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

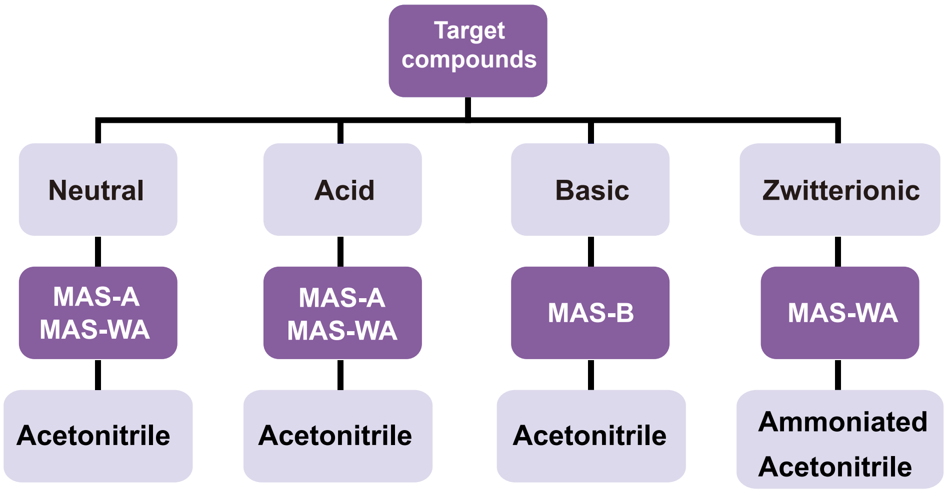
For amphoteric compounds, MAS-A should be followed by basic eluant (0.01%~1%NH_3•H_2O is suggested to be added in the elution solvent), while MAS-B should be followed by acidic eluant (0.02%-2% acetic acid is suggested to be added in the elution solvent). Because the acidity/basicity of most compounds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

Product Format

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

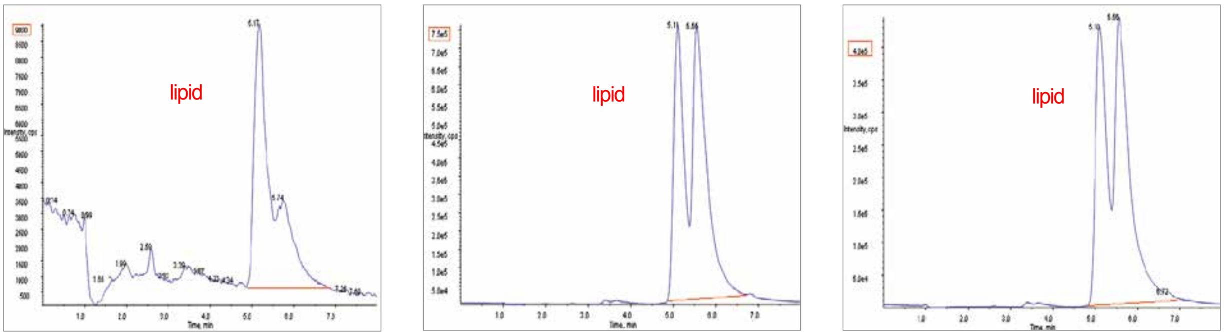
Product and Solvent Selection

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



Flow chart of solvent and products selection

Removal of Phospholipids MAS vs PPT and SPE



Response : $9*10^3$ CPS **MAS**

Response : $7.5*10^5$ CPS **PPT**

Response : $4.3*10^5$ CPS **SPE**

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

Perfect performance for protein removal

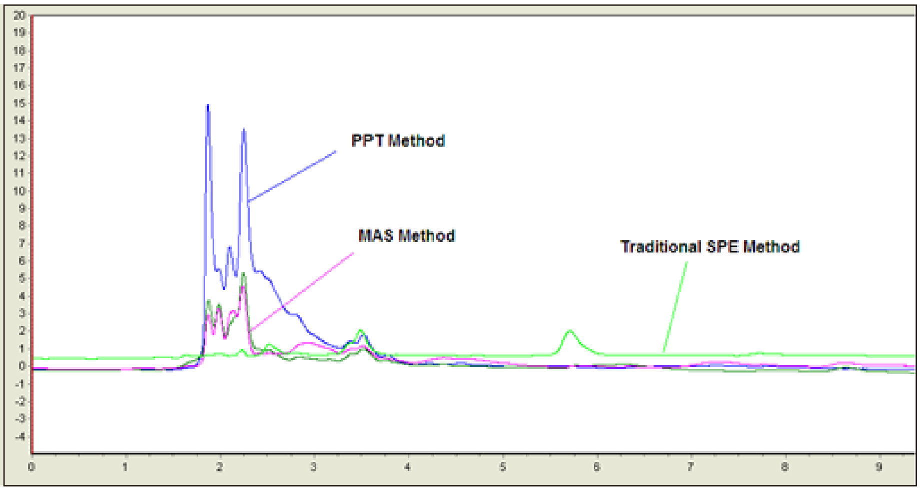


Fig 2 the comparison of protein signal after processed by PPT, SPE and MAS method

Suggested processing method

96 well Plate Format

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

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

SPE Cartridge Format

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

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

Mix well for 3 mins by pipette or vortex

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

Ordering information

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

Cleanert® SLE Products

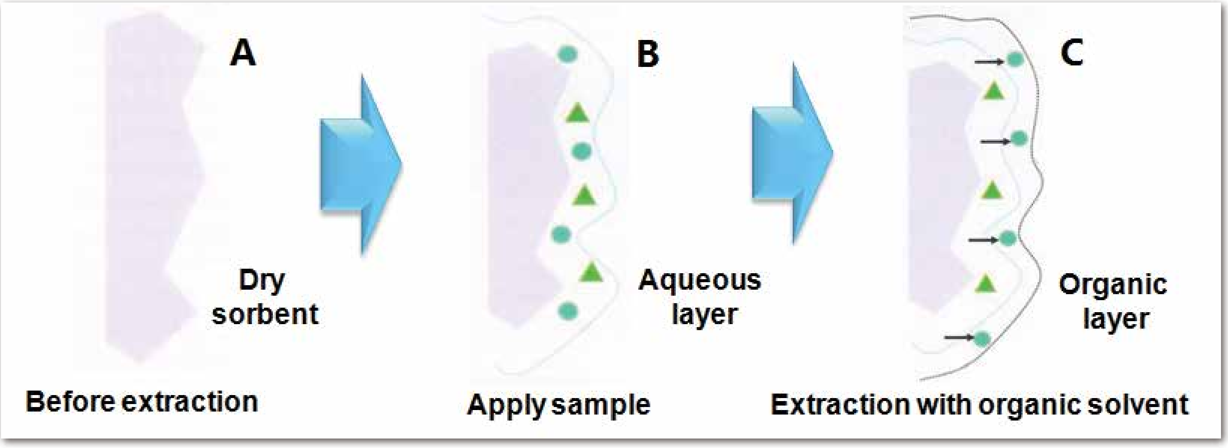
Supported Liquid Extraction Products

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

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

Cleanert® SLE cartridge

Cleanert® SLE plate



Benefits compared with LLE method

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

Fast & Simple

Cleanert SLE Plate
Supported Liquid Extraction
< 20 min

1. 100 µL of sample diluted with 100 µL of buffer and load onto Cleanert SLE plate with positive pressure or vacuum.
2. Stand for 5 min.
3. Elute with organic solvent & complete elution by positive pressure or vacuum.
4. Evaporation at 45 ℃ under N_2 and reconstitute with 200 µL methanol.

Slow & Tedious

Traditional
Liquid Liquid Extraction
> 40 min

1. 100 µL of sample diluted with 100 µL of buffer and add 200 µL of ethyl acetate.
2. Vortex & centrifuge 15 min.
3. Transfer supernatant to clean tube .
4. Evaporation at 45 ℃ under N_2 and reconstitute with 200 µL methanol.

Product Specification selection guide

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

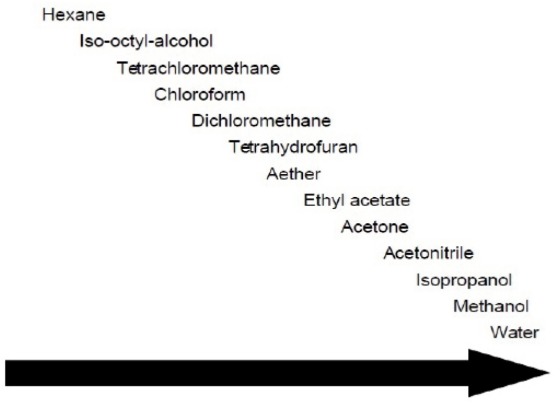
For sticky sample, SLE Plus series which using bigger particle size is suggested to improve flow rate also avoid blocking. The bigger particle size is also provide a benefit tool for filtration to get rid of particles and matrix.

Elution solvent selection guide

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

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

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



The polarity chart of common solvents

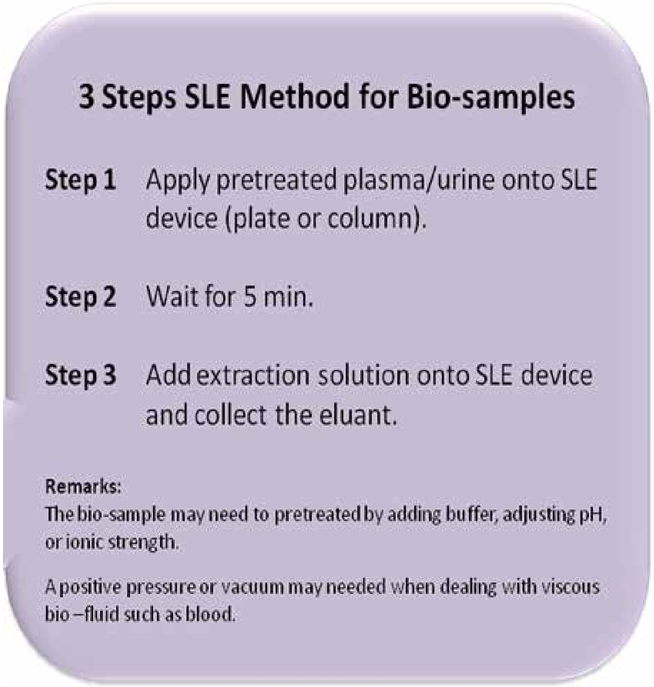
Salting-out in SLE

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

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

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

Recommended SLE Method



3 Steps SLE Method for Bio-samples

Step 1 Apply pretreated plasma/urine onto SLE device (plate or column).

Step 2 Wait for 5 min.

Step 3 Add extraction solution onto SLE device and collect the eluant.

Remarks:
The bio-sample may need to pretreated by adding buffer, adjusting pH, or ionic strength.

A positive pressure or vacuum may needed when dealing with viscous bio–fluid such as blood.

Tips:

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

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

Ordering Information

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

Cleanert® MAS-Q (QuEChERS)

Description

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

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

Slim bag package

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

Homogenous balls

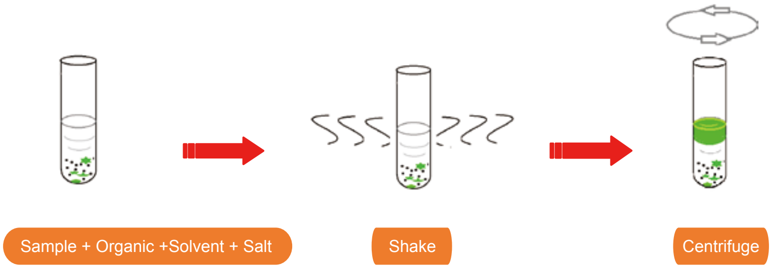
- The clean up kits are available in 2 mL, 15 mL for different sample volume requirement, and extraction kits are packed in anhydrous sealed packets;
- Superior centrifuge tubes and glass homogeneous ball provide you a simple and convenient experience.

Recommended Standard Operating Procedure for MAS-Q

- Step 1: Extraction

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

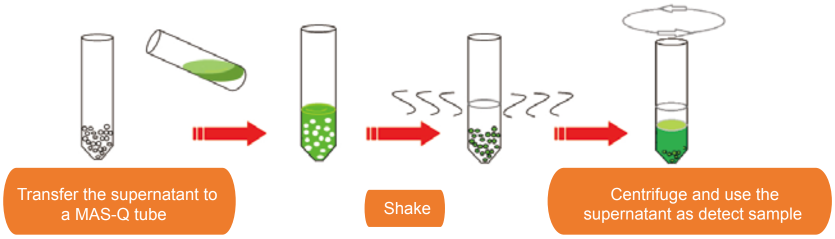
Extraction into the organic layer. Four glass homogeneous balls are suggested to add in to break the $MgSO_4$ caking caused by sample rich in water.



- Step 2: Cleanup

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

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



Production Selection Guide

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

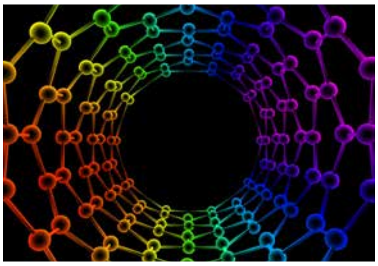
Ordering Information

Cat.No.	Centrifuge tube	Sorbent Weight	Description	Package
MS-MG5052	50 mL	6 g $MgSO_4$(anhydrous), 1.5 g anhydrous NaAc	QuEChERS extraction kit (with buffered) AOAC 2007.01 method for 15 g sample	50
MS-MG5052-1	No tube	6 g $MgSO_4$(anhydrous), 1.5 g anhydrousNaAc	QuEChERS extraction kit (with buffered) AOAC 2007.01 method for 15 g sample	50
MS-NMS5050	50 mL	NaCl 1 g, $MgSO_4$(anhydrous) 4 g, 0.5 g disodium citrate sesquihydrate (TSCD), 1 g NaCitrate (DHS)	QuEChERS extraction kit (with buffered) EN 15662 method for 10 g sample	50
MS-NMS5050-1	No tube	NaCl 1 g, $MgSO_4$(anhydrous) 4 g, 0.5 g disodium citrate sesquihydrate (TSCD), 1 g NaCitrate (DHS)	QuEChERS extraction kit (with buffered) EN 15662 method for 10 g sample	50
MS-NMS5151	50 mL tube	6 g Magnesium Sulfate, 1.5 g Sodium Chloride, 1.5 g Trisodium Citrate Dihydrate, and 0.75 g Disodium Hydrogencitrate	Extraction kit, apply to vegetables and fruit matrix, EN Method 15662	50
MS-MG5053	50 mL	6 g $MgSO_4$(anhydrous), 1 g NaCl	QuEChERS extraction kit (unbuffered) for 15 g sample	50
MS-MG5055	50 mL	4 g $MgSO_4$(anhydrous), 1g NaCl	QuEChERS extraction kit (unbuffered) for 15 g sample	50
MS-MG5051	50 mL	6 g $MgSO_4$(anhydrous), 1.5 g NaCl	QuEChERS Original extraction kit (unbuffered) for 15 g sample	50
MS-PA0251	2 mL	25 mg PSA, 150 mg $MgSO_4$(anhydrous)	EN 15662 (1 mL extract solution)	100
MS-PA0250	2 mL	50 mg PSA, 150 mg $MgSO_4$(anhydrous)	AOAC 2007.01 (1 mL extract solution)	100
MS-PA1011	15 mL	150 mg PSA, 900 mg $MgSO_4$(anhydrous)	EN 15662 (6 mL extract solution)	50
MS-PA1012	15 mL	400 mg PSA, 1.2 g $MgSO_4$(anhydrous)	AOAC 2007.01 (8 mL extract solution)	50

Part No.	Centrifuge tube	Sorbent Weight	Description	Package
MS-9PA0204	2 mL	25 mg C18, 25 mg PSA, 150 mg MgSO$_4$(anhydrous)	EN 15662 (1 mL extract solution)	100
MS-9PA0203	2 mL	50 mg C18, 50 mg PSA, 150 mg MgSO$_4$(anhydrous)	AOAC 2007.01 (1 mL extract solution)	100
MS-9PA1210	15 mL	150 mg C18, 150 mg PSA, 900 mg MgSO$_4$(anhydrous)	EN 15662 (6 mL extract solution)	50
MS-9PA1011	15 mL	400 mg C18, 400 mg PSA, 1200 mg MgSO$_4$(anhydrous)	AOAC 2007.01 (8 mL extract solution)	50
MS-PP0251	2 mL	25 mg PSA, 2.5 mg PestiCarb, 150 mg MgSO$_4$(anhydrous)	EN 15662 (1 mL extract solution)	100
MS-PP1511	15 mL	150 mg PSA, 15 mg PestiCarb, 900 mg MgSO$_4$(anhydrous)	EN 15662 (6 mL extract solution)	50
MS-PP0252	2 mL	25 mg PSA, 7.5 mg PestiCarb, 150 mg MgSO$_4$(anhydrous)	EN 15662 (1 mL extract solution)	100
MS-PP1510	15 mL	150 mg PSA, 50 mg PC, 900 mg MgSO$_4$(anhydrous)	EN 15662 (6 mL extract solution)	50
MS-PP0250	2 mL	50 mg PSA, 50 mg PC, 150 mg MgSO$_4$(anhydrous)	AOAC 2007.01 (1 mL extract solution)	100
MS-PP1550	15 mL	400 mg PSA, 400 mg PC, 1200 mg MgSO$_4$(anhydrous)	AOAC 2007.01 (8 mL extract solution)	50
MS-9PP0250	2 mL	50 mg PSA, 50 mg C18, 50 mg PC, 150 mg MgSO$_4$(anhydrous)	AOAC 2007.01 (1 mL extract solution)	100
MS-9PP0253	15 mL	400 mg PSA, 400 mg C18, 400 mg PC, 1200 mg MgSO$_4$(anhydrous)	AOAC 2007.01 (8 mL extract solution)	50
MS-SPC5001	50 mL	Ion exchange and reverse phase materials	Melamin detection in complicated Samplemeat,egg,milk powder,fish and so on	50
MS-SPM5001	50 mL		Melamin detection in milk	50
HG01	-	homogeneous balls	Glass material, 4 balls are suggested to be added for improve extraction	50/bag
HG500	-			500/box

Cleanert® NANO

—— A New Material of Carbon Nanotube



Structure of the Cleanert® Nano

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

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

Syringe Filter Format

Product Formats

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

Application Note

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

Sample Preparation Procedure

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

↓

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

↓

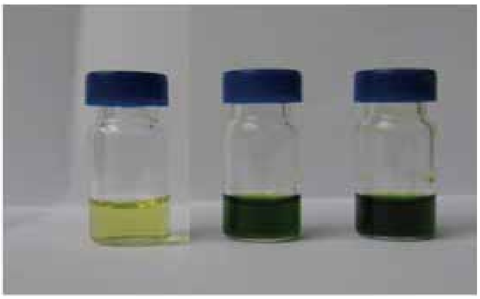
Shake by hand for 1 mins and then centrifuge at 8000 r/min

↓

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

↓

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



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

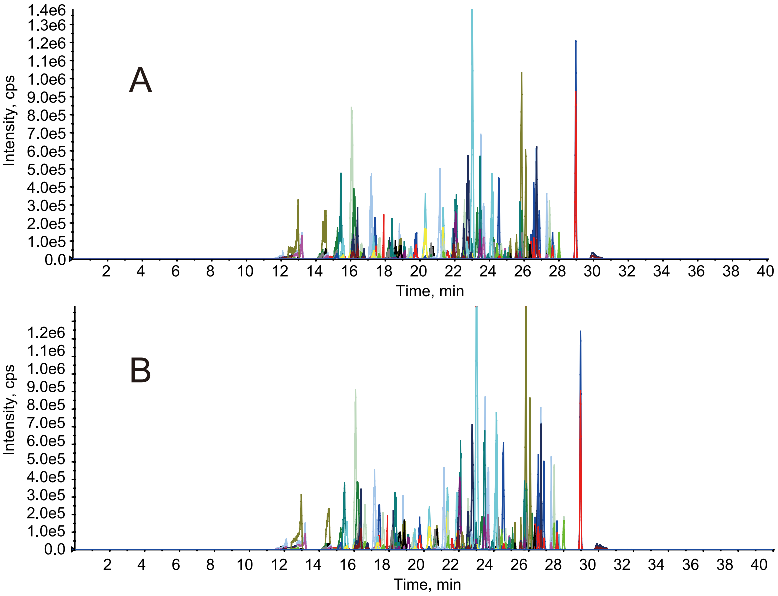


Figure 1. LC-MS/MS chromatogram of tea leaves extract. (A) 114 kinds of pesticides standard solution; (B) tea leaves extract spiked with 0.02 μg/mL 114 kinds of pesticides standard solution.

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

Ordering Information

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

Special Products

Cleanert® PAE and DEHP *NEW !*

—— Plasticizers Detection in Food and Water

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

Suggested Processing Method

Applicative sample matrix

Liquid sample such as bottled Drinking water and Soft drink

SPE Process

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

Analytical Method

Analysis by HPLC or GC-MS

Ordering Information

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

Applicative Sample

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

Sample-treatment

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

Take the supernatant , after filtration and then for analysis.

Analytical Method

GC/MS analysis

Ordering Information

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

Sample

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

Extraction

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

SPE Process

Activation: 5 mL of hexane

Sample loading: transfer all the sample upside

Elute: 3 mL×2 of ethyl acetate : hexane combination (50:50, v / v).

Evaporate the elution to nearly dryness with N_2, add 1 mL of acetonitrile for reextraction, vortex for 3 min, vortex for 3min, centrifugal for 5min at 4000rpm, take the supernatant for analysis.

Analytical Method

HPLC analysis

Ordering Information

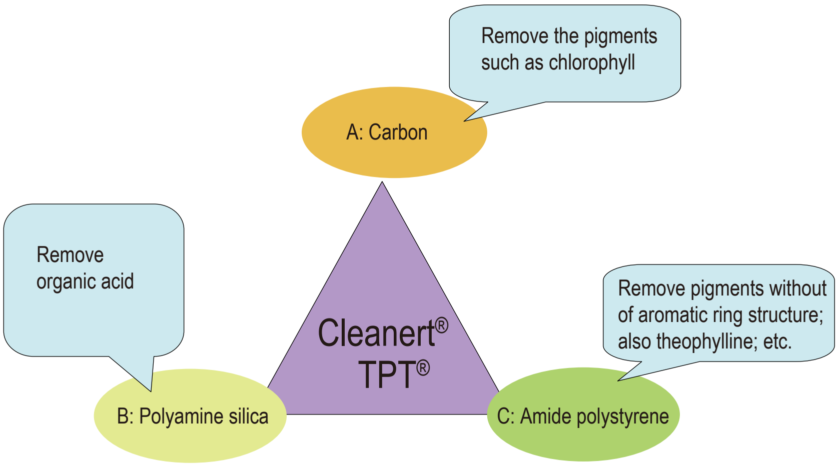
SPE Cartridge	Cleanert® PAE, Cat. No:PAE30006-G, 30/pk Cleanert® PAE (Sample with colorant); Cat.No:PAE30006-C; 30/pk

Cleanert® TPT® *NEW !*

——Triple Phases SPE for Tea Leaves

Description

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



Comparison of Clean up Effectiveness

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

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

Excellent Recoveries for Pesticide Residues

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

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

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

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

Ordering Information

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

Cleanert® TPH *NEW !*

—— Triple Phases SPE for Herb Samples

Description

Cleanert® TPH means triple phases SPE for Herb samples, and it is a composite of three types of sorbent with different mechanisms. By mixing different materials together, we achieve a better result of clean up, particularly for complicated matrices. Cleanert® TPH is mainly used for extraction and detection of pesticides from Chinese Herb Medicines such as Ramulus Mori, Honeysuckle and the Fruit of Chinese Wolfberry. It can reduce effectively the interference without adsorbing any pesticide residues.
Cleanert® TPH has been used in Chinese national standard methods, determinating of 488 Pesticide Residues and Related Chemicals Residues and 413 Pesticide Residues and Related Chemicals Residues in the Ramulus Mori, Honeysuckle and the Fruit of Chinese Wolfberry with GS-MS and LC-MS/MS respectively.
The process includes ACN extraction, loading the sample onto the pre-activated column, and the subsequent elution by ACN- toluene (3+1).

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

Ordering Information

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

Cleanert® BAP *NEW !*

—— Benzopyrene Detection in Edible Oil

Description

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

Suggested Processing Method (BAP)

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

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

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

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

Suggested Processing Method (Bap-3)

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

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

Washing: 10mL n-hexane

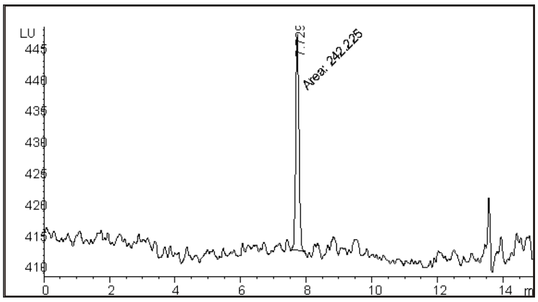
Elution: 5 mL dichloromethane

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

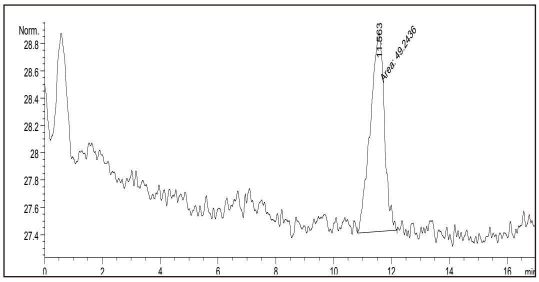
	Sorbent	Elution Volume	Max Sample Volume
BAP-3	Polymer	5 mL dichloromethane	All kind of oil sample, 0.5 g
BAP	Alumina	80 mL Hexane (concentration is needed)	0.3 g Not work for Oil with more polar component

Chromatographic condition

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



Standard solution



Edible oil sample spiked with
10 µg/Kg benzopyrene

Ordering Information

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

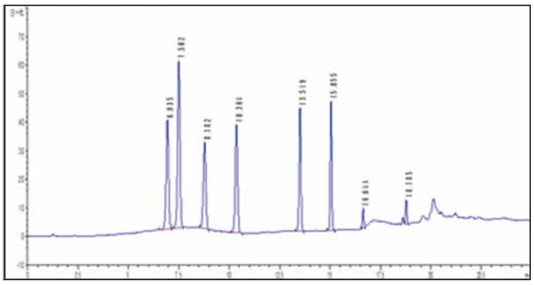
Cleanert® PA

Description

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

SPE Process

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

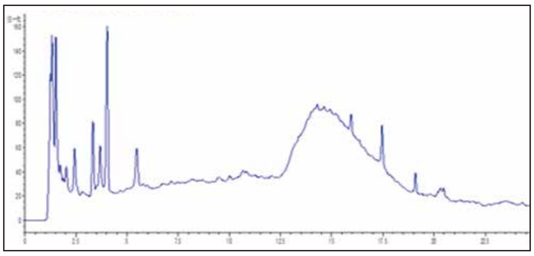


Standard solution of 8 kinds of pigment (Lemon yellow; new red; Amaranth; Indigo; Carminum; Sunset Yellow; Allura Red; Brilliant Blue)

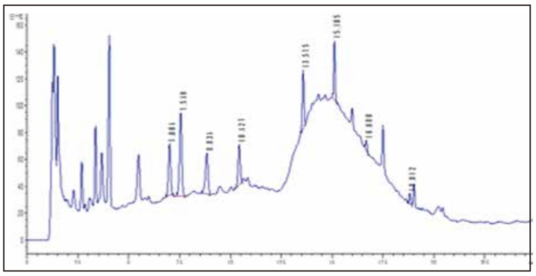
HPLC Conditions

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

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

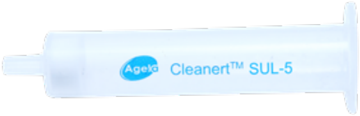


Blank sample of red wine



Blank sample spiked by 5ppm standard solution

Cleanert® SUL-5
(Specific Columns for Sulfonamides)



Description

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

Ordering Information

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

Cleanert® DNPH-Silica

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

3 mL DNPH cartridge

200 mg/1 mL (Left)
350 mg/1 mL (Right)

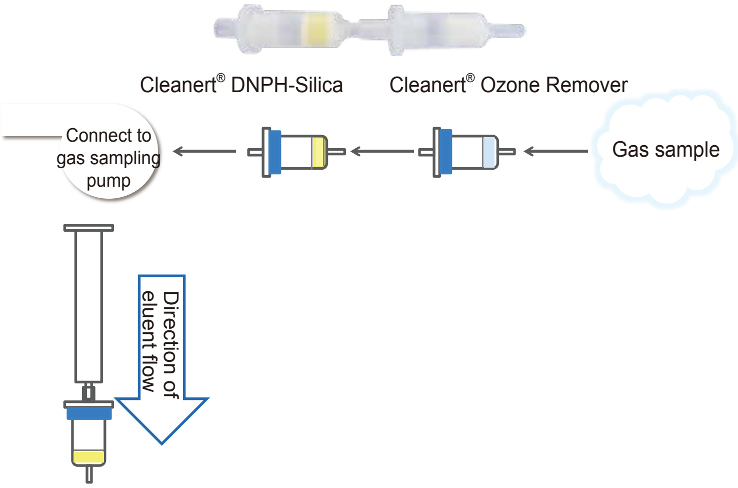
Specification

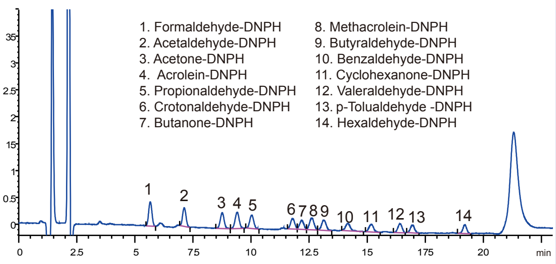
▲ Ave Background value: ≤0.1 μg (calculated by formaldehyde)
▲ Max sampling quantity: ≥75 μg (calculated by formaldehyde)
▲ Max Sampling Pressure: 0.15 MPa
▲ Recoveries: >90%

Operation

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

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

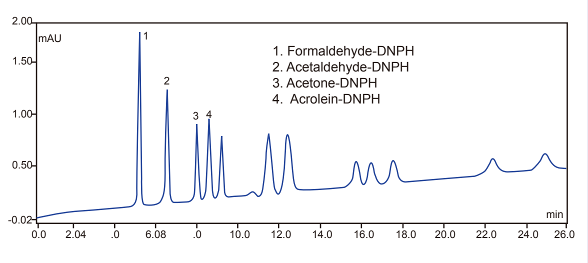




Gradient Separation of 14 kinds of Aldehyde and Ketone Derivatives

HPLC Column: Venusil® XBP C18(2), 4.6×150 mm, 3 μm 100 Å (Cat. No.:VX931505-2)
Mobile Phase: A: Water; B: Acetonitrile:THF = 66:34 (V:V);
Flow rate: 1.0 mL/min;
Column Temperature: 25 °C;
Injection: 10 μL ;
Detection: UV 360 nm;
Gradient:

Time (mins)	B%
0	55
3	55
12	63
18.5	75
19	55
23	55



Isocratic Separation of 4 kinds of Aldehyde and Ketone Derivatives

HPLC Column: Venusil® HLP, 4.6 × 200 mm, 5 μm, 100 Å (Cat. No.:VHL952005-0)
Mobile Phase: Acetonitrile : Water = 55 : 45 (V:V);
Flow rate: 1.5 mL/min;
Column Temperature: 25 °C;
Injection: 10 μL;
Detection: UV 360 nm

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat.No.
Cleanert® DNPH	200 mg	1 mL	50/PK	IC-DN2001
Cleanert® DNPH	200 mg	3 mL	50/PK	DN2003
Cleanert® DNPH	350 mg	1 mL	50/PK	IC-DN3501
Cleanert® Ozone remover	1.45 g	2.5 mL	50/PK	KI1450

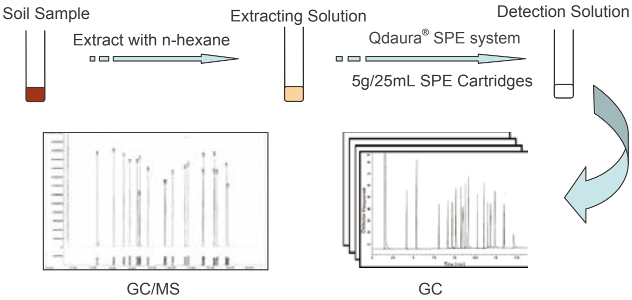
Cleanert® EPH *NEW !*

—— for Extractable Petroleum Hydrocarbons

Description

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

Please find the detailed experimental information in the application part.



Ordering Information

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

Cleanert® ACA *NEW !*

——Polar Pollutant Analysis in Water Analysis

Description

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

Ordering Information

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

Cleanert® SLE-OD

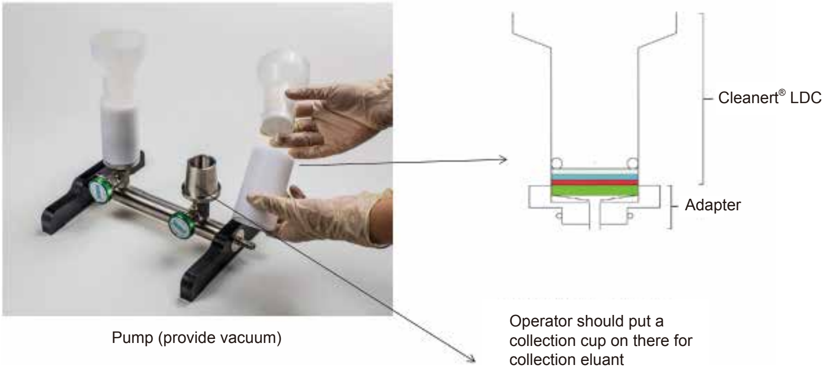
Bonna-Agela provide commercial diatomaceous column which mentioned in EN 14362-1:2012(E), and 22 kinds of aromatic amine could be detected in textile samples.

Ordering Information

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

Cleanert® LDC *NEW !*

—— Large Disk for Water Pollutant Analysis



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

Description

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

▲ The special cartridge design is suitable for water concentration, and it can replace SPE disk in most of the analysis.

▲ Cleanert® LDC uses AQ C18 sorbent to concentrate trace chemical substances in method EPA 525, show good recovery and stability for hundreds of compounds.

Ordering Information

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

Cleanert® LRC *NEW !*

—— Large Receiver Column

Description

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

Ordering Information

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

Cleanert® SPE in Glass Tubes *NEW !*

Description

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

Ordering Information

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

Conventional Products

Bonded Silica SPE

Cleanert® AQ C18

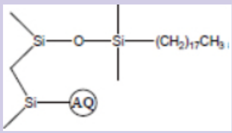
Description

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

Particle Characteristics

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

Ordering Information

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

Cleanert® S C18 (End-capped)

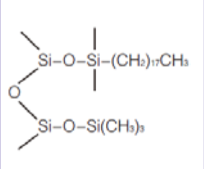
Description

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

Particle Characteristics

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

Ordering Information

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

Cleanert® S C18-N (Non-end-capped)

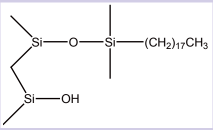
Description

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

Particle Characteristics

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

Ordering Information

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

Cleanert® S C8 (Octyl)

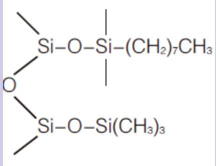
Description

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

Particle Characteristics

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

Ordering Information

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

Cleanert® CN (Cyanopropyl)

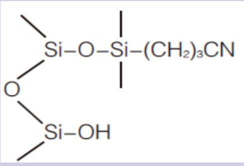
Description

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

Particle Characteristics

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

Ordering Information

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

Cleanert® NH$_2$ (Aminopropyl)

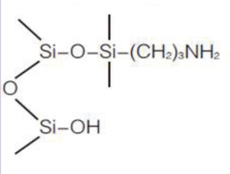
Description

Cleanert® NH$_2$ products are silica based sorbent bonded with aminopropyl functional group. This sorbent can be used in either normal phase or reversed phase mode. It retains the analytes either by a polar adsorption (from non-polar solution) or by weak anion exchange (from aqueous solution). pKa=9.8.

Particle Characteristics

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

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® NH$_2$  	100 mg	1 mL	100	NH1001
	200 mg	3 mL	50	NH2003
	500 mg	3 mL	50	NH5003
	500 mg	6 mL	30	NH5006
	1 g	6 mL	30	NH0006
	50 mg/well	2 mL	96-well plate, 1/PK	NH0502-W
	100 mg/well	2 mL	96-well plate, 1/PK	NH1002-W
	10 g	-	bottle	NH0010
	100 g	-	bottle	NH0100

Cleanert® PSA {(N-aminoethyl) Aminopropyl}

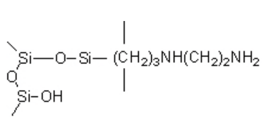
Description

Cleanert® PSA SPE is similar to Cleanert® NH$_2$. It has two amino groups with pKa = 10.1 and 10.9, respectively. This sorbent is an anion exchanger slightly stronger than Cleanert® NH$_2$. It can be used for the extraction of metal ions by chelating interactions. It is also commonly used to remove organic acids, pigments and metal ions from organic samples such as vegetables and fruits.

Particle Characteristics

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

Ordering Information

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

Cleanert® SAX (Strong Anion Exchanger)

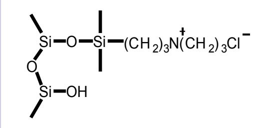
Description

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

Particle Characteristics

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

Ordering Information

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

Cleanert® COOH (Weak Cation Exchanger)

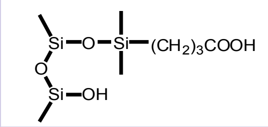
Description

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

Particle Characteristics

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

Ordering Information

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

Cleanert® PRS (Propane Sulfonic Acid)

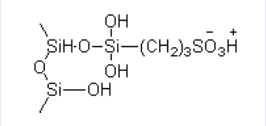
Description

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

Particle Characteristics

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

Ordering Information

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

Cleanert® SCX (Strong Cation Exchanger)

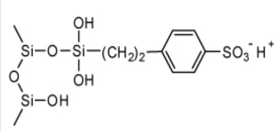
Description

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

Particle Characteristics

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

Ordering Information

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

Cleanert® Silica

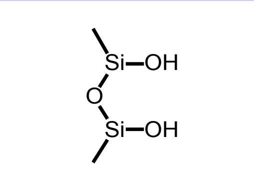
Description

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

Particle Characteristics

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

Ordering Information

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

Cleanert® Diol

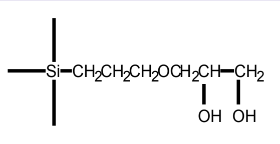
Description

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

Particle Characteristics

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

Ordering Information

Material	Sorbent	Vol	Tubes/box	Cat. Number
Cleanert® Diol  $-\mathrm{Si}-\mathrm{CH_2CH_2CH_2OCH_2\underset{OH}{CH}-\underset{OH}{CH_2}}$	100 mg	1 mL	100	DI1001
	200 mg	3 mL	50	DI2003
	500 mg	3 mL	50	DI5003
	500 mg	6 mL	30	DI5006
	1 g	6 mL	30	DI0006
	50 mg/well	2 mL	96-well plate, 1/PK	DI0502-W
	100 mg/well	2 mL	96-well plate, 1/PK	DI1002-W
	10 g	-	bottle	DI0010
	100 g	-	bottle	DI0100

Non-silica Adsorption Phase Cartridges

Cleanert® Florisil (Magnesia Silica)

Description

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

Particle Characteristics

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

Ordering Information

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

Cleanert® PestiCarb (Graphitized Carbon Black)

Description

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

Particle Characteristics

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

Ordering Information

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

Cleanert® Alumina N (Aluminium Oxide; Neutral)

Description

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

Particle Characteristics

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

Ordering Information

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

Cleanert® Alumina A (Aluminium Oxide; Acidic)

Description

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

Particle Characteristics

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

Ordering Information

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

Cleanert® Alumina B (Aluminium Oxide; Basic)

Description

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

Particle Characteristics

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

Ordering Information

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

Mixed and Layered Phases

Cleanert® Double-layer series

Cleanert® PestiCarb/NH_2

Description

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

Ordering Information

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

Cleanert® Pesticarb/PSA

Description

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

Ordering Information

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

Cleanert® SAX/PSA

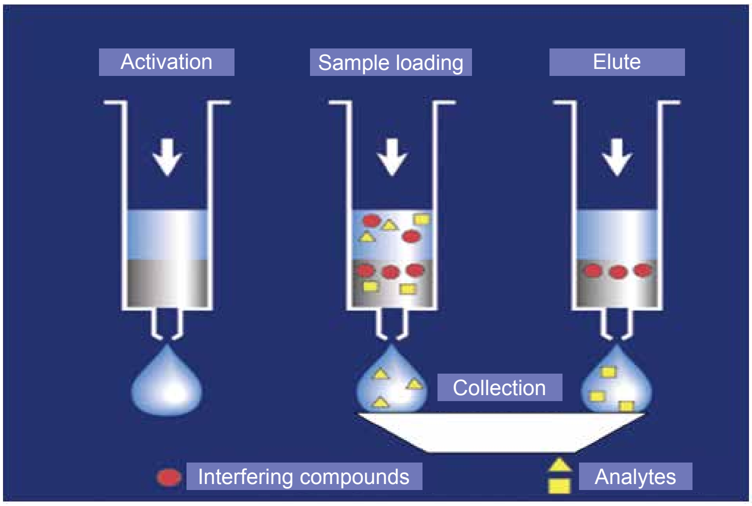
Description

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

Ordering Information

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

Suggested processing method for double layer cartridge



Tips:

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

Cleanert® C8/SCX

Description

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

Particle Characteristics

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

Ordering Information

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

Cleanert® IC:

Sample Clean-up Cartridges for Ion Chromatography

Description

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

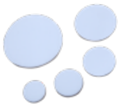
The IC cartridges are designed as luer inlet and outlet for easy operation. They also can connect several cartridges together for complex matrix. The sample solution can be passed through the IC cartridge by syringe, and also by vacuum manifold connected with an empty SPE cartridge as funnel.

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

Accessories

Empty Columns and Accessories

Ordering Information

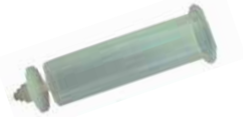
Product name	Specification, Package	Cat. No.	Picture
Empty Cartridge	1 mL, 100/PK	AZ001-H	 SPE empty tube  SPE empty tube （flangeless）  LRC empty tube  LDC empty tube
	3 mL, 100/PK	AZ003	
	6 mL, 100/PK	AZ006	
	6 mL, flangeless, 100/PK	AZ006-N	
	10 mL, LRC empty tube, 100/PK	LAZ010	
	12 mL,100/PK	AZ012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

Large Volume Sampling cartridge

Ordering Information

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

a: Water Sample Loading Pipeline



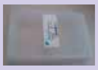
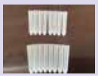
b: Large loading column

Collection Plate and Mat

Ordering Information

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

Ordering Information

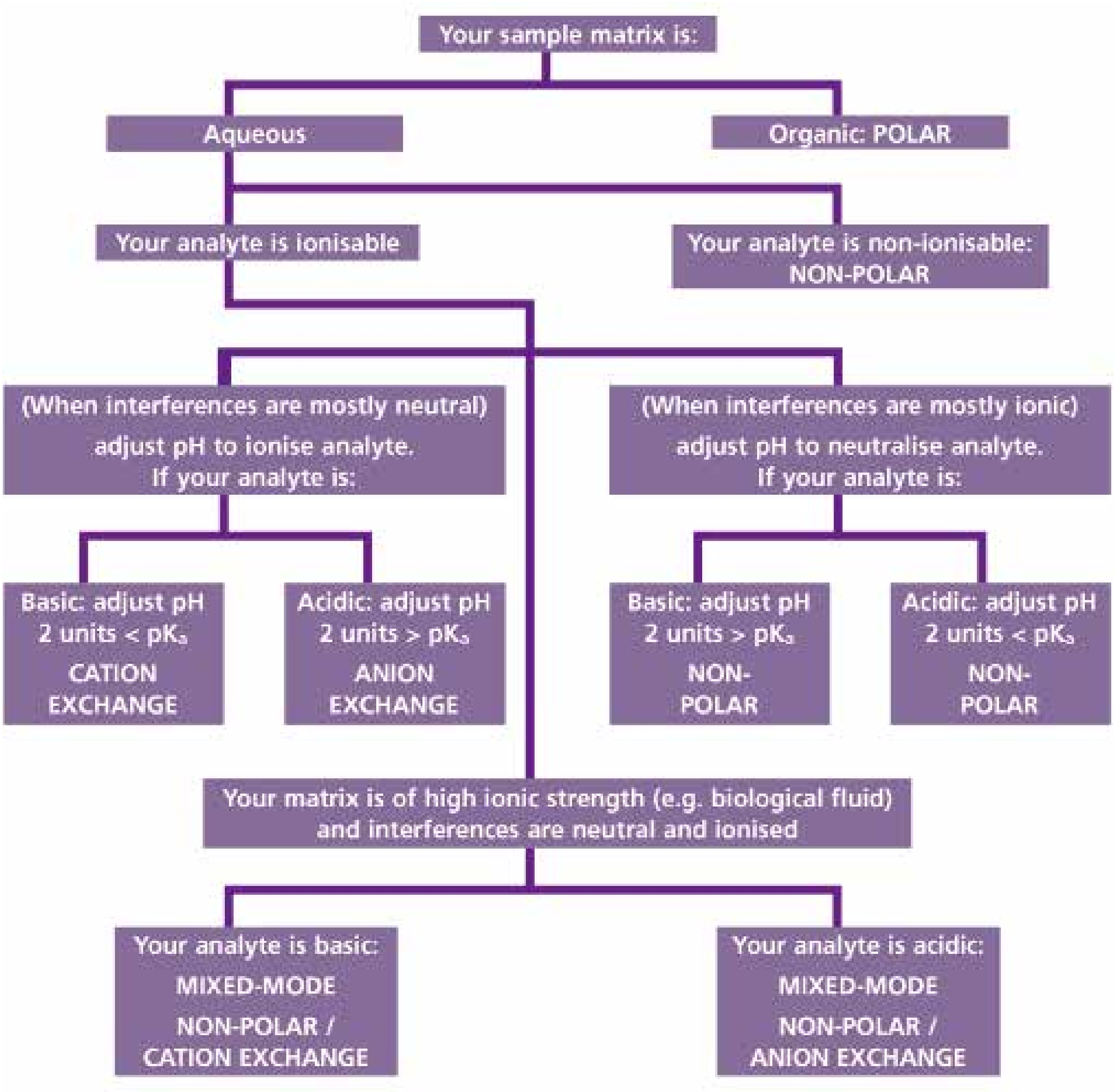
Product	Specification		Package/PK	Picture	Cat. No.
96-well collection plate	Waste collection plate		10		96WSP
Silica mat for 96-well plate	96 well silicone mat, square well, pierceable	8×12well	10		96GP2036
	96 well silicone mat, round well, pierceable	8×12well	10		96GP2036-M
8 collection tube	8well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

Method Development of SPE Procedures

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

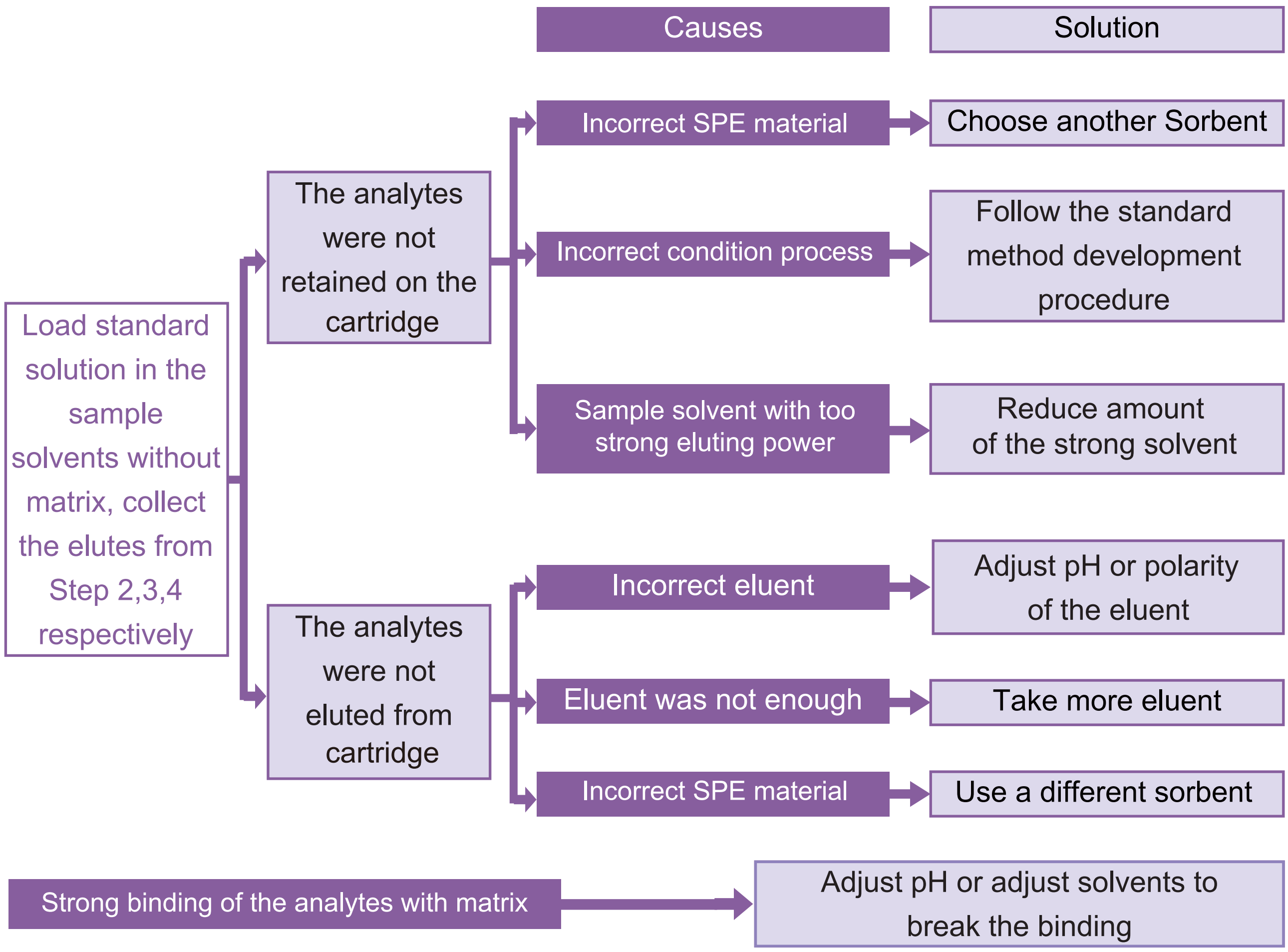
The Selection of Sorbent Retention Mechanism

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



Trouble Shooting

Low Recovery



Causes

Solution

Load standard solution in the sample solvents without matrix, collect the elutes from Step 2,3,4 respectively

The analytes were not retained on the cartridge

Incorrect SPE material → Choose another Sorbent

Incorrect condition process → Follow the standard method development procedure

Sample solvent with too strong eluting power → Reduce amount of the strong solvent

The analytes were not eluted from cartridge

Incorrect eluent → Adjust pH or polarity of the eluent

Eluent was not enough → Take more eluent

Incorrect SPE material → Use a different sorbent

Strong binding of the analytes with matrix → Adjust pH or adjust solvents to break the binding

High Deviation

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

Clogging

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

SPE Method Development Protocol

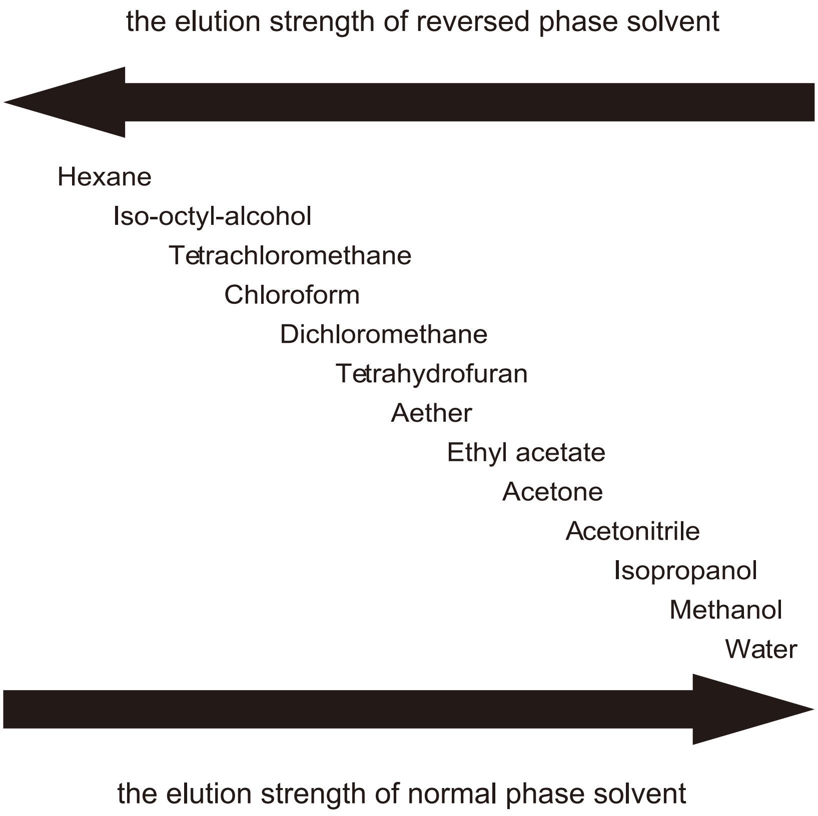
Properties	**Sorbent Phase Type**			
	Normal Phase	**Reversed-Phase**	**Lon Exchange Phase or Mix-phase**	
Typical Sorbents	Silica Florisil	PEP series	PAX	PCX
	Amine (NH$_2$)	C18 C18-N	SAX	SCX
	Cyano (CN) Diol	C8	PWAX	PWCX
Sorbent Polarity	High	Low	High	
Matrix Properties	Organic	Organic or Aqueous	Organic or Aqueous	
Analyte Properties	Slightly to Moderately Polar	Non-Polar or Polar	Acidic	Basic
Retention	Polar Non-Polar	Non-Polar polar	Ionic Strength	
Step 1: Condition	Sample solvent or other organic solvents	Water-miscible organic solvents followed by Water	Water-miscible organic solvents followed by aqueous solution with pH adjusted	
Step 2: Sample Loading	Load the sample or with dilution in low polarity solvents	Load the sample or with dilution in high polarity solvents	Load sample or with dilution in high polarity solvents with pH adjusted	
Step 3: Washing	Washing with low-polar solvents	Washing with mixture of aqueous solution or buffer with a small amount polar solvent	Washing with Polar organic solvents followed by aqueous solvents with pH adjustment to maintain analytes ionized	
Step 4: Elution	Eluting with mixture of non-polar and polar solvents	Eluting with non-polaror polar organic solvents	Eluting with polar solvents with pH adjustment	

Size, Capacity and Elution Volume in SPE Process

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

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

The Selection of Ideal Elution Solvent



the elution strength of reversed phase solvent

Hexane
Iso-octyl-alcohol
Tetrachloromethane
Chloroform
Dichloromethane
Tetrahydrofuran
Aether
Ethyl acetate
Acetone
Acetonitrile
Isopropanol
Methanol
Water

the elution strength of normal phase solvent

Food Safety Applications

Veterinary Drug Residues Detection

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

EMF10007

Experimental

Sample Preparation

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

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

SPE Procedure (Cleanert® PS, 200mg / 3mL)

- Activate the PS cartridge by sequentially adding 10 mL methanol, 10 mL water and 5 mL EDTA-2Na solution.
- Load sample solution prepared from above procedure; and then wash the cartridge with 10 mL water, and discard the eluate.
- Dry the cartridge via positive pressure.
- Elute the cartridge with 10 mL methanol and collect the eluate.
- Evaporate the sample eluate under rotovap to nearly dryness.
- Reconstitute the sample residue with 1 mL HPLC mobile phase.
- Filter sample through 0.45 um membrane before HPLC analysis.

Condition of HPLC

Column: Unisol C18, 5 μm, 4.6 × 250 mm
Mobile phase: A:B = 77:23
A: Iminazole buffer solution—dissolve 68.08 g of iminazole, 10.72 g of magnesium acetate and 0.37 g of EDTA2Na in 100 mL of water; modify pH to 7.2 with glacial acetic acid; bring the solution to 1000 mL with water.
B: Acetonitrile

Results

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

Ordering Information

Products	Specification	Cat.No.
Cleanert® PS	200 mg / 6 mL, 30/pk	PS2006
Unisol C18	5 µm, 4.6 × 250 mm, 100 Å	UO952505-0
Syringe	2 mL without needle	LZSQ-2ML
1.5 mL vials	Screw neck vials, 12 × 32 mm	AV1001-6
Caps and Septa	Screw neck cap, center hole; red silicone/white PTEE septa, slitted	AV2200-0

Detection of Chloramphenicol, Florfenicol and Thiamphenicol in Fish by LC/MS/MS (Cleanert® Silica, P/N: SI2003)

EAF10144

Introduction

Phenicols, a kind of amide alcohol broad-spectrum antibiotic, includes chloramphenicol and its derivatives. Chloramphenicol, thiamphenicol and florfennicol all belong to phenicols. Chloramphenicol can lead to aplastic anemia with its side effect, and the side effect is irreversible and can't be reduced by the use of dose and frequency. The United States, European Union, Japan and many other countries have prohibited the existence of chloramphenicol in animal-derived food. China also classified it as a forbidden drug and prohibited the existence of it in animal food in 2002. Thiamphenicol is less toxic to the blood system than chloramphenicol, but it can damage the formation of body's immune system, red blood cells and platelet. The European Community and the United States have banned the thiamphenicol on food animals except China and Japan. Florfenicol can impact animals' embryos though it is not the cause of aplastic anemia.

In this paper, the method of simultaneous determination of chloramphenicol, florfenicol and thiamphenicol in fish was established by the reappearance of the national standard.

Experimental

Instruments, Reagents and Materials

SCIEX API 4000+ Liquid Chromatography Tandem Mass Spectrometer
HPLC purity of acetonitrile, n-hexane and acetone; experimental water (ultra-pure water); analytical pure n-propanol; mixed standard solution: chloramphenicol, thiamphenicol, florfenicol, methanol solution;
Cleanert® Silica: 200 mg/3 mL.

Sample Preparation

Sample extraction

Firstly, transfer 5 g homogenized fish sample and 30 mL acetonitrile into a 50 mL centrifuge tube. Then vortex 1 min, and sonicate for 5 min then centrifuge at 8000 rpm for 5 min. Secondly, all supernatant in the centrifuge tube was put into a liquid funnel. Add 15 mL acetonitrile saturated n-hexane solution into the same liquid funnel and shake for 5min, stratified it. Thirdly, the acetonitrile layer was transferred into a heart-shaped bottle. Another 30mL acetonitrile was added into the residue to extract another acetonitrile layer according to the above process. Fourthly, the two acetonitrile layers and 5mL n-propyl alcohol were mixed in the heart-shaped bottle by shaking. Rotary evaporate under 40°C to near dry and dry with N2 flow. Add 5mL acetone-n-hexane (1+9) into the mixture to dissolve, shake and wait for sample preparation.

Sample preparation

The Cleanert® Silica was activated and equilibrated with 5mL acetone / n-hexane (V/V, 1+9). Load the extraction onto the cartridge. Elute the cartridge with 5mL acetone hexane (V/V, 6+4). Collect elution and rotary evaporate under 40°C to near dry. Reconstitute into 1 mL of water for further analysis.
Above steps was operated on Qdaura® Automated SPE Workstation.

HPLC conditions

HPLC column: Unisol C18, 3 μm, 100 Å, 3.0×50 mm
Mobile phase: 0.1% formic acid aqueous (A), 0.1% Formic acid acetonitrile (B)
Column temperature: 30 °C
Injection volume: 5 μL

Table 1 HPLC Gradient Condition

Time (min)	Flow (mL/min)	A%	B%
0.0	0.4	95	5
1.0	0.4	95	5
1.1	0.4	85	15
9.5	0.4	25	75
9.6	0.4	5	95
11.5	0.4	5	95
11.6	0.4	95	5
16.0	0.4	95	5

Mass spectrometer conditions
Ion source: ESI-; ESI voltage: -4500 V; atomizer pressure: 50 psi; air curtain pressure: 10 psi;
Auxiliary gas pressure: 60 psi; ion source temperature: 550 °C; collection method: multiple-reaction monitoring (MRM).

Table 2 MS/MS Parameters

Phenicols	Q1	Q3	DP/V	CE/V
Chloramphenicol	321.0	152.1	-75	-24
		256.9	-75	-17
Florfenicol	353.9	289.9	-75	-18
		184.9	-75	-28
Thiamphenicol	356.0	119.0	-80	-23
		184.9	-80	-12

Notes: The quantitive ions are underlined.

Results and Discussion

Table 3 shows that the recovery of spike sample was 70%-150% by LC/MS/MS using Cleanert Silica and the method can meet the demand of fish testing. It can also be seen from Figure 1 that the peak shape is sharp and the retention time of phenicols is stable using Unisol C18 column.

Table 3 The Recoveries of the 3 kinds of Phenicols in Fish

Phenicols	Retention time/min	Average recovery/%	CV/%
Chloramphenicol	6.78	114.3	0.4
Florfenicol	5.18	78.2	8.9
Thiamphenicol	6.51	98.4	5.0

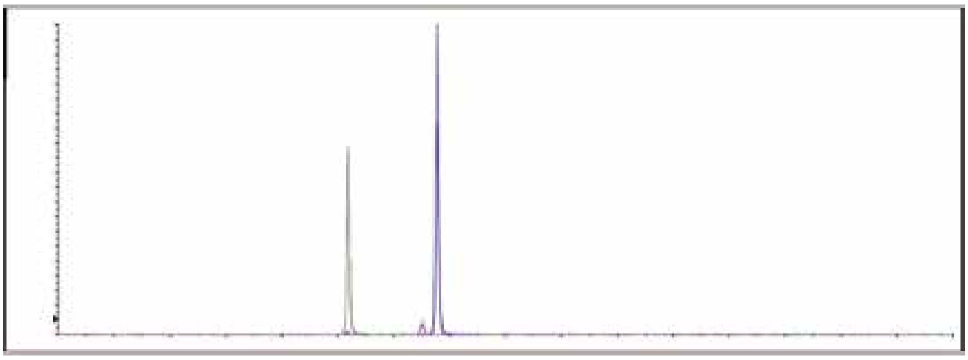


Figure 1. Chromatogram of 0.02 μg/mL Standard Solution of Phenicols Mix

Conclusion

In this study, the sample preparation methods of 3 kinds of phenicols in fish were established, and the samples were detected by LC-MS / MS. The result shows that the overall recoveries ranged from 70% to 115% of 4.0 μg/kg spiking fish samples, which meets the testing requirements. This study indicated that the method was suitable for simultaneous detection of phenicols in fish.

Ordering Information

Products	Specification	Cat.No.
Cleanert® Silica	200 mg/3 mL	SI2003
Unisol C18	3 μm, 100 Å, 3.0 × 50 mm	UO930503-0
Qdaura® Automated SPE Workstation	4 channel, 24 position	SPE-40

Determination of five Nitroimidazoles Residues in Fish (Cleanert® S C18, P/N: S180006)

EAF10147

Introduction

With better activity of ani-anaerobic bacteria and anti-protozoan, Nitroimidazoles are used in anaerobic infections and protozoal diseases of livestock. Vivo and in vitro toxicological experiments have confirmed that nitroimidazoles can lead to deformity, mutation and cancer. Ministry of Agriculture in China has forbidden the use of metronidazole and dimetridazole as feed additives on food animals and the use of ronidazole on food animals for any purpose.
In this study, the simultaneous detection of 5 nitroimidazoles in fish was established by optimizing preparation methods.

Experimental

Material and Reagents

HPLC purity of methanol, acetone, n-hexane, ethyl acetate, ammonium acetate; analytical pure sodium chloride; experimental water (ultra-pure water);
Standard solution mix of 5 nitroimidazoles: hydroxyl-methylimidazole, dimetridazole, metronidazole, ronidazole, hydroxy dimetridazole, dissolved in methanol;
Cleanert® S C18: 1000 mg/6 mL.

Sample Extraction

Transfer 20 g comminuted fish sample and 10 g diatomite into a 250 mL conical flask with cover, mix well. Add into 5mL saturated sodium chloride aqueous solution and 70 mL methanol : acetone = 3:1(V/V) in proper order and homogenize at high speed for 3 min. Transfer extract into a centrifuge tube, centrifuge at 10000 rpm for 5 min. Then put supernatant into a 250 mL concentrate bottle, extract the residue with methanol: acetone = 3:1(V/V) for twice. Put the two extract together, rotary evaporate under 40°C until only aqueous phase left and transfer it into a separatory funnel. Add 50 mL saturated sodium chloride and 25 mL ethyl acetate, shake for 3min, stratify and collect ethyl acetate phase. Repeatedly extract the aqueous phase with ethyl acetate for twice and mix the two ethyl acetate phase. Dehydrate the ethyl acetate phase with 3g anhydrous sodium sulfate, collect and rotary evaporate under 40°C to near dry. Add 5 mL methanol into the residue and filter through 0.45 μm nylon filter for sample preparation.

Sample Preparation

Activate and equilibrate Cleanert® C18 with 5 mL methanol. Apply all the extract onto the cartridge and collect the eluent. Then elute the cartridge with 10 mL methanol, collect the extract and merge the two eluents. Rotary evaporate under 45°C to dry and reconstitute into 1 mL of mobile phase for further analysis.

Instrumentation

Column: Unisol C18, 3 μm, 100 Å, 3.0 × 50 mm;
Mobile phase: A: 5 mmol ammonium acetate; B: methanol;
Flow rate: 0.3 mL/min;
Column temperature: 30 °C;
Injection: 5 μL;
Ion source: ESI+;
Electrospray voltage:5500 V;
Nebulizer pressure: 50 psi;
Curtain gas pressure: 25 psi;
Auxiliary gas pressure: 50 psi;
Ion source temperature: 500 °C;
Scan mode: MRM.

Table 1 Gradient

Time (min)	A%	B%
0.0	95	5
1.0	95	5
3.5	10	90
6.0	10	90
6.1	95	5
10.0	95	5

Table 2 MS Parameters

Analytes	Q1	Q3	DP/V	CE/V
Hydroxyl-methylimidazole	187.7	126.2	64.0	25.0
		123.3	64.0	18.0
Dimetridazole	128.2	82.2	88.8	24.7
		111.2	88.8	23.6
Metronidazole	172.0	128.2	63.0	21.2
		82.2	63.0	36.7
Ronidazole	201.4	140.2	80.0	17.0
		145.0	80.0	35.0
Hydroxy dimetridazole	157.8	140.2	50.0	17.0
		55.1	50.0	27.0

* underlined were quantitative ions

Results and Discussion

Table 3 shows that the recoveries of spiked samples ranged from 90% to 110% and the CVs were under 10% using Cleanert® S C18 by LC-MS/MS to determinate five Nitroimidazoles in fish. Typical method performance results were well within acceptable criteria. Figure 1 shows a good separation was obtained by using Unisol C18 column to detect five Nitroimidazoles simultaneously.

Table 3 The Recoveries of the 3 kinds of Phenicols in Fish

Analytes	Retention Time/min	Recoveries/%	RSD/%
Hydroxyl-methylimidazole	3.19	108.0	4.0
Dimetridazole	5.14	102.3	6.6
Metronidazole	5.14	104.4	5.0
Ronidazole	4.99	100.3	5.5
Hydroxy dimetridazole	4.48	92.0	2.7

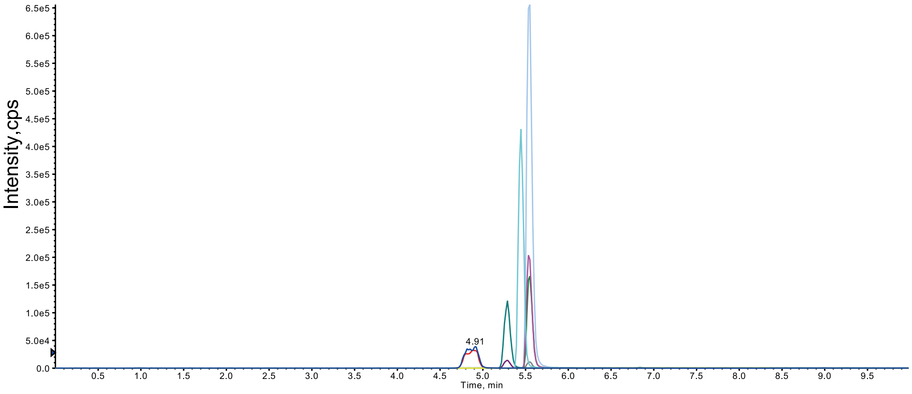


Figure 1 Chromatogram of 0.1 μg/mL standard sample mix of five Nitroimidazoles

Conclusion

In this study, the sample preparation methods of five Nitroimidazoles in fish were established, and the samples were detected by LC-MS / MS. The result shows that the overall recoveries ranged from 90% to 110% and the CVs were lower than 10% for 1.0 μg/kg spiking fish samples, which meets the testing requirements. This study indicates that the method was suitable for detection of five Nitroimidazoles in fish.

Ordering Information

Products	Specification	Cat.No.
Cleanert® S C18	1000 mg/6 mL	S180006
Qdaura® Automated SPE Workstation	4 channels, 24 positions	SPE-40
Unisol C18	3 μm, 100 Å, 3.0 × 50 mm	VA930503-0
Nitrogen evaporator	Maximal 15 samples	NV15-G
1.5 mL vials	Screw neck vials, 12 × 32 mm	AV1001-6
Caps and Septa	Screw neck cap, center hole; red silicone/ white PTEE septa, slitted	AV2200-0
Syringe Filter (Nylon)	Monofilm, 13 mm, 0.22 μm	AS021320
Disposable Needle-Free injection systems	2 mL, 100/pk	LZSQ-2ML

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

EMF10010

Material

(1) Quinolone standards: Enrofloxacin (ENR), Ciprofloxacin (CIP), Norfloxacin (NOR), Ofloxacin (OFL), Flumequine (FLU), Oxolinic acid (OXO), Difloxacin HCl (DIF), Sarafloxacin HCl (SAR), Sparfloxacin (SPA), Danofloxacin (DAN), Fleroxcain (FLE), Marbofloxacin (MAR), Enoxacin (ENO), Orbifloxacin (ORB), Pipemidic acid (PIP), Pefloxacin (PEF), Lomefloxacin (LOM), Cinoxacin (CIN), Nalidixic acid (NAL). The purities of all above standards are all ≥99%.

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

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

Experimental

Cleanert® PAX Procedure

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

Determination

(1) LC Conditions:
  a) Column: Unisol C18, 3 µm, 150 mm × 2.0 mm i.d or equivalent;
  b) Mobile phase: methanol + water containing 0.1% formic acid;
  c) Flow rate: 0.20 mL/min;
  d) Gradient elution procedure: (omitted);
  e) Temperature: ambient;
  f) Injection volume: 25 µL;
(2) MS Conditions:
  Ion source: ESI (+);
  Scan mode: SIM;
  Electrospray voltage: 4100 V;
  Capillary temperature: 350 °C;

Table 1 Gradient

Time (min)	A%	B%
0	10	90
2	10	90
4	90	10
8	90	10
9	10	90
12	10	90

Ordering Information

Products	Specification	Cat.No.
Cleanert® PAX	60 mg / 3 mL, 50/pk	AX0603
Unisol C18	3 µm, 2.1 × 150 mm, 100 Å	UO931502-0
Syringe	2 mL Without Needle	LZSQ-2ML
1.5 mL vials	Screw neck vials, 12 × 32 mm	AV1001-6
Caps and Septa	Screw neck cap, center hole; red silicone/white PTEE septa, slitted	AV2200-0

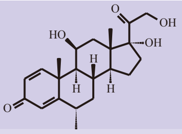
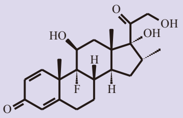
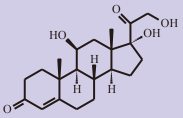
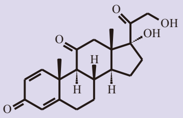
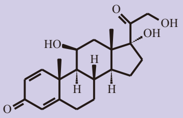
Detection of Glucocorticoids Drugs Residues in Animal-derived Foods(Cleanert® Silica, P/N: SI5006)

EMF10009

Introduction

With the anti-inflammatory and anti-allergic effect of glucocorticoid, it can provide feed conversion ratio, which will help stimulate the growth of livestock and poultry. Therefore, it is widely used in the animal husbandry industry. However, the overuse of glucocorticoid in the process of animal growth will lead to the residue of glucocorticoid in the animal-derived food, which means the extreme harm on human health. In this sense, all countries in the world attach higher importance to the monition of glucocorticoid in animal-origin food and stipulate the maximum residual amount.

Table 1 Information of Analytes

Compounds	Chemical Structure	CAS Number
MethyLprednisoLone		83-43-2
Dexamethasone		50-02-2
Hydrocortisone		0-23-7
Prednisone		53-03-2
PrednisoLone		50-24-8

Experimental

Preparation of standard stock solution

Precisely measure certain volume of prednisone, prednisolone, hydrocortisone, methyl-prednisolone, and dexamethasone. Dissolve the sample into methanol and make up to 1000 g/mL stock solution respectively. Reserve the standard solutions in the environment below minus 20 ºC.

Preparation of mixed standard working solution

Standard stock solutions of glucocorticoid drugs are accurately measured, 20% acetonitrile aqueous solution is used for preparation the mixed standard working solution of 0.2 μg, 0.5 μg/L, 1 μg/L, 2 μg/L, 5 μg/L respectively.

Sample extraction

Meat or tissue: transfer 2 g homogenized sample (+/- 0.05 g) into 50 mL centrifuge tube, add 15 mL ethyl acetate, vortex, centrifuge at 8000 rpm for 15 min, and transfer the ethyl acetate layer. Add 10 mL of 0.1 moL/L sodium hydroxide solution into the residue and mix it. Add 20 mL ethyl acetate into the tube, vortex well and centrifuge at 8000 rpm for 15 min. Extract the ethyl acetate layer and combine the two extracting solutions. Rotary evaporate at 40 ºC to near dry, dissolve the residue into constant volume using 1 mL ethyl acetate and 5 mL n-hexane for purification.

Milk or eggs: transfer 2 mL milk sample or 2 g egg sample (+/- 0.05 mL or g) into 50 mL centrifuge tube, add 20 mL ethyl acetate, vortex well and centrifuge at 8000 rpm for 15 min. Aspirate the ethyl acetate layer. Rotary evaporate at 40 ºC to near dry. Reconstitute into 1 mL ethyl acetate and 5 mL n-hexane.

Sample phase extraction

Condition: 6 mL of n-hexane;

Sample addition: apply the extract to the Cleanert® Silica and vacuum the cartridge;

Analytes Elution: 6 mL acetone / n-hexane (6/4, V/V);

Dry under nitrogen below 50 ºC and reconstitute into 0.5 mL of 20% acetonitrile in water. Centrifuge at 15000 r/min for 20 min. Filter the supernate through 0.22 membrane followed by LC-MS/MS analysis.

Instrumentation

LC columns: Venusil® ASB C18, 5 μm, 150 Å, 2.1 × 150 mm;
Mobile phases: A: Water of 0.1% formic acid; B: Acetonitrile;
Flow rate: 0.2 mL/min;
Column temperature: 30 ºC;
Injection: 10 μL;
Ion source: ESI (-);
IS: -4500 V;
GS1: 50 Psi;
GS2: 45 Psi;
TEM: 550 ºC;
Collecting mode: MRM.

Table 2 LC Gradient

Time (min)	A%	B%
0.00	62	38
2.00	62	38
8.00	55	45
8.01	10	90
10.00	10	90
10.01	62	38
15.00	62	38

Table 3 MS parameters

Analytes	Retention(min)	Ion pair monitoring	DP	EP	CE	CXP
Prednisone	4.70	403.0/327.0	-45	-10	-18	-9
		403.0/457.0	-40	-10	-18	-9
PrednisoLone	4.55	405.1/329.1	-50	-10	-22	-13
		405.1/359.1	-50	-10	-22	-13
Hydrocortisone	4.65	407.1/331.1	-45	-10	-24	-9
		407.1/361.1	-45	-10	-24	-9
MethyLprednisoLone	6.44	419.2/343.1	-45	-10	-22	-9
		419.2/373.1	-40	-10	-22	-9
Dexamethasone	7.23	437.1/361.1	-55	-10	-22	-9
		437.1/391.1	-60	-10	-22	-9

Results and Discussion

Calibration Curve

5-point calibration curve was plotted from 0.2 to 5 µg/L. Excellent method linearity is demonstrated for each analyte from 0.2 to 5 µg/L at five concentration levels.

Table 4 LC Gradient

Analytes	Regression equation	R^2
Prednisone	Y=30225x+731.14	R^2=0.9999
PrednisoLone	Y=25436x+706.91	R^2=0.9999
Hydrocortisone	Y=69494x+1167.0	R^2=0.9999
MethyLprednisoLone	Y=56491x+2313.4	R^2=0.9999
Dexamethasone	Y=33437x+952.7	R^2=0.9999

Method stability

Inject the 5 µg/L standard solution 6 times continuously and calculate the RSDs of the peak areas and retention times to evaluate the method stability. Excellent stability of the method was obtained with RSD range of 1.58-2.96% and 1.76-3.19% for retention times and peak areas.

Table 5 RSDs of peak areas and retention times of 5 kinds of glucocorticoids working solution

Analytes	RSD of retention time	RSD of peak area
Prednisone	1.85%	1.85%
PrednisoLone	1.70%	2.06%
Hydrocortisone	1.58%	1.76%
MethyLprednisoLone	2.65%	2.11%
Dexamethasone	2.96%	3.19%

Sensitivity

Table 6 LODs of 5 kinds of glucocorticoids in animal-derived food matrix

Analytes	LOD Milk matrix, µg/Kg (S/N≥10)	LOD Eggs matrix, µg/Kg (S/N≥10)	LOD Eggs matrix, µg/Kg (S/N≥10)
Prednisone	0.1	0.1	0.2
PrednisoLone	0.2	0.2	0.5
Hydrocortisone	0.1	0.1	0.2
MethyLprednisoLone	0.1	0.1	0.2
Dexamethasone	0.1	0.1	0.2

Accuracy

Table 7 Recoveries and accuracies of 1.0 µg/kg spiked pork liver samples

Analytes	Sample 1	Sample 2	Sample 3	Average recoveries	RSD
Prednisone	71.00%	65.49%	69.35%	68.61%	4.12%
PrednisoLone	66.80%	68.82%	70.23%	68.62%	2.51%
Hydrocortisone	71.16%	74.29%	79.51%	74.99%	5.63%
MethyLprednisoLone	62.38%	64.44%	63.38%	63.40%	1.62%
Dexamethasone	74.81%	79.38%	79.66%	77.95%	3.49%

Table 8 Recoveries and accuracies of 1.0 μg/kg spiked eggs samples

Analytes	Sample 1	Sample 2	Sample 3	Average recoveries	RSD
Prednisone	57.40%	61.00%	64.58%	60.99%	5.89%
PrednisoLone	43.72%	39.43%	45.99%	43.05%	7.74%
Hydrocortisone	57.06%	52.56%	56.45%	55.36%	4.41%
MethyLprednisoLone	47.60%	45.73%	47.78%	47.04%	2.41%
Dexamethasone	65.05%	57.45%	66.75%	63.08%	7.85%

Table 9 Recoveries and accuracies of 1.0 μg/kg spiked milk samples

Analytes	Sample 1	Sample 2	Sample 3	Average recoveries	RSD
Prednisone	65.02%	71.03%	58.90%	64.98%	9.33%
PrednisoLone	53.86%	50.97%	58.39%	54.41%	6.87%
Hydrocortisone	55.09%	61.38%	52.67%	56.38%	7.97%
MethyLprednisoLone	51.39%	55.28%	53.91%	53.53%	3.69%
Dexamethasone	59.03%	58.75%	62.02%	59.93%	3.02%

Table 10 Recoveries and accuracies of 1.0 μg/kg spiked pork samples

Analytes	Sample 1	Sample 2	Sample 3	Average recoveries	RSD
Prednisone	90.47%	88.03%	90.71%	89.74%	1.65%
PrednisoLone	76.09%	76.35%	80.38%	77.61%	3.10%
Hydrocortisone	84.34%	81.45%	85.76%	83.85%	2.62%
MethyLprednisoLone	83.77%	86.08%	88.24%	86.03%	2.60%
Dexamethasone	85.52%	88.40%	84.37%	86.10%	2.41%

Conclusion

This application note describes the analysis of glucocorticoids residues in animal-derived food using sample phase extraction coupled to LC-MS/MS detection. SPE for simultaneous extraction of all five analytes from animal-derived food is shown with excellent recoveries and accuracies.

Ordering Information

Products	Specification	Cat.No.
Venusil® ASB C18	5 μm, 150 Å, 2.1 × 150 mm	VS951502-0
Cleanert® Silica	500 mg/ 6 mL	SI5006
1.5 mL vials	Screw neck vials, 12 × 32 mm	AV1001-6
Caps and Septa	Screw neck cap, center hole; red silicone/white PTEE septa, slitted	AV2200-0
Syringe Filter (Nylon)	Monofilm, 13 mm, 0.22 μm	AS021320
Disposable Needle-Free injection systems	2 mL, 100/pk	LZSQ-2ML

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

EMF10008

Experimental

Muscle tissue sample

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

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

Liver tissue sample

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

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

Bovine milk sample

(1) Extraction: add 5.0 mL of sample to a 50 mL centrifuge tube. Add 0.1 mL of 18% H_2SO_4 solution and vortex mix to uniformity.

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

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

Egg sample

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

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

Ordering Information

Products	Specification	Cat.No.
Cleanert® PAX	500 mg / 6 mL, 30/pk	NH5006
Cleanert® NH_2	150 mg / 6 mL, 30/pk	AX1506

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

EAF10011

Experimental

Extraction

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

Cleanert® C18 cleanup

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

Derivatization

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

GC/ MS conditions

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

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

Ordering Information

Products	Specification	Cat.No.
Cleanert® C18	500 mg / 3 mL, 50/pk	S185003

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

EMF10033

Abstract

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

Keywords

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

Introduction

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

Experimen

Instruments, reagents and materials

(1) Main instruments and equipment
SCIEX API 4000+ HPLC-MS
(2) Reagents material
Methanol, acetonitrile, n-hexane, ethyl acetate are chromatography pure; ultrapure water; Sodium chloride, hydrochloric acid, ammonia are analytically pure; standard substance Sulfanilacetamide, Sulfachlorpyridazine, Sulfisoxazole, Sulfisoxazole, Sulfamethoxazole, Sulfamethizole, Benzenesulfonamide, Sulfapyridine, Sulfathiazole, Sulfamonomethoxine, Sulfadiazine, Sulfamerazine, Sulfamethazine, Sulfaquinoxaline, Sulfadoxine, Sulfamethoxypyridazine, Sulfameter, Sulfamonomethoxine and so on(Not less than 98% purity); 0.1mol/Lhydrochloric acid aqueous solution:add water to dilute 9 mL concentrated hydrochloric acid and capacity to 100 mL; Disposable sterilized syringe; Microporous membrane filter (0.22 μm, 13 mm in diameter); Cleanert® PCX SPE cartridge:150mg / 6mL

Sample preparation

(1) Sample extraction

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

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

(2) Sample clean-up

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

Experiment condition

(1) Liquid condition

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

Table 1. HPLC gradient elution conditions

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

(2) Mass spectrometry conditions

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

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

Results and discussion

Experimental results

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

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

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

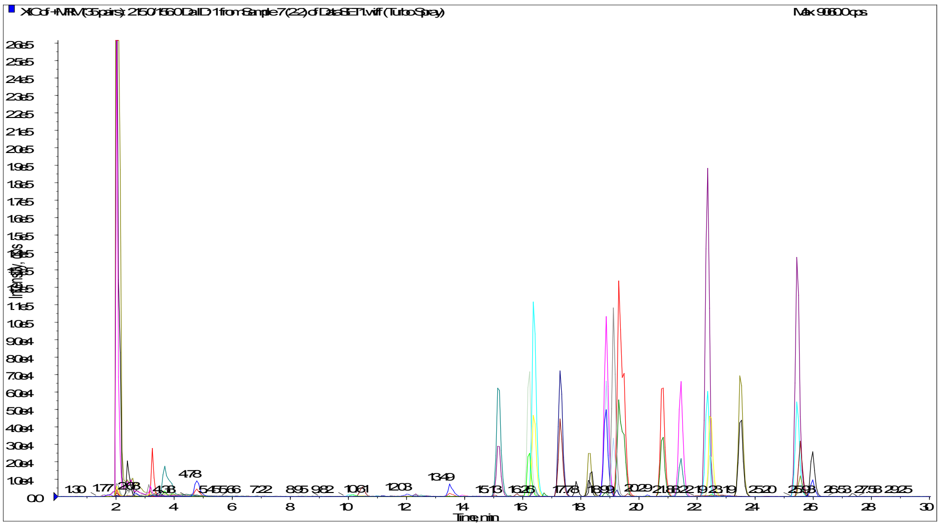


Fig 1. Liquid chromatography/tandem mass spectrometry mass spectrometry of sulfa samples with add amount of 10 μg/kg

Conclusion

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

Ordering Information

Products	Specification	Cat.No.
Venusil® ASB C18 column	3 μm, 150 Å; 2.1×150 mm	VS931502-0
Cleanert® PCX	150 mg/6 mL	CX1506
Guard cartridge holder	Suitable for 4.6×10 mm and 2.1×10 mm	SH-100
Direct-connection Guard Cartridge	3 μm, 150 Å, 2.1×10 mm	DC930102-LS
1.5 mL vial	Screw neck vial, graduated, 32×11.6 mm 9 mm center hole;	AV1001-6
Cap for 1.5 mL vial	red silicone/ white PTFE septa, slitted 45 Shore A 1.0 mm	AV2200-0
Micropore Membrane	Monofilm, 13 mm, 0.22 μm	AM021320
Syringe	2 mL Without Needle	LZSQ-2ML

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

EAF10036

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

Sample extraction

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

Purification

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

Analytical method

(1) Chromatographic conditions

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

Table 1 Gradient elution conditions of HPLC chromatography

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

Table 2 Mass spectrum parameters of fluoroquinolones

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

Results

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

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

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

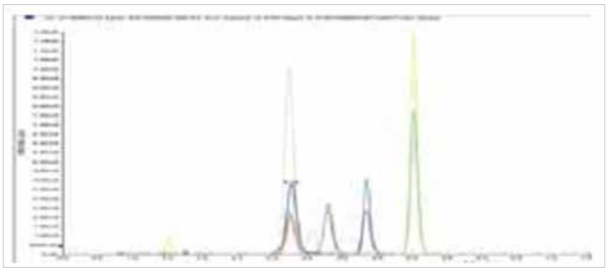


Fig.1 Chromatogram of 2.0 µg/kg fluoroquinolones in spiked pork sample (adding standards 2.0 µg/kg)

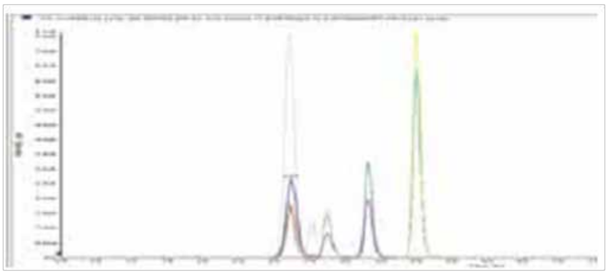


Fig.2 Chromatogram of 20 µg/kg fluoroquinolones in spiked pork sample (adding standards 20 µg/kg)

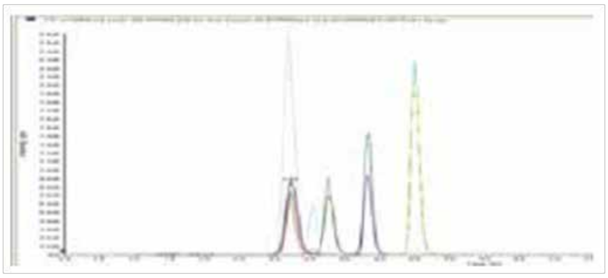


Fig.3 Chromatogram of 100 µg/kg fluoroquinolones in spiked pork sample (adding standards 100 µg/kg)

Conclusion

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

Ordering Information

Products	Specification	Cat.No.
Unisol C18	2.1×50 mm, 5 µm, 100 Å	VA950502-0
Cleanert® PEP-2	200mg / 6mL,30/pk	PE2006-2
Qdaura® Automatic SPE Workstation	4 channel, 24 position	SPE-40
Guard Column Holder	For 4.6×10 mm and 2.1×10 mm	SH-100
Direct-connected Guard Column	5 µm,150 Å, 2.1×10 mm	DC950102-LS
1.5mL Vial	Short-thread and transparent, 32×11.6 mm, 100/pk	AV1001-6
1.5mL Vial Cap	9mm center bore, blue cap, red rubber/beige PTFE pad, 45. Shore A 1.0 mm, 100/pk	AV2200-0
Syringe Filter (Nylon)	0.22 µm, diameter 13 mm, 200/pk	AS021320
Disposable Syringe	2 mL, 100/pk	LZSQ-2ML

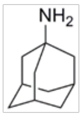
The Detection of 1-Aminoadamantane in Meat and Tissues Food with SPE-LC/MS/MS Method (Cleanert® PCX 60mg / 3mL P/N: CX0603)

EAF10025

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

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

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

Compound	CAS	Structure
1-Aminoadamantane	768-94-5	

Sample Extraction

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

Purification

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

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

Analytical Method

(1) Chromatographic conditions:

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

Table 1 Mobile phase conditions of HPLC chromatography

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

(2) Mass spectrum conditions:

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

Table 2 Mass spectrum parameters

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

"_" stands for ion detected for quantify.

Results

Table 3 Linearity and detection limit

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

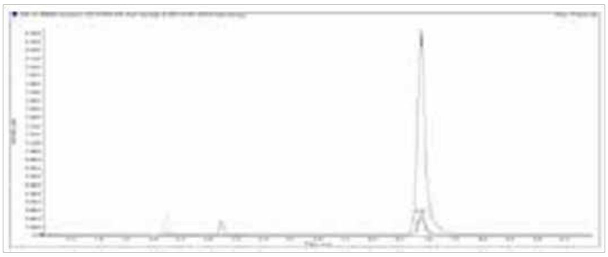


Fig.1 Standard mass spectrogram of 1-Aminoadamantane

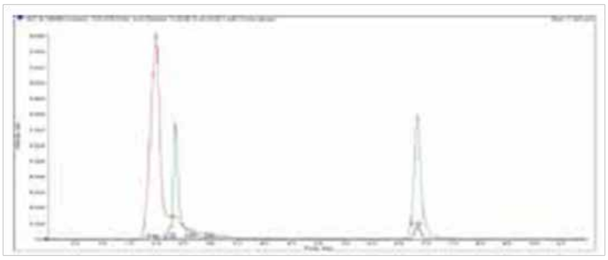


Fig.2 Mass spectrogram of 50 μg/mL 1-Aminoadamantane in spiked sample

Ordering Information

Products	Specification	Cat.No.
Qdaura®Automated SPE Workstation	4 channels, 24 positions	SPE-40
Cleanert®PCX	60mg / 3mL, 50/pk	CX0603
Venusil®ASB C18	2.1×150 mm, 3 μm, 150 Å	VS931502-0
Syringe Filter(PTFE, prefilter, double membrane)	1μm, diameter 25 mm, 100/pk	AS082501-G
Syringe Filter (Nylon)	0.22μm, diameter 13mm, 200/pk	AS021320
Disposable Needle-Free injection systems	5 mL,100/pk	ZSQ-5ML
1.5mL vial	Short-thread and transparent,32×11.6 mm, 100/pk	AV1001-6
1.5mL vial cap	9mm center bore, blue cap, red rubber/beige PTFE pad, 45.Shore A1.0 mm,100/pk	AV2200-0

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

EAF10037

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

Sample extraction

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

Purification

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

Analytical method

(1) Chromatographic conditions

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

Table 1 Gradient elution conditions of HPLC chromatography

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

(2) Mass spectrum conditions:

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

Table 2 Mass spectrum parameters of lincomycin

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

Results

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

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

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

Conclusion

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

Ordering Information

Products	Specification	Cat.No.
Venusil® ASB C18	2.1×100 mm, 5 μm, 150 Å	VS951002-0
Cleanert® PEP-2	500 mg / 6 mL, 30/pk	PE5006-2
Guard column sleeve	For 4.6×10 mm and 2.1×10 mm	SH-100
Direct-coupled protect column core	5 μm, 150 Å, 2.1×10 mm	DC950102-LS
1.5 mL vial	Short-thread and transparent, 32×11.6 mm, 100/pk	AV1001-6
1.5 mL vial cap	9 mm center bore, blue cap, red rubber/beige PTFE pad, 45. Shore A 1.0 mm, 100/pk	AV2200-0
Syringe Filter (Nylon)	0.22 μm, diameter 13 mm, 200/pk	AS021320
Disposable Needle-Free injection systems	2 mL, 100/pk	LZSQ-2ML

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

EAF10040

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

Sample Extraction

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

Purification

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

Analytical method

(1) Chromatographic conditions

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

Table 1 Gradient elution conditions of HPLC chromatography

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

(2) Mass spectrum conditions:

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

Table 2 Mass spectrum parameters of Tylosin

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

Results

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

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

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

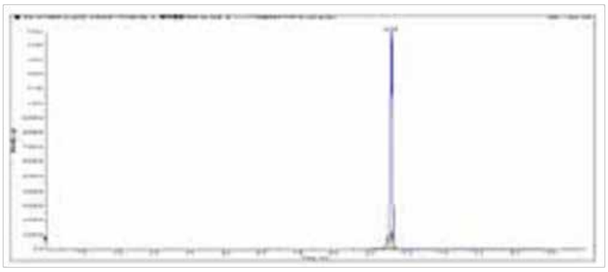


Fig.1 Chromatogram of spiked 4.0 μg/kg Tylosin in pork

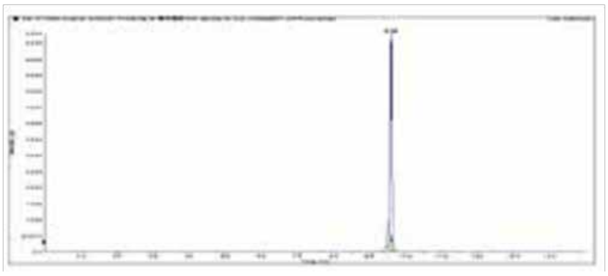


Fig.2 Chromatogram of spiked 20 μg/kg Tylosin in pork

Conclusion

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

Ordering Information

Products	Specification	Cat.No.
Unisol C18	2.1× 50 mm, 5 μm, 100 Å	UO950502-0
Cleanert® PEP-2	500 mg / 6 mL, 30/pk	PE5006-2
Guard column sleeve	For 4.6×10 mm and 2.1×10 mm	SH-100
Direct-coupled protect column core	5 μm, 150 Å, 2.1×10 mm	DC950102-LS
1.5mL vial	Short-thread and transparent, 32×11.6 mm, 100/pk	AV1001-6
1.5mL vial cap	9mm center bore, blue cap, red rubber/beige PTFE pad, 45. Shore A 1.0 mm, 100/pk	AV2200-0
Syringe Filter (Nylon)	0.22 μm, diameter 13 mm, 200/pk	AS021320
Disposable Needle-Free injection systems	2 mL, 100/pk	LZSQ-2ML

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

EAF10029

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

Sample extraction

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

Purification

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

Analytical method

(1) Chromatographic conditions:

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

Table 1 Gradient elution conditions of HPLC chromatography

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

(2) Mass spectrum conditions:

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

Table 2 Mass spectrum parameters of Tilmicosin

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

Results

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

Table 3 Recoveries and retention time for Tilmicosin (n=3)

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

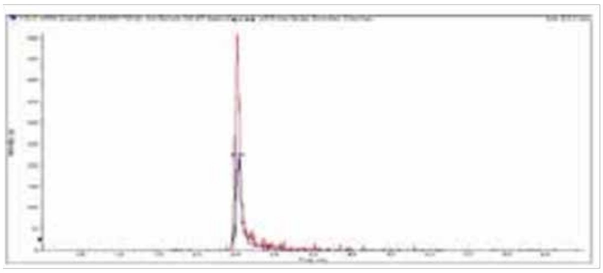


Fig.1 Chromatogram of spiked 1.0 μg/kg Tilmicosin in meat

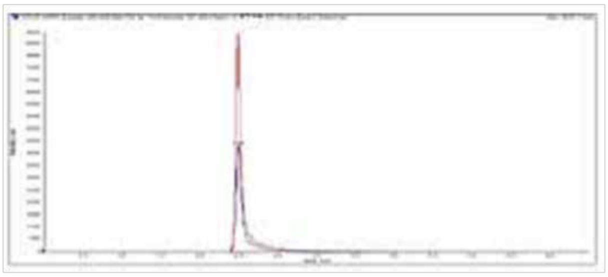


Fig.2 Chromatogram of spiked 20.0 μg/kg Tilmicosin in meat

Conclusion

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

Ordering Information

Products	Specification	Cat.No.
Venusil® ASB C18	2.1×100 mm, 5 μm, 150 Å	VS951002-0
Cleanert® PEP-2	500 mg / 6 mL,30/pk	PE5006-2
Guard Column Holder	For 4.6×10 mm and 2.1×10 mm	SH-100
Direct-connected Guard Column	3 μm, 150 Å, 2.1×10 mm	DC930102-LS
1.5 mL vial	Short-thread and transparent, 32×11.6 mm, 100 /pk	AV1001-6
1.5 mL vial cap	9mm center bore, blue cap, red rubber/beige PTFE pad, 45. Shore A 1.0 mm, 100/pk	AV2200-0
Syringe Filter (Nylon)	0.22 μm, diameter 13 mm, 200/pk	AS021320
Disposable Needle-Free injection systems	2 mL, 100/pk	LZSQ-2ML

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

EAF10034

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

Sample extraction

Weighed 5.00 g (accurate to 0.01 g) homogenized sample, then put it into 50.0 mL centrifuge tube, and 15.0 mL t-butyl methyl ether was added, vortex for 1 min and oscillation for another 10 min. Centrifuged the solution for 5 min at 6000 r/min speed at -4 °C and transfered the supernate to the condensed bottle. Extracted the residue with another 15.00 mL t-butyl methyl ether then combined the twice extracts. The solution was evaporated in the water bath at 45 °C. Then added 80 % acetonitrile solution 2 mL to the concentrated bottle, making the residue dissolved, and transferred it to the 10 mL centrifuge tube. The concentrated bottle was washed with 5 mL n-hexane and the n-hexane was transferred to the 10 mL centrifuge tube. Then centrifuged the mixed solution for 10 min at 6000 r/min at 15 °C and the supernate was removed.The lower layer would be used finally.

Purification

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

Analytical method

(1) Chromatographic conditions:

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

Table 1 Gradient elution conditions of HPLC chromatography

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

(2) Mass spectrum conditions:

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

Table 2 Mass spectrum parameters ofDiethylstilbestrol

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

Results

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

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

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

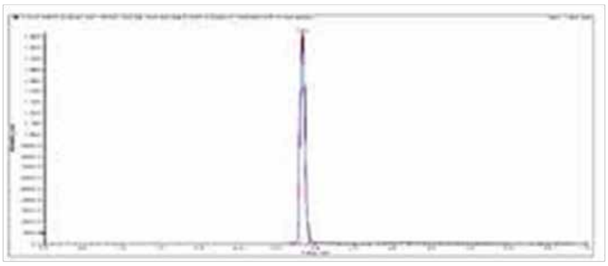


Fig.1 Chromatogram of Diethylstilbestrol
(spiked 2.5 μg/kg)

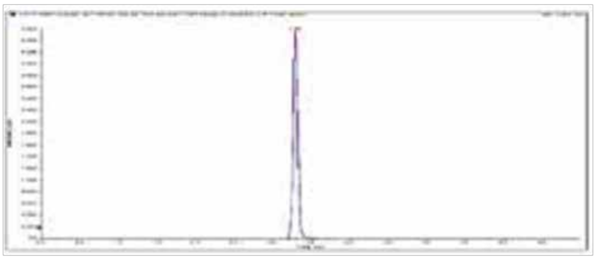


Fig.2 Chromatogram of Diethylstilbestrol
(spiked 25 μg/kg)

Conclusion

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

Ordering Information

Products	Specification	Cat.No.
Unisol C18	2.1×50 mm, 2.5 μm, 100 Å	UO920502-0
Cleanert® PEP-2	500 mg / 6 mL,30 /pk	PE5006-2
Guard Column Holder	For 4.6×10 mm and 2.1×10 mm	SH-100
Direct-connected Guard Column	3 μm, 100 Å, 2.1×10 mm	DC930102-LS
1.5 mL Vial	Short-thread and transparent, 32×11.6 mm, 100/pk	AV1001-6
1.5 mL Vial Cap	9mm center bore, blue cap, red rubber/beige PTFE pad, 45. Shore A 1.0 mm, 100/pk	AV2200-0
Syringe Filter (Nylon)	0.22 μm, diameter 13 mm, 200/pk	AS021320
Disposable Needle-Free Injection Systems	2 mL, 100/pk	LZSQ-2ML
Diethylstilbestrol	Standard, 100 mg	GBW(E)060984

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

EAF10039

Abstract

In this study, solid phase extraction combined with liquid chromatography - tandem mass spectrometry (SPE-LC/MS/MS) method were used to established the detection method of tetracycline drugs such as tetracycline, oxytetracycline and chlortetracycline in port and chicken under the multiple reaction monitoring (MRM) mode according to GB/T 21317-2007 standard. Tetracycline drugs in the samples were extracted by Na_2-EDTA-Mcllvaine buffered solution, cleaned by Cleanert® PEP-2 SPE cartridge, separated by Durashell C18 HPLC Column (2.1×150 mm, 2.5 μm, 100 Å) and quantitated by external standard method with 0.3 % formic acid and 0.3 % formic acid acetonitrile as mobile phase in gradient elution. The result indicated that when the sample amount of tetracycline drugs is 10 μg/kg, 50 μg/kg, 200 μg/kg, the recovery rate is 70%-110%, which meet the National standard.

Keywords

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

Introduction

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

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

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

Experiment

(1) Instruments, reagents and materials

1) Main instruments and equipment
SCIEX API 4500 HPLC-MS

2) Reagents material

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

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

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

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

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

5% methanol aqueous solution;

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

Acycline hydrochloride are no less than 97%;

Cleanert® PEP-2 cartridge: 60mg / 3mL

(2) Sample preparation

1) Sample extracts

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

2) Sample purification

Condition the PEP-2 cartridge with 5 mL methyl alcohol and 5 mL activated water. Take 10 mL extracting solution to pass the SPE cartridge with the speed of 1 drop/s. Wash the cartridge with 5 mL water and 5 mL 5% methanol aqueous solution after all the extracting solution outflow and discard all the effluent. Reduced pressure and swab off the cartridge for 5 min and finally elution with 10 mL methyl alcohol + ethyl acetate (1:9,v/v). Dry the eluent with N_2 under 40°C and then dissolve solution residue with 1 mL methyl alcohol solution (3:7, v/v). Filtered with 0.45 μm filter membrane for future examination.

(3) Experiment condition

1) Liquid condition

HPLC Column: Durashell C18, 2.5 μm; 100 Å; 2.1×50 mm

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

Column temperature: 30°C

Sample size: 5 μL

Gradient elution: See table 1

Table 1. HPLC gradient elution conditions

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

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

Table 2. Spectrometry conditions of tetracycline drugs

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

Results and discussion

(1) Experimental results

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

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

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

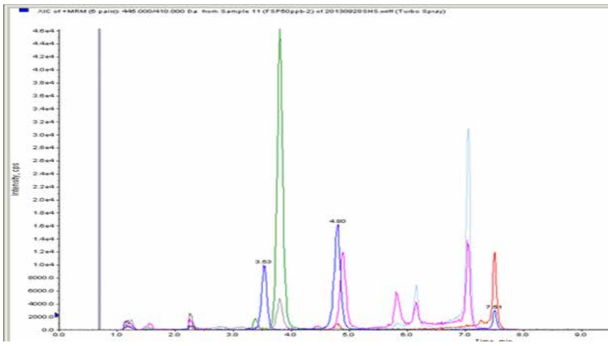


Fig 1. LC/MS spectrometry of spiked 10 μg/kg Tetracycline

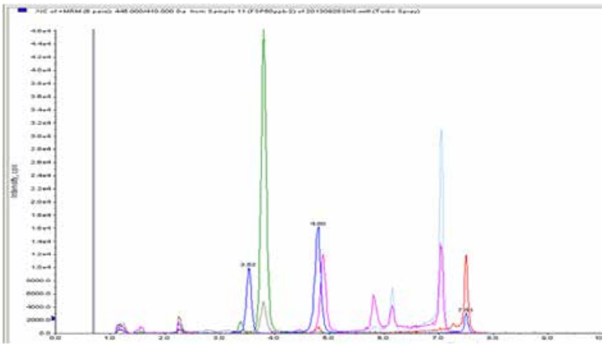


Fig 2. LC/MS spectrometry of spiked 50 μg/kg Tetracycline

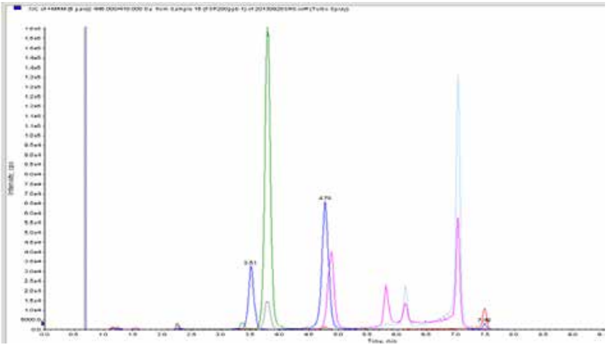


Fig 3. LC/MS spectrometry of spiked 200 μg/kg Tetracycline

Conclusion

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

Ordering Information

Products	Specification	Cat.No.
Durashell C18	2.5 μm, 100 Å; 2.1×50 mm	DC930502-0
Cleanert® PEP-2	60 mg/3 mL	PE0603-2
Qdaura® Automated SPE System	6 channels	SPE-40
Guard cartridge holder	Suitable for 4.6×10 mm and 2.1×10 mm	SH-100
Direct-connection Guard Cartridge	3 μm, 150 Å, 2.1×10 mm	DC930102-LS
1.5 mL vial	Screw neck vial, graduated, 32×11.6 mm	AV1001-6
Cap for 1.5 mL vial	9 mm center hole; blue cap; Red silicone/ white PTFE septa. 45. Shore A 1.0 mm	AV2200-0
Micropore Membrane	Monofilm, 13 mm, 0.45 μm	AS021345
Syringe	2 mL Without Needle	LZSQ-2ML

Pesticide Residues

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

EMF10004

Experimental

Extraction

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

Cleanert® TPT Cleanup

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

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

Ordering Information

Products	Specification	Cat.No.
Cleanert® TPT	2 g / 12 mL, 20/pk	TPT200010
Syringe	2 mL Without Needle	LZSQ-2ML
1.5 mL vials	Screw neck vials, 12 × 32 mm	AV1001-6
Caps and Septa	Screw neck cap, center hole; red silicone/white PTEE septa, slitted	AV2200-0

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

EMF10005

Experimental

Extraction

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

Sample Preparation

(1) Extraction

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

(2) Cleanup

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

Ordering Information

Products	Specification	Cat.No.
Cleanert® TPH	2 g / 12 mL, 20/pk	TPH200010
Syringe	2 mL Without Needle	LZSQ-2ML
1.5 mL vials	Screw neck vials, 12 × 32 mm	AV1001-6
Caps and Septa	Screw neck cap, center hole; red silicone/white PTEE septa, slitted	AV2200-0

Analysis of Multi-Pesticide Residues in Strawberry Using MAS-QuEChERS Kits by LC-MS/MS (AOAC 2007.01) (MAS-QuEChERS Extraction Kits,P/N: MS-MG5052)

EAF10140

Introduction

This experiment establishes a method for 45 kinds of pesticide residues in strawberry using MAS-QuEChERS Kits by LC-MS/MS. In this experiment, the sample is mixed with acetic acid-acetonitrile solution (V/V: 0.1%), extracted with a QuEChERS extraction kits, cleanup with a QuEChERS cleanup kits, and then detected by LC-MS/MS method, finally quantified by external standard method. The experimental results show that when the pesticide addition is 0.03 mg/kg, among the 45 kinds of pesticide residues, the recoveries of 42 kinds of pesticide residues are between 70% and 120%, conforming to the detection requirement.

Experimental

Sample Extraction

Weight 10 g homogenized strawberry sample into a 50 mL centrifuge tube. Add 10 mL 0.1% acetic acid acetonitrile for extraction. Transfer all the salts from MAS-QuEChERS Extraction Kits (P/N: MS-MG5052) and add four glass homogenizers into the sample. Shake vigorously for 1 min, and centrifuge at 6000 r/min for 5 min.

Sample Cleanup

Take 1 mL supernatant and transfer into a 2 mL MAS-QuEChERS Cleanup Tube (P/N: MS-9PP0265). Shake for 1 min and centrifuge at 6000 r/min for 5 min. Transfer the supernatant and dilute with water to 1:2 (V/V). The solution was then filtered by Nylon filter for LC-MS/MS analysis.

Instrumentation

Chromatographic columns: Unisol C18 (3 μm, 100 Å, 3.0 × 50 mm);
Mobile phases:
Phase A: Ammonium formate aqueous solution (concentration: 5 mM)
Phase B: Formic acid -methanol solution (concentration: 5 mM);
Column temperature: 30 °C;
Sample size: 5 μL;
Gradient elution conditions (see Table 1)
Ion source: ESI+; ESI voltage: 4500 V;
Atomized gas pressure: 50 psi; Curtain gas pressure: 10 psi;
Auxiliary gas pressure: 50 psi; Ion source temperature: 400 °C;
Collecting mode: MRM.

Table 1 Gradient Condition

Time (min)	Flow (mL/min)	A%	B%
0.0	0.4	95	5
0.5	0.4	95	5
1.0	0.4	60	40
3.0	0.4	15	85
4.5	0.4	5	95
6.0	0.4	5	95
6.1	0.4	95	5
12.0	0.4	95	5

Table 2 MS Parameters

Analytes	Q1	Q3	DP/V	CE/V
Methamidophos	142	94	54	19
		25	54	18
Acephate	184	143	50	10
		125	50	26
Omethoate	214	182.9	56	16
		109	56	36
Aldicarb sulfoxide	207	132	51	10
		89	51	20
Aldicarb Sulfone	223	148	63	12
		76.1	63	10
Methomyl	163	106	38	13
		88	38	13
Thiamethoxam	292	211	60	18
		181	60	32
Imidacloprid	256.1	209	60	23
		175	60	26
3-Hydroxycarbofuran	238	181	65	14
		163	65	20
Dichlorvos	221	109	70	23
		127	70	25
Trichlorfon	274	109	32	25
		220.9	32	22
Acetamiprid	223	126	70	27
		99	70	47
Dimethoate	230	125	56	29
		199	56	13
Carbendazim	192	160	80	25
		132	80	41
Aldicarb	116.1	89	51	10
		70	51	20
Carbofuran	222.1	165	70	17
		123.1	70	29
Carbaryl	202.1	145	54	15
		127	54	40
Isofenphos-methyl	273	231	39	13
		121.1	39	33
Azoxystrobin	404.1	372	70	20
		344.1	70	34
Phosmet	318	160	38	16
		133	38	49
Dimethomorph	388.1	301	115	29
		165	115	43
Malathion	331	127	64	17
		285	64	13
Triadimefon	294	197	81	21
		225	81	17

Analytes	Q1	Q3	DP/V	CE/V
Pyrimethanil	200	107	91	34
		82	91	37
Triazophos	314	162	70	25
		119.1	70	47
Fipronil	454	436.9	50	15
		367.9	50	31
Iprodione	330.1	245	50	21
		288	50	18
Diflubenzuron	311	158	72	21
		141.2	72	47
Isocarbophos-H2O	273.1	231	67	15
		121	67	32
Chlorbenzuron	309	156	75	20
		139	75	44
Diazinon	305	169	80	27
		153	80	28
Fenthion	279.1	169	78	23
		247	78	18
Phoxim	299.1	77	67	46
		129	67	16
Phosalone	368	182	71	20
		322	71	13
Prochloraz	376.2	308	65	17
		70.1	65	43
Phorate	261.1	75.2	34	14
		199	34	11
Difenoconazole	406.1	251	120	37
		337	120	23
Profenofos	373	302.9	80	25
		345.2	80	18
Emamectin B1a	886.5	158.1	120	37
		302.1	120	39
Fenpropathrin	350.2	97.1	71	43
		125.1	71	15
Chlorpyrifos	350	198	82	29
		97	82	49
Pendimethalin	282.1	212	45	15
		194	45	25
Chlorfluazuron	540	383	80	27
		158	80	24
Pyridaben	365	309	110	17
		147	110	31
Bifenthrin	440.3	181.1	40	17.6
		166.2	40	56

Results and Discussion

Table 3 Recoveries and reproducibility of pesticides in strawberry samples (spiked sample 0.03 mg/kg)

Pesticides	Retention Time/min	Recoveries/%	RSD/%
Methamidophos	1.99	77.6	17.4
Acephate	3.15	100.6	1.7
Omethoate	3.50	91.8	1.1
Aldicarb sulfoxide	3.61	93.0	2.2
Aldicarb Sulfone	3.75	93.7	1.6
Methomyl	3.96	105.2	1.2
Thiamethoxam	4.04	101.7	2.0
Imidacloprid	4.31	75.4	0.7
3-Hydroxycarbofuran	4.43	114.3	1.0
Dichlorvos	5.01	98.6	4.8
Trichlorfon	4.47	103.1	1.9
Acetamiprid	4.49	124.7	13.8
Dimethoate	4.50	85.6	10.0
Carbendazim	4.77	88.3	0.7
Aldicarb	4.82	109.8	5.4
Carbofuran	5.04	98.0	3.5
Carbaryl	5.15	96.9	2.4
Isofenphos-methyl	5.33	103.9	0.8
Azoxystrobin	5.44	99.2	2.3
Phosmet	5.51	101.3	0.4
Dimethomorph	5.63	110.4	3.3
Malathion	5.64	96.4	4.6
Triadimefon	5.67	102.2	8.4
Pyrimethanil	5.67	89.2	4.0
Triazophos	5.74	106.3	6.1
Fipronil	5.84	96.1	7.2
Iprodione	5.89	52.4	5.4
Diflubenzuron	5.95	79.5	1.4
Isocarbophos-H2O	5.95	90.6	3.8
Chlorbenzuron	6.04	63.5	4.9
Diazinon	6.09	93.3	3.7
Fenthion	6.09	81.0	10.2
Phoxim	6.13	98.2	2.6
Phosalone	6.15	92.8	3.3
Prochloraz	6.18	96.0	0.4
Phorate	6.23	102.3	5.3
Difenoconazole	6.26	100.2	10.7
Profenofos	6.48	94.4	0.2
Emamectin B1a	6.52	86.8	1.2
Fenpropathrin	6.77	101.2	6.3
Chlorpyrifos	6.80	98.3	0.4
Pendimethalin	6.87	102.6	7.2
Chlorfluazuron	7.00	84.5	3.7
Pyridaben	7.24	100.5	3.6
Bifenthrin	7.76	86.3	12.0

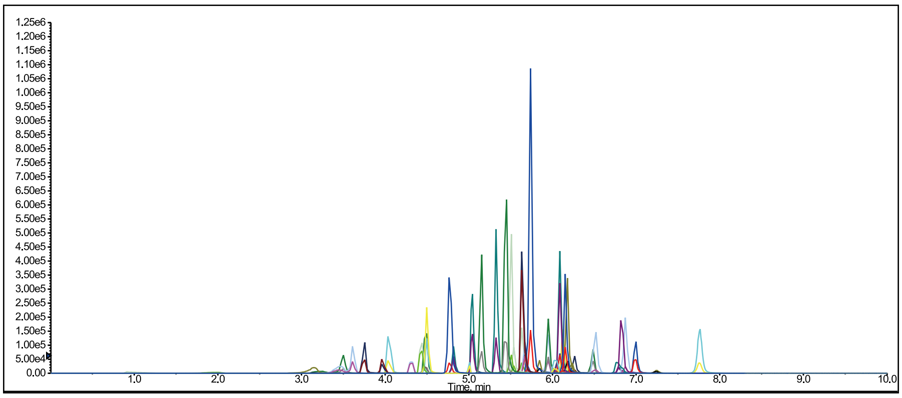


Figure 1 Chromatogram of 0.01 μg/mL standard solution of pesticides

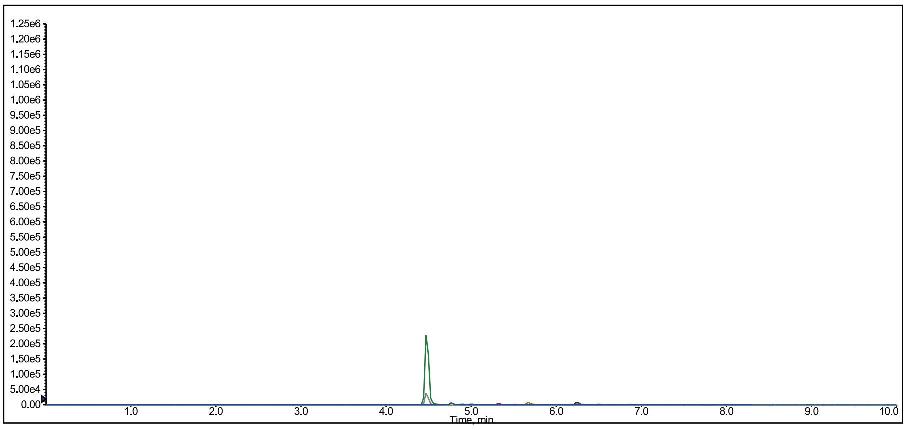


Figure 2 Chromatogram of strawberry blank sample

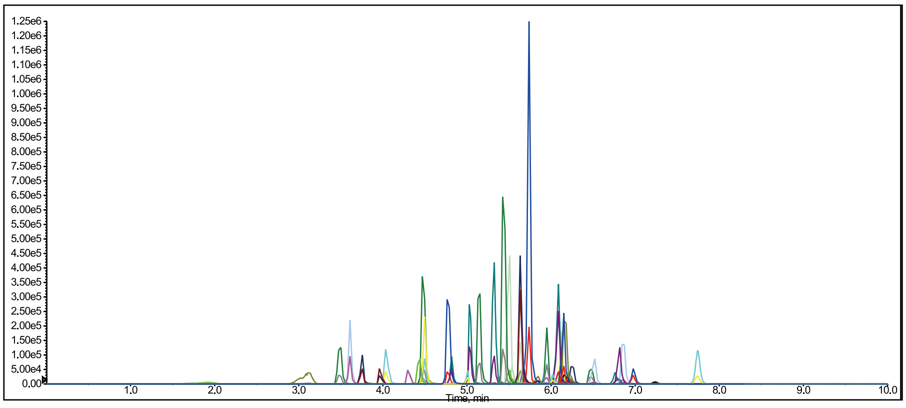


Figure 3 Chromatogram of spiked strawberry sample (0.03 mg/kg)

Conclusion

The detection results show that, the recoveries of 42 kinds of pesticide residues among the 45 kinds are conforming to the requirement and RSD is no more than 20% using MAS-QuEChERS Kits, proving this method is stable and applicable to the detection of the 42 kinds of pesticide residues in the strawberry.

Ordering Information

Products	Specification	Cat.No.
MAS-QuEChERS Extraction Kits	6 g $MgSO_4$ (Anhydrous), 1.5 g NaAc (Anhydrous) with 50 mL centrifuge tube	MS-MG5052
MAS-QuEChERS Cleanup Tube	50 mg PSA, 50 mg C18, 8 mg PC, 150 mg $MgSO_4$ (Anhydrous) with 2 mL centrifuge tube	MS-9PP0265
Glass homogenizers	Diameter: 10 mm	HG-01
Unisol C18	3 μm, 100 Å, 3.0 × 50 mm	UO930503-0
1.5 mL vials	Screw neck vials, 12 × 32 mm	AV1001-6
Caps and Septa	Screw neck cap, center hole; red silicone/white PTEE septa, slitted	AV2200-0
Nylon Syringe Filters	13 mm, 0.22 μm	AS021320
Disposable Syringe	2 mL, no needle	LZSQ-2ML

Simultaneous Detection of 152 Kinds of Pesticide Residues in Cabbage and Apple by LC-MS/MS using QuEChERS (MAS-QuEChERS Extraction Kits, P/N: MS-MG5052)

EAF10002

Introduction

The QuEChERS method is established according to AOAC standard to extract and detect 152 types of pesticides in two matrices - cabbages and apples - at the same time.

Experimental

Sample Extraction

Cabbage: Weigh 15 g of cabbage sample with a good homogeneity, and transfer it into a 50 mL vegetable & fruit extraction tube. Add 15mL of acetic acid-acetonitrile solution (concentration: 1%), Bonna-Agela MAS-QuEChERS vegetable & fruit extraction package (P/N: MS-MG5052) and 4 glass beads to the tube in turn. Keep violently oscillating the tube manually for 1min and keep centrifuging the tube at 8000 rpm for 5 min, and then reserve 1 mL of supernate for purification.

Apple: Weigh 15 g of apple sample with a good homogeneity, and transfer it into a 50 mL vegetable & fruit extraction tube. Add 15mL of acetic acid-acetonitrile solution (concentration: 1%), Bonna-Agela MAS-QuEChERS vegetable & fruit extraction package (P/N: MS-MG5052) and 4 glass beads to the tube in turn. Keep violently oscillating the tube manually for 1min and keep centrifuging the tube at 8000 rpm for 5 min, and then reserve 1 mL of supernate for purification.

Sample Purification

Cabbage: Add the aforesaid cabbage supernate to be purified to a 2 mL Bonna-Agela MAS-QuEChERS vegetable & fruit purification tube (P/N: MS-PA0250). Keep oscillating the tube manually for 1min and keep centrifuging the tube at 8,000rpm for 5min. Then filter proper amount of supernate with a 0.22 μm nylon membrane for LC-MS detection.

Apple: Add the aforesaid apple supernate to be purified to a 2 mL Bonna-Agela MAS-QuEChERS vegetable & fruit purification tube (P/N: MS-9PA0203). Keep oscillating the tube manually for 1min and keep centrifuging the tube at 8000 rpm for 5 min. Then filter proper amount of supernate with a 0.22 μm nylon membrane for LC-MS detection.

Preparation of matrix-mixed standard working solution

Dilute the high-concentration pesticide-mixed standard solution with blank sample matrix solution to the matrix-mixed standard working solution (concentration: 0.03 μg/mL).

Instrumentation

Chromatographic columns: Venusil® ASB C18 (5 μm, 150 Å, 2.1 × 150 mm);
Mobile phases:
A: Formic acid aqueous solution (concentration: 0.1%)
B: Formic acid -acetonitrile solution (concentration: 0.1%);
Column temperature: 30 °C;
Sample size: 10 μL;
Gradient elution conditions (see Table 1)
Ion source: ESI+; ESI voltage: 5500 V;
Atomized gas pressure: 50 psi; Curtain gas pressure: 15 psi;
Auxiliary gas pressure: 50 psi; Ion source temperature: 400 °C;
Collecting mode: MRM.

Table 1 LC Gradient Elution

Time (min)	Flow (mL/min)	A%	B%
0.00	0.3	99	1
3.00	0.3	70	30
6.00	0.3	60	40
9.00	0.3	60	40
15.00	0.3	40	60
19.00	0.3	1	99
23.00	0.3	1	99
23.01	0.3	99	1
40.00	0.3	99	1

Results and Discussion

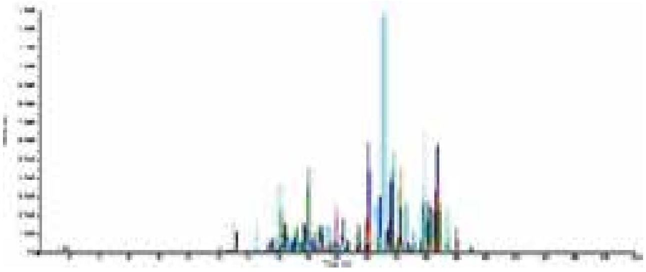


Figure 1 LC-MS/MS Chromatogram of Cabbage Matrix-mixed Standard Working Solution (concentration: 0.03 μg/mL)

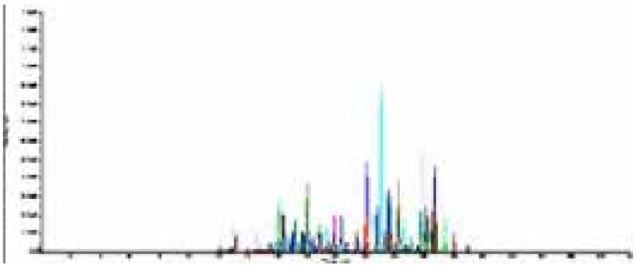


Figure 2 LC-MS/MS Chromatogram of Cabbage Matrix Sample with Standard Addition (content: 0.03 mg/kg)

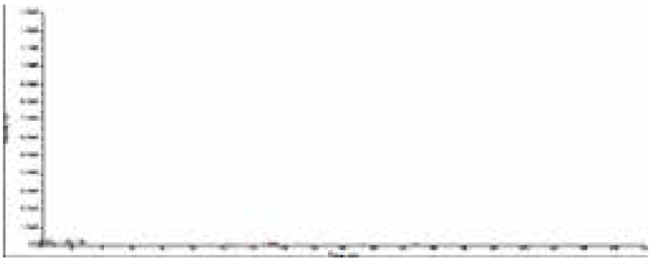


Figure 3 Blank LC-MS/MS Chromatogram of Apple Matrix

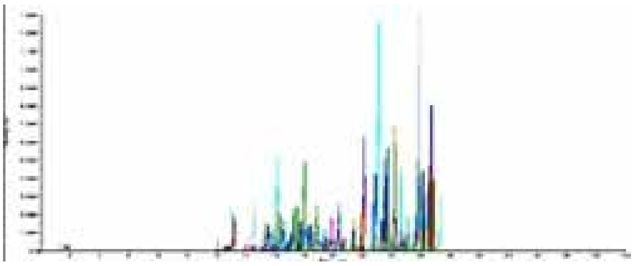


Figure 4 LC-MS/MS Chromatogram of Apple Matrix-mixed Standard Working Solution (concentration: 0.03 μg/mL)

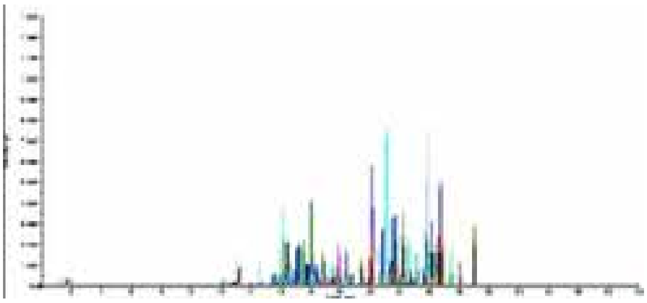


Figure 5 LC-MS/MS Chromatogram of Apple Matrix Sample with Standard Addition (concentration: 0.03 mg/kg)

Table 2 Accuracies and reproducible of 152 kinds of Pesticide Residues in Apple and Cabbage (n=3) (spiked level: 0.03 mg/kg)

Analytes	Apple		Cabbage		
	Recoveries (%)	RSD/%	Recoveries (%)	RSD/%	RT/min
Chlordimeform	81.5	7.0	88.6	7.1	1.4
Aldicarb-sulfoxide	85.6	4.4	83.5	5.1	12
Dinotefuran	99.3	1.6	94.6	8.6	12.1
Aldicarb-sulfone	81.0	8.9	73.2	7.1	12.2
Oxamyl	86.0	4.0	84.0	8.3	12.7
Demeton-S-methylsulfoxide	105.1	4.9	97.7	9.1	12.9
Monocrotophos	86.2	5.3	93.5	8.7	13.1
Trichlorfon	81.3	3.9	91.3	9.7	13.3
Thiofanox-sulfoxide	85.7	6.9	85.0	3.2	13.9
Clothianidin	79.6	3.5	67.0	9.1	14.3
Dimethoate	91.5	3.2	75.2	8.4	14.5
Thiofanox-sulfone	81.9	4.0	74.0	9.1	14.6
ImazamethabenzMethyl	97.4	7.7	66.4	7.5	15
Acetamiprid	69.5	2.2	64.4	5.6	15.2
Cymoxanil	71.0	8.2	72.8	4.9	15.2
Flumetsulam	93.8	9.0	106.2	6.9	15.3
Oxycarboxin	94.5	2.8	63.0	5.0	15.5
Aldicarb	97.2	2.5	83.5	8.7	15.8
Carbetamide	66.0	6.2	68.6	5.5	16
Imazethapyr	83.0	5.8	60.6	8.6	16
Tebuthiuron	101.2	5.0	75.3	6.2	16.1
Thiacloprid	101.1	8.4	65.1	6.3	16.1
Fenpropimorph	108.2	8.9	88.1	3.7	16.2
Phosphamidon	97.9	8.2	98.4	8.8	16.2
Phoxim	75.5	8.0	99.6	9.5	16.2
Spiroxamine	85.4	6.4	102.9	7.9	16.2
Metolcarb	90.5	4.2	107.4	6.9	16.3
Isouron	87.3	6.6	74.5	5.8	16.6
Thidiazuron	83.2	5.2	70.3	9.4	16.6
Cyanazine	109.1	3.9	69.9	7.9	16.7
Demeton-S-methyl	79.0	9.3	88.7	7.9	16.9
Foramsulfuron	87.0	4.1	94.5	7.7	16.9
Azamethiphos	78.2	7.1	101.7	5.0	17
Thiophanate-methyl	90.0	4.3	92.7	9.3	17.2
Lenacil	78.6	9.8	82.6	8.6	17.2
Imazaquin	85.9	6.9	74.0	9.0	17.3
Thifensulfuron-methyl	81.3	6.7	75.6	6.5	17.3
Carbofuran	61.9	4.0	75.8	8.8	17.4
Cinosulfuron	93.0	4.3	77.2	9.0	17.7
Metsulfuron-methyl	90.9	3.1	75.3	9.9	17.7
Florasulam	78.8	6.8	78.9	4.5	17.8

Analytes	Apple		Cabbage		
	Recoveries (%)	RSD/%	Recoveries (%)	RSD/%	RT/min
Methabenzthiazuron	77.4	7.0	74.8	9.8	18
Thiodicarb	96.5	1.8	60.3	7.0	18.1
Triasulfuron	74.1	9.4	69.7	8.6	18.1
Chlortoluron	87.5	9.8	69.7	6.0	18.2
Carbaryl	78.0	5.8	67.4	2.2	18.2
Diuron	83.4	6.2	75.0	8.7	18.3
Fluometuron	78.8	0.5	75.0	5.3	18.3
Carboxin	78.6	9.6	70.7	9.3	18.4
Amidosulfuron	92.0	9.6	86.1	7.1	18.4
Chlorsulfuron	82.3	7.1	79.7	6.9	18.5
Monolinuron	92.2	7.3	86.9	8.1	18.5
Cyprodinil	79.5	4.6	90.9	8.9	18.5
Ethiofencarb	96.0	9.0	107.2	6.3	18.5
Rimsulfuron	79.3	4.9	106.4	7.1	18.5
Forchlorfenuron	83.9	7.0	66.6	7.7	18.7
Ethametsulfuron-methyl	90.4	9.0	75.3	9.9	18.8
Furametpyr	84.5	6.4	80.2	7.0	18.9
Isoproturon	104.4	7.5	71.9	6.8	19.1
Dimethirimol	84.2	1.8	109.7	7.6	19.3
Metosulam	82.3	7.9	79.6	5.4	19.3
Bupirimate	93.4	2.5	109.1	8.0	19.3
Azimsulfuron	91.2	5.7	75.5	7.4	19.4
Mesosulfuron-methyl	92.0	0.4	88.2	9.2	19.4
Azaconazole	76.7	7.1	83.0	0.7	19.8
Flumorph	95.8	8.3	82.2	8.6	19.8
Demeton	83.8	2.5	75.6	9.9	19.9
Thiophanate	88.2	5.8	77.1	8.4	20.3
Cloransulam-methyl	83.4	9.1	77.8	5.2	20.3
Sulfosulfuron	101.8	9.8	92/8	3.4	20.3
Chlorantraniliprole	97.3	5.2	69.0	7.8	20.6
Bensulfuron-methyl	66.8	2.3	70.9	6.5	20.6
Nicosulfuron	99.2	5.5	81.2	9.0	20.6
Fensulfothion	86.3	9.1	74.4	8.2	20.7
Flazasulfuron	97.3	9.8	77.8	7.2	20.8
Diclosulam	85.3	2.6	84.3	4.0	20.9
Imazosulfuron	103.8	9.6	62.0	6.9	20.9
Spinetoram	80.8	4.5	81.0	8.5	21.1
Propoxycarbazone	76.3	2.9	91.8	7.3	21.3
Methiocarb	105.3	9.2	74.6	8.4	21.3
Iodosulfuron-methyl	70.6	7.8	76.4	8.3	21.4
Diethofencarb	97.8	1.9	68.9	5.6	21.4
Linuron	82.0	0.2	68.9	4.3	21.5

Analytes	Apple		Cabbage		
	Recoveries (%)	RSD/%	Recoveries (%)	RSD/%	RT/min
Prochloraz	97.4	9.4	75.7	9.4	21.5
Desmedipham	90.1	3.0	66.4	5.3	21.7
Phenmedipham	109.4	2.2	66.4	8.3	21.8
Ethiprole	76.0	3.6	75.4	6.5	21.9
Promecarb	76.6	0.8	86.9	8.8	21.9
Iprovalicarb	84.1	0.9	84.7	7.5	22.1
Prosulfuron	92.6	8.2	74.1	8.3	22.2
Pyrazosulfuron-ethyl	97.0	8.6	80.6	8.0	22.2
Paraoxon-methyl	89.7	5.7	85.9	3.6	22.5
Fenamiphos	76.4	1.0	81.0	8.9	22.6
Spirotetramat	78.6	8.9	78.9	9.1	22.6
Chloroxuron	66.3	6.4	77.5	5.2	22.7
Cumyluron	99.5	3.6	74.5	7.7	22.7
Mefenacet	92.9	3.1	82.3	7.4	22.7
Fenhexamid	82.6	4.8	78.2	6.2	22.7
Mepanipyrim	95.8	6.1	75.5	8.2	22.8
Halosulfuron Methyl	80.3	8.2	80.6	7.6	22.9
Azoxystrobin	69.3	1.4	74.2	1.7	23
Boscalid	95.4	5.1	76.4	9.6	23
Fluopicolide	86.0	5.7	85.7	4.5	23.3
Mandipropamid	90.9	9.7	74..9	5.8	23.4
Metolachlor	87.7	9.2	86.9	7.9	23.5
Triflusulfuron-methyl	89.9	9.7	69.8	9.5	23.5
Diflubenzuron	79.5	3.3	76.1	7.7	23.7
Methoxyfenozide	78.3	2.8	76.5	4.9	23.7
Cyclosulfamuron	79.6	5.0	83.2	9.5	23.9
Ametoctradin	92.9	7.1	75.5	7.1	24
Cafenstrole	107.4	8.7	80.9	6.2	24.1
Thenylchlor	89.5	5.8	80.6	5.0	24.1
Fenbuconazole	95.4	5.1	78.8	9.5	24.2
Chlorbenzuron	97.8	4.5	66.7	7.2	24.2
Fenoxycarb	104.9	3.1	76.5	8.0	24.3
Rotenone	98.7	6.1	67.4	8.3	24.4
Edifenphos	88.6	1.6	72.5	3.5	24.5
Alanycarb	66.8	2.4	61.0	4.3	24.6
Tebufenozide	109.1	9.3	80.6	5.0	24.7
Propisochlor	81.1	4.1	88.7	7.2	24.8
Fluthiacet-Methyl	99.1	2.3	74.5	6.1	24.9
Pyrazoxyfen	101.9	9.9	84.4	7.0	24.9
Isoxadifen-Ethyl	91.0	4.3	71.7	9.4	25.1
Flubendiamide	91.2	1.0	77.9	6.1	25.1
Triflumuron	103.4	7.3	83.9	9.0	25.2

Analytes	Apple		Cabbage		
	Recoveries (%)	RSD/%	Recoveries (%)	RSD/%	RT/min
Pyraclofos	83.9	3.5	78.6	7.9	25.3
Bensulide	74.7	2.6	73.5	7.6	25.6
Chlorimuron-ethyl	72.4	4.8	72.8	6.5	25.6
Pyraclostrobin	97.8	3.3	75.1	7.5	25.7
Clofentezine	70.9	9.6	68.8	8.6	25.8
Pencycuron	87.8	4.7	81.9	6.0	25.8
Difenoconazole	89.1	1.3	77.2	9.1	25.8
Coumaphos	87.8	7.2	81.6	7.0	25.9
Cycloxydim	85.8	7.0	69.3	9.8	26
Haloxyfop-R-methyl	91.8	9.0	85.9	7.4	26.1
Diflufenican	71.7	7.5	79.4	6.9	26.2
Hexaflumuron	90.2	8.1	89.1	8.8	26.2
Isoxathion	96.7	8.0	77.3	4.6	26.2
Indoxacarb	95.8	8.3	67.2	9.0	26.3
Quizalofop-ethyl	86.9	5.5	76.9	6.5	26.3
Clethodim	74.1	2.0	67.1	3.8	26.4
Sethoxydim	92.6	6.3	68.3	1.6	26.5
Imibenconazole	89.2	8.2	70.7	9.9	26.6
Cinidon-ethyl	78.5	1.3	72.3	9.4	26.7
Furathiocarb	81.9	3.0	86.9	7.7	26.7
Tralkoxydim	74.0	2.8	68.3	8.9	26.9
Flucarbazone-sodium	75.3	9.8	69.2	9.1	27.3
Temephos	74.2	2.2	65.6	8.0	27.3
Flufenoxuron	73.2	9.8	72.7	5.9	27.3
Butralin	75.5	8.0	107.1	6.6	27.4
Spiromesifen	79.6	3.0	74.8	9.5	28
Carbosulfan	84.5	5.3	77.0	9.9	29

Conclusion

This experiment establishes the QuEChERS pretreatment method for pesticide residues in cabbage and apple in accordance with standard AOAC2007.01, and detects the samples (standard addition amount: 0.03mg/kg) on the basis of LC-MS/MS method. The resulted recovery rates are between 60% and 110%, conforming to the national standard requirements. Moreover, the data obtained by QuEChERS method are relatively stable, showing this method can be applied to the detection of pesticide residues in cabbage and apple.

Ordering Information

Products	Specification	Cat.No.
Venusil® ASB C18	5 µm, 150 Å, 2.1 × 150 mm	VS951502-0
MAS-QuEChERS Extraction Kits	6 g $MgSO_4$ (Anhydrous), 1.5 g NaAc (Anhydrous) with 50 mL centrifuge tube	MS-MG5052
MAS-QuEChERS Cleanup Kits	50 mg C18, 50 mg PSA, 150 mg $MgSO_4$ (Anhydrous), with 15 mL centrifuge tube	MS-9PA0203
MAS-QuEChERS Cleanup Kits	2 mL Centrifuge tube for 1 mL sample, 50 mg PSA, 150 mg $MgSO_4$	MS-PA0250
Guard cartridge holder	Suitable for 4.6 × 10 mm and 2.1 × 10 mm	SH-100
Direct-coupled protect column core	5 µm, 150 Å, 2.1×10 mm, 4/pk	VS950102-0S
1.5 mL vials	Screw neck vials, 12 × 32 mm	AV1001-6
Caps and Septa	Screw neck cap, center hole; red silicone/white PTEE septa, slitted	AV2200-0
Syringe Filter (Nylon)	Monofilm, 13 mm, 0.22 µm	AS021320
Disposable Needle-Free injection systems	2 mL, 100/pk	LZSQ-2ML

Simultaneous Detection of 112 Kinds of Pesticide Residues in the Black Tea using Extraction-type QuEChERS (Cleanert® NANO, P/N: IC-NN1010-V)

EAF10004

Introduction

To increase production and income, the tea gardens have long been using highly effective pesticides to control diseases and pests, leading to excessive pesticide residues in some of the tea products. Black tea as a kind of drink is a target for strict regulation of detection limit in many countries. And this article also introduces the QuEChERS-LC-MS/MS method for extracting and testing 112 types of pesticide residues in black tea.

Experimental

Sample Extraction

Weigh 2.0 g of tea powder, and transfer it in a 50 mL centrifuge tube. Add 10 mL of water into the tube, and leave it stand still for 1h. Add 15 mL of acetonitrile, 4.0 g of magnesium sulfate and 1.0 g of sodium chloride (P/N: MS-MG5055). Keep violently oscillating the tube manually for 1min and keep centrifuging the tube at 8,000 rpm for 5min, and then reserve 1 mL of supernate for purification.

Sample Purification

Connect Bonna-Agela Cleanert® NANO special purification columns (P/N: IC-NN1510-C) for agricultural complex matrixes in series with 10 mL one-time needle tubes. Extract 1 mL of extracting liquid, and then push out the liquid. Repeat this step for 3 times. Filter the liquid with a 0.22 μm organic membrane for the next LC-MS/MS determination.

Preparation of matrix-mixed standard working solution

Dilute the high-concentration pesticide-mixed standard solution with blank sample matrix solution to the matrix-mixed standard working solution (concentration: 0.02 μg/mL).

Instrumentation

Chromatographic columns: Venusil® ASB C18 (5 μm, 150 Å, 2.1 × 150 mm);
Mobile phases:
    A: Formic acid aqueous solution (concentration: 0.1%)
    B: Formic acid -acetonitrile solution (concentration: 0.1%);
Column temperature: 30 °C;
Sample size: 10 μL;
Gradient elution conditions (see Table 1);
Ion source: ESI source;
Scanning mode: Positive ion scanning;
ESI voltage: 5500 V;
Atomized gas pressure: 50 psi;
Curtain gas pressure: 15 psi;
Auxiliary gas pressure: 50 psi;
Ion source temperature: 400 °C;
Collecting mode: MRM.

Table 1 LC Gradient Elution Conditions

Time (min)	Flow (mL/min)	A%	B%
0.00	0.30	99	1
3.00	0.30	70	30
6.00	0.30	60	40
9.00	0.30	60	40
15.00	0.30	40	60
19.00	0.30	1	99
23.00	0.30	1	99
23.01	0.30	99	1
40.00	0.30	99	1

Results and Discussion

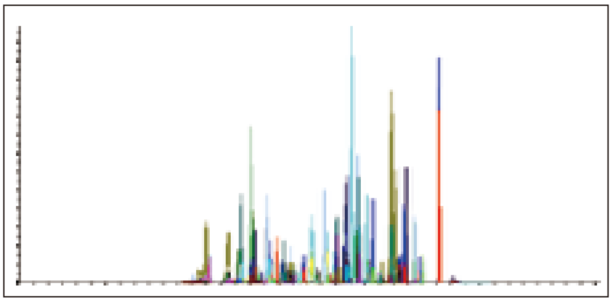


Figure 1 LC-MS/MS Chromatogram of Mixed Standard Solution (concentration: 0.02 μg/mL)

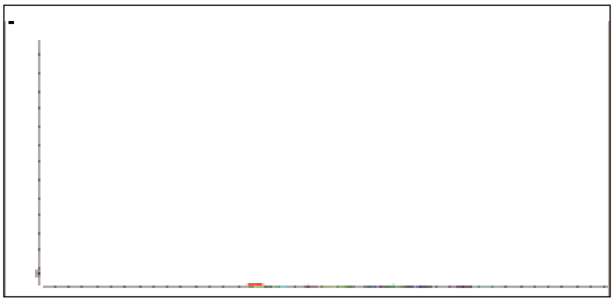


Figure 2 LC-MS/MS Chromatogram of Blank Black Tea Sample

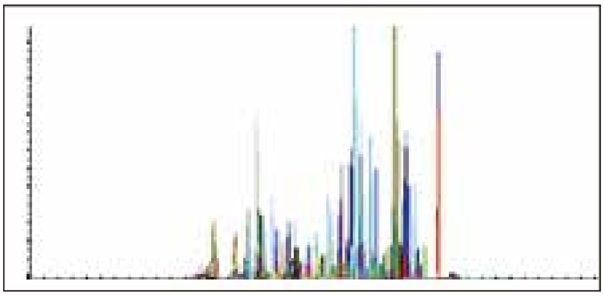


Figure 3 LC-MS/MS Chromatogram of Black Tea Matrix–mixed Standard Working Solution (concentration: 0.02 μg/mL)

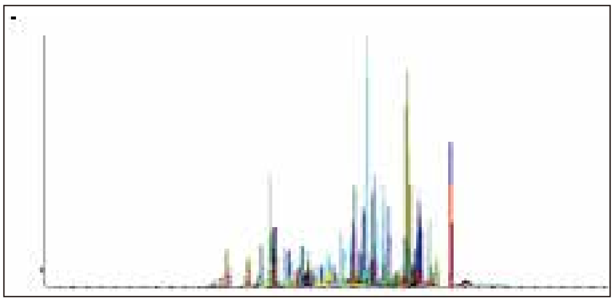


Figure 4 LC-MS/MS Chromatogram of Black Tea Matrix Sample with Standard Addition (content: 0.15 mg/kg)

Conclusion

This experiment establishes the LC-MS/MS method for detecting pesticide residues, and determines the contents of pesticide residues in the black tea on the basis of solid phase extraction technology. The samples (standard addition amount: 0.15 mg/kg) are detected, and the resulted recovery rates are between 60% and 110%, conforming to requirements. The stable QuEChERS effect and good reproducibility of chromatographic columns show this method can be applied to the detection of pesticide residues in the black tea.

Ordering Information

Products	Specification	Cat.No.
Venusil® ASB C18	5 µm, 150 Å, 2.1 × 150 mm	VS951502-0
Cleanert® NANO MAS-QuEChERS Cleanup Kits	10 mg Nano, 150 mg $MgSO_4$	IC-NN1010-V
Cleanert® NANO MAS-QuEChERS Cleanup Kits	15 mg Nano, 150 mg $MgSO_4$	IC-NN1510-C
Guard cartridge holder	Suitable for 4.6 × 10 mm and 2.1 × 10 mm	SH-100
Direct-coupled protect column core	5 µm, 150 Å, 2.1×10 mm, 4/pk	VS950102-0S
1.5 mL vials	Screw neck vials, 12 × 32 mm	AV1001-6
Caps and Septa	Screw neck cap, center hole; red silicone/white PTEE septa, slitted	AV2200-0
Syringe Filter (Nylon)	Monofilm, 13 mm, 0.22 µm	AS021320
Disposable Needle-Free injection systems	2 mL, 100/pk	LZSQ-2ML

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

EMF10003

Experimental

Sample Preparation

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

Extraction

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

Florisil Cleanup

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

Results

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

Table 1 Recoveries of tomato and cole samples

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

Ordering Information

Products	Specification	Cat.No.
Cleanert® Florisil	1 g / 6 mL, 30/pk	FS0006

Detection of Food Additives

Detection Phthalates in Different Food Matrices

EMF10012

Introduction

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

Experimental

Suggested Processing Method

Applicative Sample

Bottled Drinking water and Soft drink

SPE Process

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

Analytical Method

HPLC analysis

Ordering Information

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

Applicative Sample

Milk, jam,syrup, milk powder, biscuits, cakes, jelly or candy

Sample-treatment

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

Take the supernatant , after filtration and then for analysis.

Analytical Method

GC/MS analysis

Ordering Information

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

Sample

Vegetable oil, cheese or other animal tissues

Extraction

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

SPE process

activation: 5 mL of hexane

Sample loading: transfer all the sample upside

Elute: 3 mL×2 of ethyl acetate : hexane combination (50:50, v / v).

Evaporate the elution to nearly dryness with N_2, add 1 mL of acetonitrile for re-extraction, vortex for 3 min, vortex for 3 min, centrifugal for 5 min at 4000 rpm, take the supernatant for analysis.

Analytical Method

HPLC analysis

Ordering Information

SPE Cartridge	Cleanert® PAE, Cat. No:PAE30006-G, 30/pk Cleanert® PAE (Sample with colorant); Cat.No:PAE30006-C; 30/pk

Method 1 The detection of 15 pthalate esters with HPLC

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

Elution gradient:

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

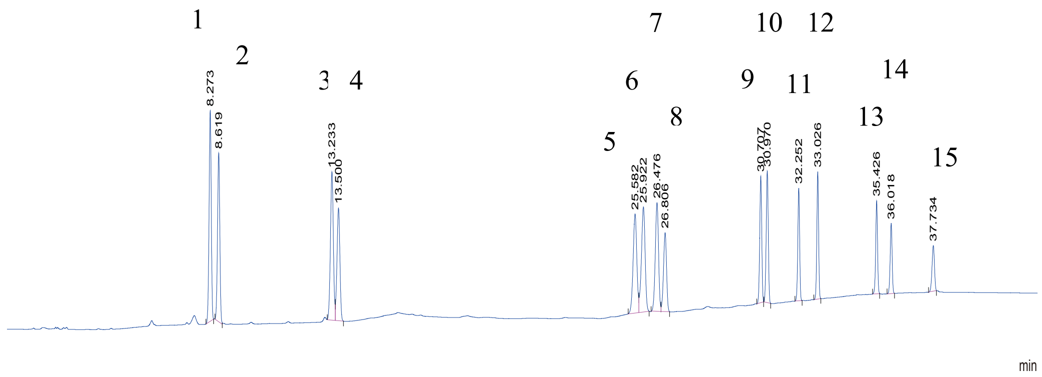


Fig 1. The chromatogram for pthalate esters standard (sample concentration: 10 ppm)
(1-DMP, 2-DEP, 3-DBP, 4-DEHP, 5-BBP, 6-DEHP, 7-DMEP, 8-DBEP, 9-DPP, 10-BMPP, 11-DEEP, 12-DCHP, 13-DIBP, 14-DNP, 15-DINP)

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

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

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

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

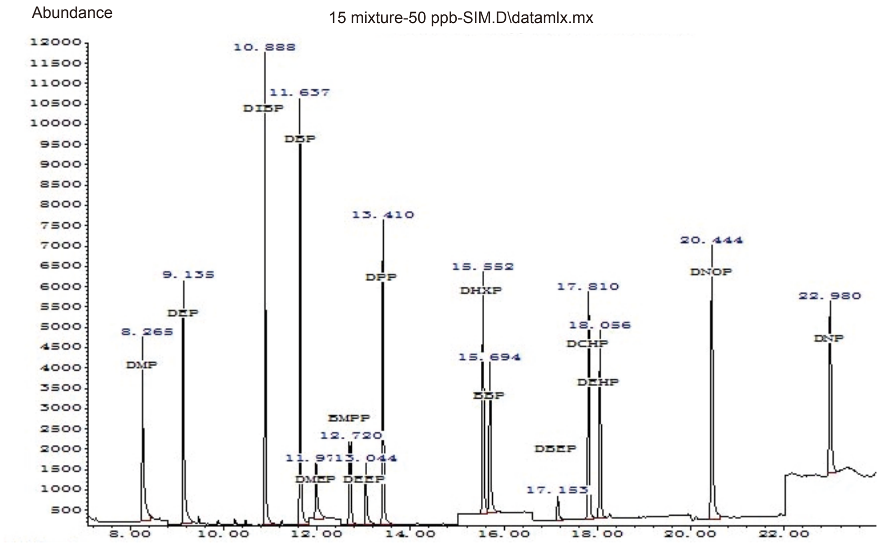


Figure 2. Detection of 15 pthalates using GC-MS method

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

The recoveries of 15 phthalate esters in vegetable oil

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

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

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

EAF10095

Introduction

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

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

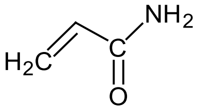


Figure 1. Structural formula of acrylamide

Experimental

Instruments, Reagents and Materials

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

Sample preparation

Sample extract

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

Sample purification

Manual operation

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

Qdaura® automatic solid phase extraction apparatus operation procedure

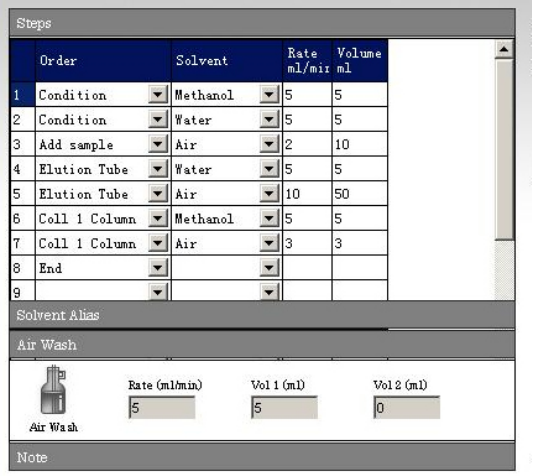
First install Cleanert® ACA solid phase extraction cartridge on the instrument, and inject samples into the sample loading tube, automatic operation according to the procedure in the following figure:

HPLC Method

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

Mass spectrometry conditions

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



Steps

	Order	Solvent	Rate ml/mir	Volume ml
1	Condition	Methanol	5	5
2	Condition	Water	5	5
3	Add sample	Air	2	10
4	Elution Tube	Water	5	5
5	Elution Tube	Air	10	50
6	Coll 1 Column	Methanol	5	5
7	Coll 1 Column	Air	3	3
8	End			
9				

Solvent Alias

Air Wash

Rate (ml/min): 5
Vol 1 (ml): 5
Vol 2 (ml): 0

Figur 2. Automatic solid phase extraction operation procedure

Table 1 Mass spectrum parameters of acrylamide

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

Results

Linear relation

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

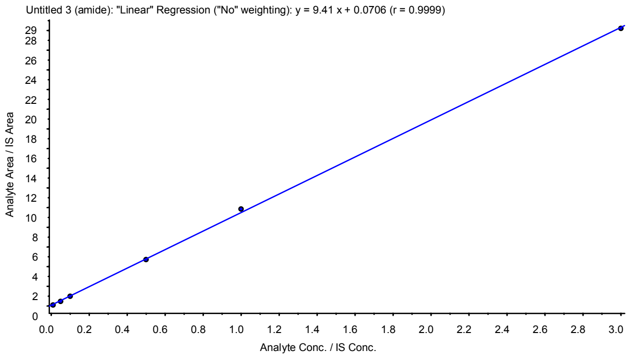


Figure 3. Standard solution curve of acrylamide

Table 2 Standard curve and the detection limit of acrylamide

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

Experimental results

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

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

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

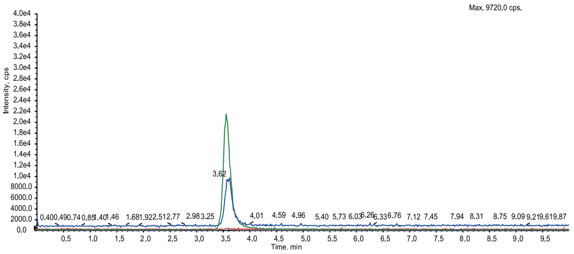


Figure 4. 0.05 mg/L LC-MS/MS spectra of acrylamide standard solution

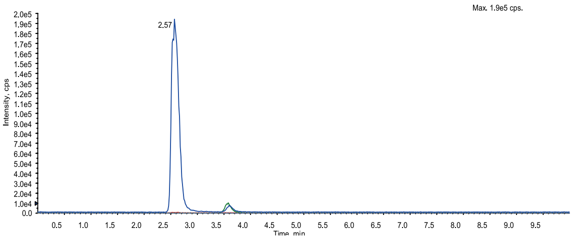


Figure 5. Matrix blank LC - MS/MS spectra of chips sample

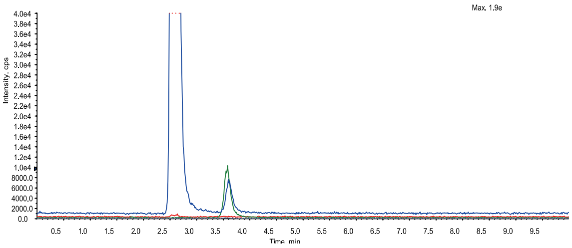


Figure 6. Enlarged spectra of chips sample

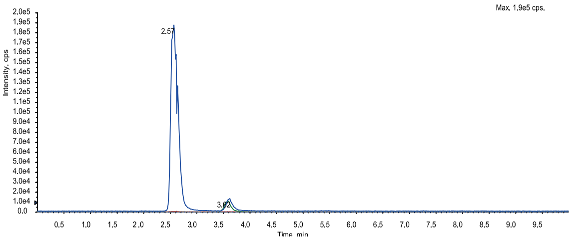
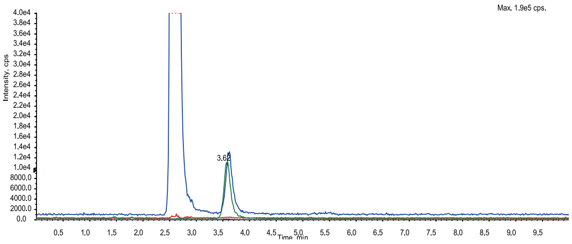


Figure 7. LC-MS/MS spectra of matrix spiked 0.1 mg/kg chips sample



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

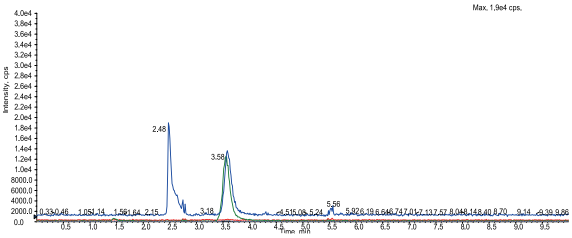
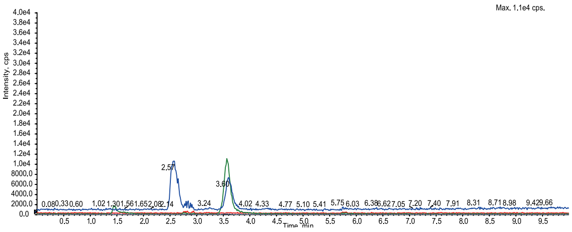


Figure 9. LC-MS/MS spectra of fried bread stick sample matrix standard work solution



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

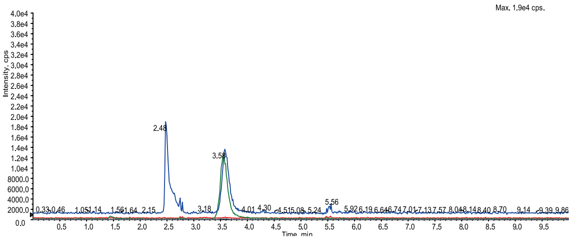


Figure 11. LC-MS/MS spectra of spiked 0.1 mg/k fried bread stick sample matrix

Ordering Information

Products	Specification	Cat.No.
Venusil® AQ C18	5 μm,100 Å, 2.1 × 150 mm	VA951502-0
Cleanert® ACA	200 mg/6mL	ACA2006
Qdaura® automatic solid-phase extraction apparatus	Maximal 24 sample, four channels	SPE-40
Nitrogen blowing instrument	Maximal 15 position	NV15-G
1.5 mL sample bottle	short thread transparent belt with writing place 32 × 11.6 mm	AV1001-6
1.5 mL sample bottle cap	9 mm center hole with blue cover, red rubber/beige PTFE insulation pad, 45°Shore A; 1.0 mm	AV2200-0
Syringe Filter(Nylon)	monofilm, 13 mm, 0.22 μm	AS021320
disposable syringe	2 mL needle-free	LZSQ-2ML

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

ERF10007

Introduction

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

Material

SPE cartridge

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

Standard solution preparation

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

Saturated sodium chloride solution

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

Experimental

GC-MS operating conditions

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

Sample preparation

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

Result

Chromatogram of standard solution

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

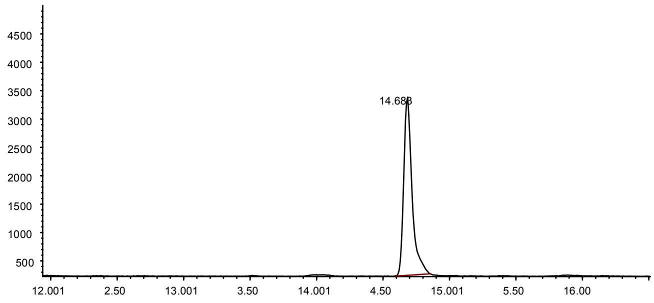


Figure1 Chromatogram of standard solution
(concentration of 50 ng/mL)

Chromatogram of samples

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

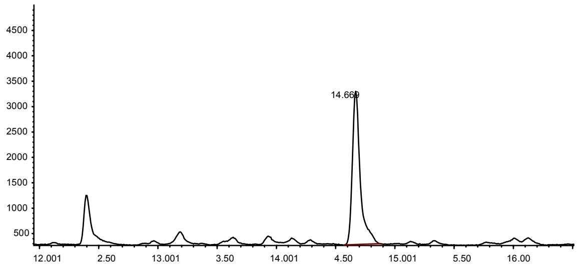


Figure 2 Chromatogram of spiked soy sauce sample
(concentration of 50 ng/mL)

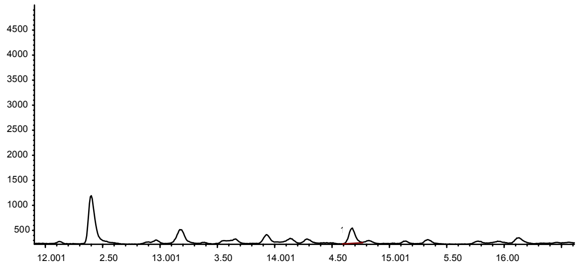


Figure 3 Chromatogram of soy sauce sample

Table 1 Recovery rate and precision

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

Ordering Information

Products	Specification	Cat.No.
Vacuum manifold	12 positions	VM12
Cleanert® MCPD column	4 g/30 mL, 15/pk	LBC400030
Cleanert® MAS-MCPD	2 g, 50/pk	MS-MCPD01
DA-5MS GC columns	30 m × 0.25 mm × 0.25 μm	1525-3002
1.5 mL sample bottle	short thread transparent belt with writing place 32 × 11.6 mm, 100/pk	AV1001-6
1.5 mL sample bottle cap	9 mm center hole with blue cover, red rubber/beige PTFE insulation pad, 45. Shore A1.0 mm, 100/pk	AV2200-0

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

ERF10001

Introduction

As a strong carcinogen, Benzopyrene is able to cause cancers in skin, lung and digestive tract. Among 200 discovered polycyclic aromatic hydrocarbons and their derivatives, benzopyrene has the highest carcinogenicity and pollution degree. Oil crops in the drying, baking and refining processes and animal and vegetable oil after heated by high temperature are extremely vulnerable to benzopyrene contamination. The contaminated animal and vegetable oil seriously poses a serious threat to human health. In this paper, a simple and fast sample pretreatment method is created through the Cleanert® BAP solid-phase extraction column to guarantee accuracy and reproducibility of the detection of benzopyrene in the oil.

Experimental

Sample Preparation

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

SPE Method

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

Experimental

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

Results

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

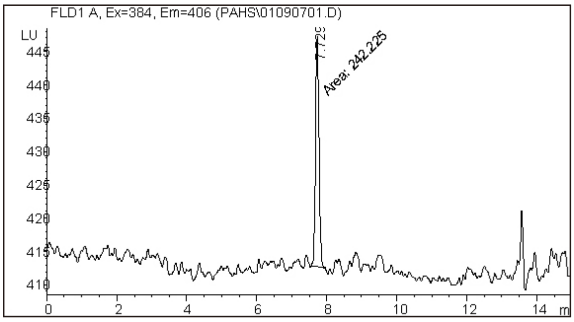


Figure1 Chromatogram of 5 μg/L benzo (a) pyrene standard sample

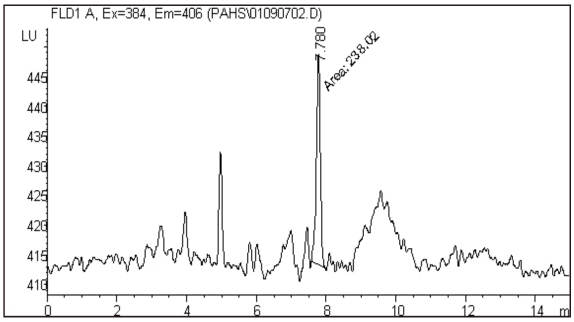


Figure 2 Actual sample chromatogram of a vegetable oil

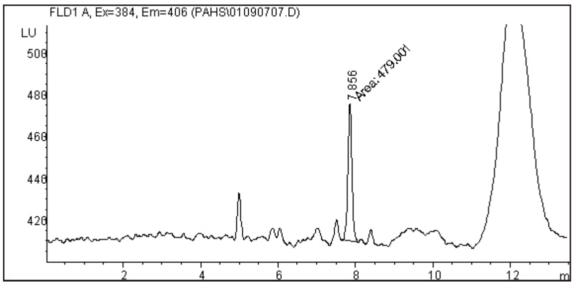


Figure 3 Chromatogram of a vegetable oil with the spiked matter amount of 5 μg/kg

Table1 Recovery rate of benzo (a) pyrene

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

Ordering Information

Products	Specification	Cat.No.
Cleanert® BAP	22 g/60 mL	BAP2260-0
Venusil® ASB C18	4.6×250 mm, 5 μm	VS952505-0

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

EMF10032

Overview

The determination of benzo(a)pyrene in oil generally used prepacked alumina column, and big volume of elution is needed and take lots of labor and time.

Bonna-Agela develop a new SPE product with much more convenient method. The main challenge is to extract hydrophobic Benzo(a)pyrene from oil matrix. Hexane was chosen as the best solvent to dissolve vegetable oil after investigation.

In the new method, 0.5 g vegetable oil was dissolved in 3 mL hexane and loaded onto the Cleanert® BAP-3 cartridge. 10mL hexane was used to wash the cartridge and 5mL methylene chloride to elute the Benzo(a)pyrene out. After drying the eluant with nitrogen blowing, 1mL acetonitrile was used to reconstitute the sample. The detection was carried out on HPLC with fluorometric detection. In addition, sesame oil contained more impurities which was need to be treated with Cleanert® SI (500 mg/6 mL) firstly. This new method show better performance, and could be used for many kinds of vegetable oil.

Reagents and apparatus

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

Experimental

Sample Preparation

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

SPE Process:
(1) Condition: 5 mL Dichloromethane and 5ml Hexane;
(2) Load all the 0.5 g sample onto the cartridge;
(3) Washing: 10ml Hexane;
(4) Elution: 5 mL Dichloromethane.

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

HPLC Condition

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

Results

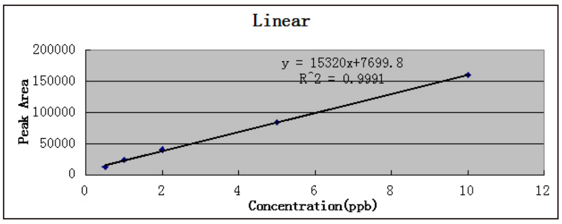


Figure 1 Benzo(a)pyrene calibration curve

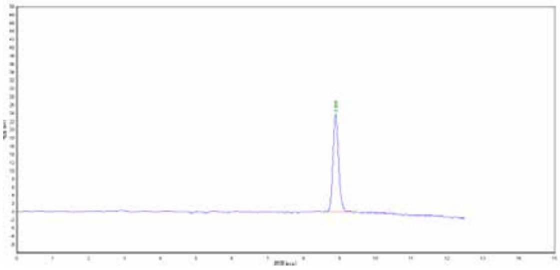


Figure 2 Benzo(a)pyrene standard solution (10μg/L)

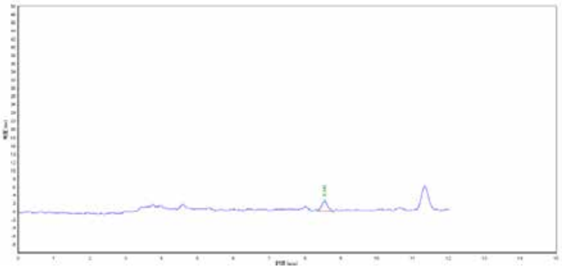


Figure 3 HPLC chromatogram of blank oil sample

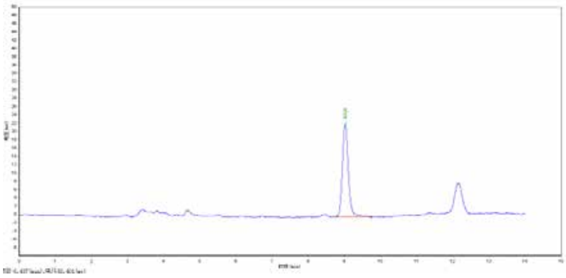


Figure 4 HPLC chromatogram of sample spiked with Benzo(a)pyrene(10μg/L)

Table1. Recoveries of Benzo(a)pyrene

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

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

Ordering Information

Products	Specification	Cat.No.
Cleanert® BAP-3	500 mg/6 mL	BAP5006
Cleanert® Si	500 mg/6 mL	Si5006
Venusil® ASB C18	4.6×250 mm, 5 μm	VS952505-0
NV-8G Evaporator	8 holes, 0-10 L/min (0-100 psi), 5-80 °C	NV-8G
Vacuum manifold	12 Positions	VM12

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

EAF10048

Material

(1) Instruments and reagents

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

(2) HPLC condition

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

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

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

Introduction

In this paper, the Cleanert® PCX is used for pretreatment of egg samples; Venusil® ASB C8 is used for the detection of chromatographic column; the proportion of buffer salt used as mobile phase (10 mM citric acid +10mM sodium 1-octanesulfonate, pH=3.0) and acetonitrile is 93:7; the detection wavelength is 240nm and the flow rate 1.0mL/min. The results show that this method has a recovery rate of 97~121% and good linear relationship (R2=0.999), thus it can be widely used in the detection of melamine in eggs.

Experimental

Sample preparation

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

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

SPE Process Cleanert® PCX (60mg / 3mL)

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

Results

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

Table1 stable data of retention time and peak area ratio

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

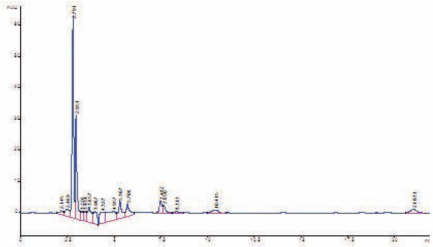


Figure 1 blank sample

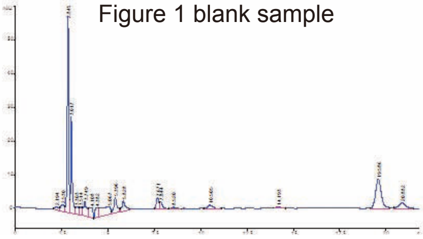


Figure 2 Profile of sample spiked with 10 ppm melamine.

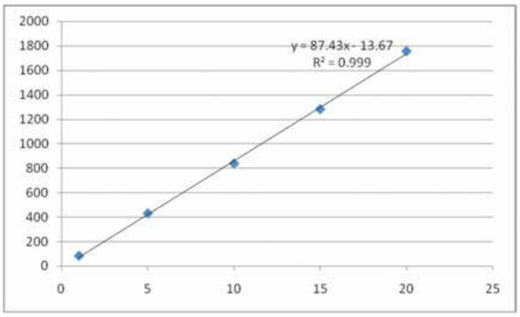


Figure 3 Regression curve of density and peak area ratio

Table 2 Experimental data of standard adjusted curve

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

Table 3 Recoveries of melamine in eggs

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

The application note indicated that excellent separation was gained along with good recoveries in all three concentration levels.

Ordering Information

Products	Specification	Cat.No.
Cleanert® PCX	60 mg/3 mL, 50/pk	CX0603
Venusil® ASB C8	4.6 × 250 mm, 5 μm	VS852505-0
LC-10F HPLC	10 mL/min, 200-800 nm	FL-LC010GS
Column Oven	5 – 70 °C; Max. 2 columns of 300mm	CC-100
Qdaura® Automated SPE Workstation	4 channels 24 positions	SPE-40
Disposable Needle-Free injection systems	5 mL,100/pk	ZSQ-5ML
1.5 mL vials	Screw neck vials, 12 × 32 mm	AV1001-6
Caps and Septa	Screw neck cap, center hole; red silicone/white PTEE septa, slitted	AV2200-0
Syringe Filter(Nylon)	0.45 μm, diameter 13 mm, 200/pk	AS021345

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

EAF10049

Experimental

Red chili and other powder samples

Put 1.00 to 5.00 g sample in a flask and then add 15 mL n-hexane. Extract the sample under sonication for 5 minutes followed by filtration. Use additional 10 mL hexane by batch to rinse the sample residue several times to colorless. Rotovap the combined supernatant to obtain sample concentrate in less than 5 mL.

Cleanert® Alumina N Cleanup: Slowly transfer the concentrate to Alumina N cartridge to reduce broadening of the pigment band. Wash the loaded cartridge with 10-30 mL hexane until the eluate is colorless, and discard the eluate. Elute the cartridge with 60 mL n-hexane/acetone (95:5, v/v) and collect the eluate. Evaporate the collected eluate in a rotovap. Transfer the final concentrate to a vial and bring up to 5 mL using acetone. Filter the solution through 0.45 μm membrane before analysis.

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

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

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

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

Sausage and other meat products

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

Ordering Information

Products	Specification	Cat.No.
Cleanert® Alumina-N	500 mg / 6 mL, 30/pk	AL5006-N

Detection of Malachite Green and Crystal Violet Residues in Fish (Cleanert® Alumina-N,P/N: AL0003-N)

EAF10146

Introduction

Malachite green, green triphenylmethane dyes and toxic triphenylmethane chemicals, can be used as drugs to kill fungi, bacteria and parasites. China's government has forbidden the use of malachite green in pollution-free aquaculture due to its carcinogenicity in long-term overuse.

In this paper, the simultaneous detection of malachite green, leuco malachite green, crystal violet and leuco crystal violet in fish was established.

Experimental

Material and Reagents

HPLC purity of acetonitrile; experimental water (ultra-pure water); analytical pure ammonium acetate;

Standard solution mix: solution of malachite green, leuco malachite green, crystal violet, leuco crystal violet in acetonitrile;

Internal standard solution: dissolve 2 ng/mL deuterated malachite green and deuterated leuco malachite green in acetonitrile: 5 mmol/L ammonium acetate solution (1:1, V/V);

Cleanert® Alumina N, 1000 mg/3 mL.

Sample Extraction

Transfer 5 g homogenized fish sample, 200 µL 2 ng/mL internal standard solution and 11 mL acetonitrile into a 50 mL centrifuge tube. Then sonicate for 2 min, homogenize at 8000 rpm for 30 s and centrifuge at 4000 rpm for 5 min. Put all the supernatant into a 25 mL colorimetric tube. Wash the homogenate knife head with 11 mL acetonitrile in another centrifuge tube for 10 s and transfer the liquid to the centrifuge tube of sample residue. Pound the residue with glass rod, vortex for 30 s, sonicate for 5 min, then centrifuge at 4000 rpm for 5 min. Blend the two supernatant, reconstitute into 1 mL of acetonitrile and shake for sample preparation.

Sample Preparation

Activate Cleanert® Alumina-N with 5 mL acetonitrile. Load 5 mL of extraction onto the cartridge, collect the eluent into a KD concentrate bottle, elute the cartridge with 4 mL acetonitrile, and then collect the eluent into the same KD concentrate bottle. Rotary evaporate under 45°C to less than 1 mL, reconstitute into 1 mL of acetonitrile and sonicate for 5 min. Then add into 1 mL 5 mmol/L ammonium acetate, sonicate for 1 min and filter residue through 0.22 µm nylon filter.

Above steps were operated on Qdaura® Automated SPE Workstation.

Instrumentation

Column: Unisol C18, 3 μm, 100 Å, 3.0 × 50 mm;
Mobile phase: A: 0.1% formic acid; B: Acetonitrile;
Flow rate: 0.3 mL/min;
Column temperature: 30 °C;
Injection: 5 μL;
Ion source: ESI+;
Electrospray voltage:5500 V;
Nebulizer pressure: 50 psi;
Curtain gas pressure: 25 psi;
Auxiliary gas pressure: 50 psi;
Ion source temperature: 500 °C;
Scan mode: MRM.

Table 1 Gradient

Time (min)	A%	B%
0.0	60	40
1.0	60	40
2.0	10	90
6.0	10	90
6.1	60	40
10.0	60	40

Table 2 MS Parameters

Analytes	Q1	Q3	DP/V	CE/V
Malachite green	329.1	313.2	95.1	49.7
		208.2	95.1	46.9
Leuco malachite green	331.1	239.3	105.1	42.0
		316.3	105.1	30.5
Crystal violet	372.4	251.4	112.1	53.4
		358.4	112.1	47.3
Leuco crystal violet	374.2	239.2	114.8	40.2
		318.2	114.8	49.1
D5- Malachite green	334.0	318.2	97.0	53.1
		213.3	97.0	48.9
D6- Leuco malachite green	337.4	240.2	107.4	40.8
		322.4	107.4	31.5

* underlined were quantitative ions

Results and Discussion

Table 3 shows that the recoveries of spiked samples ranged from 90% to 115% and the CVs were under 10% using Cleanert® Alumina-N by LC-MS/MS to determinate malachite green and crystal violet in fish. Typical method performance results were well within acceptable criteria. Figure 1 shows a good separation was obtained by using Unisol C18 column to detect malachite green and crystal violet simultaneously.

Table 3 Recoveries of malachite green and crystal in spike samples (2.5 μg/kg)

Analytes	Retention Time/min	Recoveries/%	RSD/%
Malachite green	1.65	90.3	4.4
Leuco malachite green	4.17	98.1	1.8
Crystal violet	3.87	113.7	2.7
Leuco crystal violet	1.46	97.0	1.5

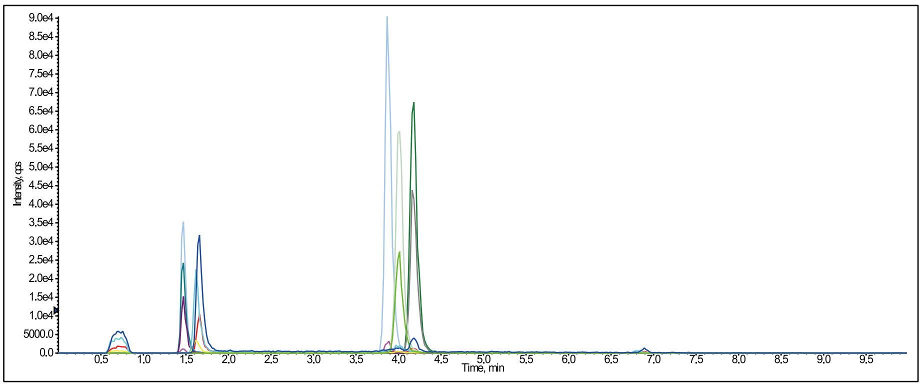


Figure 1 Chromatogram of 0.01 μg/mL standard sample mix

Conclusion

In this study, the sample preparation methods of malachite green and crystal violet in fish were established, and the samples were detected by LC-MS / MS. The result shows that the overall recoveries ranged from 90% to 115% and the CVs were lower than 10% for 2.5 μg/kg spiking fish samples, which meets the testing requirements. This study indicates that the method was suitable for detection of malachite green and crystal violet in fish.

Ordering Information

Products	Specification	Cat.No.
Cleanert® Alumina-N	1000 mg/3 mL	AL0003-N
Qdaura® Automated SPE Workstation	4 channels, 24 positions	SPE-40
Unisol C18	3 μm, 100 Å, 3.0 × 50 mm	UO930503-0
Nitrogen evaporator	Maximal 15 samples	NV15-G
1.5 mL vials	Screw neck vials, 12 × 32 mm	AV1001-6
Caps and Septa	Screw neck cap, center hole; red silicone/ white PTEE septa, slitted	AV2200-0
Syringe Filter (Nylon)	Monofilm, 13 mm, 0.22 μm	AS021320
Disposable Needle-Free injection systems	2 mL, 100/pk	LZSQ-2ML

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

EAE10063

Introduction

Chlorophenols (CPs) exist in waste water of chemical and paper-making industries. Most of CPs with strong toxicity are difficult to be biodegraded, and can accumulate in organisms. Therefore, CPs have been listed as one of main organic pollutants by EPA of America. This paper conducts the sample pretreatment to five kinds of CPs in river water by Qdaura® Automatic SPE System, and compares it with the manual pretreatment method.

Experimental

Materials

SPE Cartridge: Cleanert® PEP (500 mg / 6 mL)
Adjust the pH of 500 mL of water sample with 0.5 mol/L H2SO4 to 1.5~2.0.

SPE Method

Activation: activate the PEP cartridge with 5 mL of methanol and 5 mL of water sequentially.
Sample loading: load water sample onto the cartridge at a flow rate of below 5 mL/min.
Washing: Wash the cartridge with 5 mL of pure water and dry the cartridge under nitrogen stream for 20 min. Wash the cartridge with 0.8 mL of methanol (stand for 2 min to ensure methanol to soak packing material thoroughly) and discard the eluate.
Elution: Elute the cartridge with 5 mL of furanidine. Collect the eluate and concentrate under nitrogen stream at 30 °C to 1 mL for HPLC determination.

Instrumentation

Column: Unisol C18, 4.6 mm × 150 mm, 5 μm, 100 Å
Mobile phase: A, water with 0.1% formic acid; B, methanol with 0.1% formic acid
Injection: 20 μL
Temperature: 30 °C
Detection UV: 285 nm

Table 1 Gradient Elution Condition

Time (min)	Flow Rate (mL/min)	A%	B%
0	1	35	65
6	1	35	65
15	1.8	5	95
15.1	1	35	65
22	1	35	65

Results and Discussion

Table 2 Recoveries and reproducibility of spiked water sample (n=3, 1 μg/L)

Analytes	Manual Method		Qdaura Method	
	Recoveries/%	RSD/%	Recoveries/%	RSD/%
1-Chlorophenol	78.89	6.76	76.38	4.46
2-Chlorophenol	82.44	7.47	89.51	1.99
3-Chlorophenol	60.96	10.24	71.15	3.29
4-Chlorophenol	86.04	9.07	76.63	2.3
5-Chlorophenol	86.63	10.01	89.01	4.81

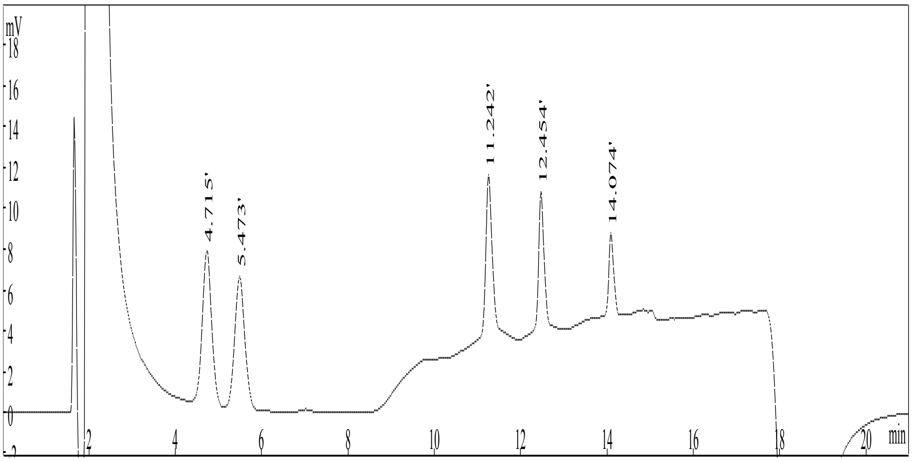


Figure 1 Chromatogram of spiked water sample (1 μg/L)

Conclusion

It is shown by the test results that the recovery and RSD of spiked samples from river water through the sample pretreatment by a full-automatic solid-phase extractor are significantly superior to those through the manual solid-phase extraction.

Ordering Information

Products	Specification	Cat.No.
Cleanert® PEP	500 mg / 6 mL	PE5006
Qdaura® Automatic SPE System	4 channels, 24 positions	SPE-40

Analysis of 11 Kinds of Pesticide Residues in Water (Cleanert® PEP, P/N: PE5006)

EAF10112

Introduction

Pesticides can be used for killing insects, fungi and other bionts which are hazardous to crop growth. The earliest pesticides used include DDT, BHC, etc., which can kill plenty of pests. However, they were eliminated because they could exist in the environment for a long term and continuously accumulate in animals, plants and human bodies due to their good stability. Later the earliest pesticides were replaced by organophosphorus pesticides (such as Dichlorvos). However, the phosphorus contained in the organo-phosphorus pesticides is easy to result in water eutrophication. During agricultural production, pesticides enter the water environment via sedimentation, surface runoff and other ways, causing a series of problems including water resource pollution, water ecosystem destruction, food security, etc. Therefore it is necessary to monitor pesticide residues contained in water.

Experimental

Materials and Reagents

Methanol, dichloromethane and acetone were HPLC grade; High purity water;
Cleanert® PEP: 150 mg/6 mL;
11 kinds of pesticide-mixed standard working solutions: Dichlorvos, Dimethoate, Methyl Parathion, Chlorpyrifos, Malathion, α-BHC, β-BHC, γ- BHC, δ- BHC, o,p`DDT, p,p`DDT

Sample Preparation

Sample 500 mL of water, add methanol to the water until the proportion between methanol and water reaches 5%; mix the solution uniformly, and keep it as the solution to be purified.
Activate and balance Cleanert® PEP cartridge with 5 mL of methanol and 5 mL of water in turn; load all the aforesaid solution to be purified to the cartridge at the flow rate of 10 mL/min; discard the effluent; keep blowing the cartridge with nitrogen for 30 min; elute the cartridge with 10 mL of acetone and 5 mL of dichloromethane; collect the effluent, blow it to nearly dry with nitrogen at 40 °C and add n-hexane into it until the total volume reaches 1 mL; divide the sample into two: one for GC-ECD detection and the other one for GC-FPD detection.

Instrumentation

GC-ECD
DA-5MS chromatographic column, 30 m × 0.25 mm × 0.25 μm
Injection port temperature: 250 °C
Column temperature: 110 °C (kept for 0.5 min) to 320 °C (kept for 5 min)(15 °C/min rise)
Carrier gas: nitrogen (purity ≥ 99.999%, flow rate: 3.5 mL/min)
Sample size: 1 μL
Injection mode: Splitless injection

GC-FPD
DB-17 chromatographic column, 30 m × 0.25 mm × 0.25 μm
Injection port temperature: 250 °C
Detector temperature: 300 °C
Column temperature: 150 °C (kept for 2 min) to 210 °C (8 °C/min rise), then to 250 °C (5 °C/min rise) and kept for 15 min
Carrier gas: nitrogen (purity ≥ 99.999%, line speed: 70 cm/sec)
Combustion gas: hydrogen (purity ≥ 99.999%, flow rate: 90 mL/min)
Oxidizing gas: air (flow rate: 90 mL/min)
Sample size: 1 μL
Injection mode: Splitless injection

Results and Discussion

As is shown in Table 1, the standard addition recoveries of the 11 kinds of in-water pesticide residues are detected by GC-ECD and GC-FPD methods based on SPE technology are between 70% and 110%, conforming to the detection requirement. As shown in Figure 1 ~ Figure 6, the detection results of the 11 kinds of in-water pesticide residues detected with DA-5MS and DB-17 chromatographic columns show good peak shapes and stable retention time.

Table 1 Recoveries and reproducibility of pesticides in water samples (n=3, spiked sample 0.1 mg/kg)

Pesticide	Retention time/min	Recoveries/%	RSD/%
Dichlorvos	2.105	82.5	4.1
Dimethoate	9.278	95.8	6.3
Methyl Parathion	10.405	108.9	3.2
Chlorpyrifos	10.921	93.0	0.4
Malathion	11.232	95.3	9.2
α-BHC	5.879	93.0	4.0
β-BHC	6.341	97.9	2.7
γ- BHC	6.486	108.6	2.6
δ- BHC	6.716	88.2	1.4
o,p`DDT	9.120	73.9	12.5
p,p`DDT	9.654	85.0	11.6

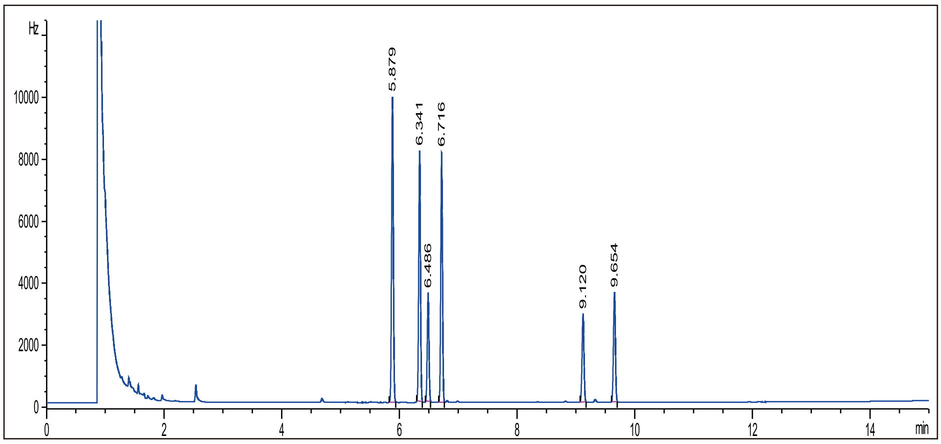


Figure 1 GC-ECD chromatogram of 0.1 μg/mL standard solution of α-BHC, β-BHC, γ-BHC, δ- BHC, o,p`DDT, p,p`DDT mixed

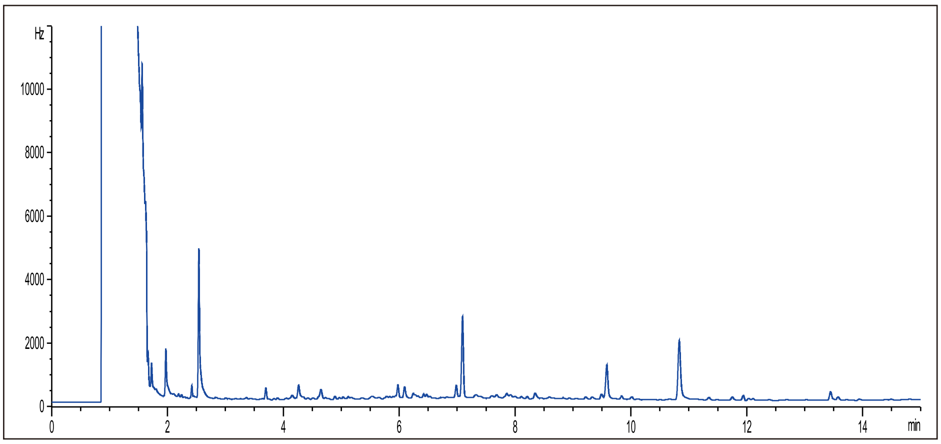


Figure 2 GC-FPD chromatogram of blank sample

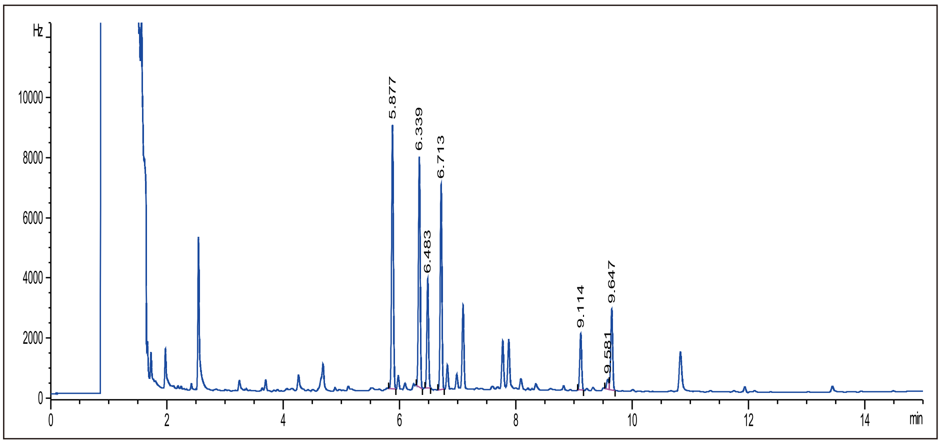


Figure 3 GC-ECD chromatogram of spiked water sample (0.1 μg/mL)

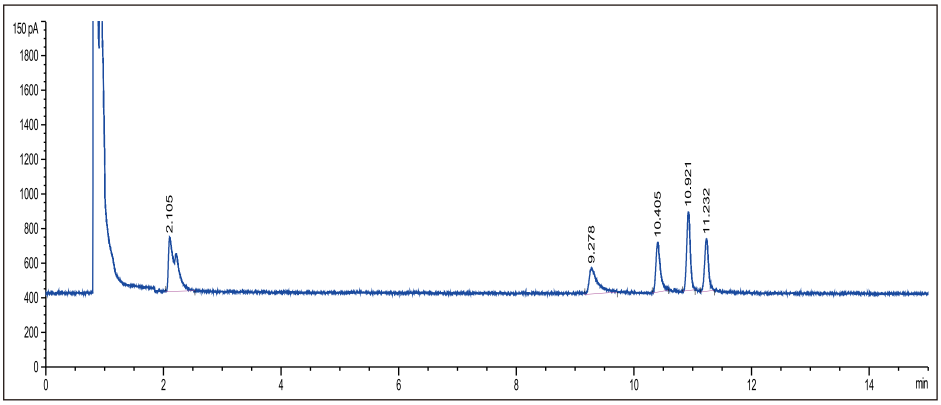


Figure 4 GC-FPD chromatogram of standard solution of 5 kinds of organophosphorus pesticides mixed (0.1 μg/mL)

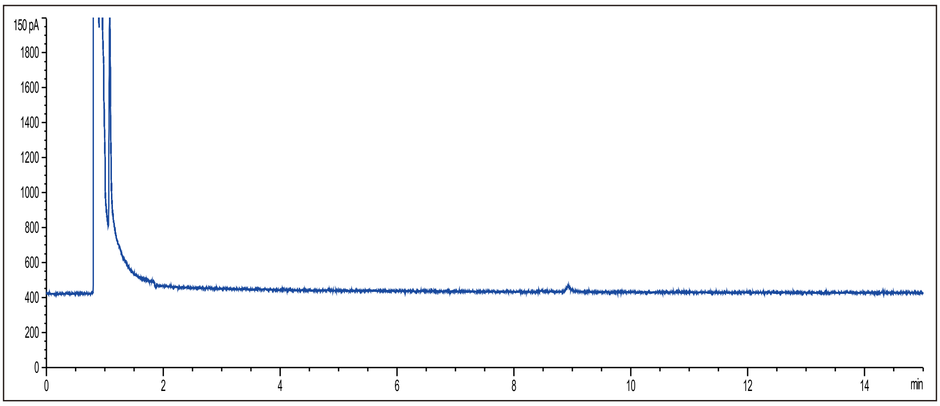


Figure 5 GC-FPD chromatogram of blank sample

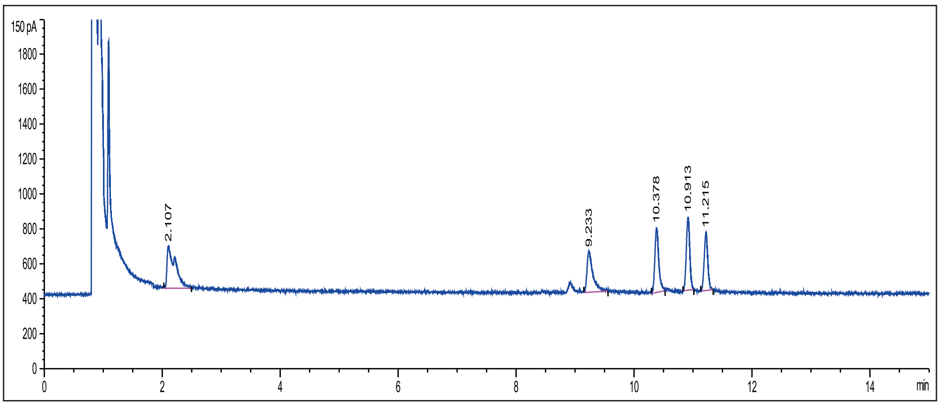


Figure 6 GC-FPD chromatogram of spiked water sample (0.1 μg/mL)

Conclusion

This experiment establishes a pretreatment method for the 11 kinds of pesticide residues contained in water and respectively detects the sample by GC-ECD and GC-FPD methods. The detection results show that, for the water sample added with standard solution (concentration: 0.1 μg/mL), the recoveries of the pesticide residues are between 70% and 110% (conforming to the detection requirement) and RSD is no more than 15% (showing the good detection stability), proving Cleanert® PEP cartridge can be used for detecting the 11 kinds of pesticide residues contained in water.

Ordering Information

Products	Specification	Cat.No.
Cleanert® PEP	150 mg / 6 mL, 30/pk	PE1506
SPE-M08 Positive Pressure SPE Device	8 positions	SPE-M08
Syringe Filter (Nylon)	0.22 μm, diameter 13 mm, 200/pk	AS021320
Syringe	2 mL Without Needle	LZSQ-2ML
1.5 mL vials	Screw neck vials, 12 × 32 mm	AV1001-6
Caps and Septa	Screw neck cap, center hole; red silicone/white PTEE septa, slitted	AV2200-0

Determination of Antibiotics in Water using Cleanert® LDC(PEP-2)

EAF10078

Introduction

Antibiotics were called as anti-bacteria elements before. Actually they not only can kill bacteria, but also have excellent bacteriostatic and killing effects on moulds, mycoplasmas, chlamydiae, spirochaete, rickettsiae and other pathogenic microorganisms, so anti-bacteria elements are renamed as antibiotics. Antibiotics may be the substances generated during the growth and reproduction of certain microorganisms. For the curative antibiotics, a part of them are directly extracted from the microorganisms, the other part of them are completely or partially artificially synthesized. Popularly speaking, antibiotics are the drugs for treating various non-virus infections. However, clinical application has shown many side effects of antibiotics. The heavy usage of antibiotics would bring relatively toxic side effects and direct damage to human bodies, especially for children's hearing. The abuse of antibiotics would result in drug-resistance of bacteria and kill a lot of normal bacteria in human bodies, consequently leading to dysbacteriosis of normal flora in human bodies.

Therefore monitoring antibiotics in water through effective experimental technical means is of great importance to ensuring people's health.

Based on the detection methods for antibiotics in water, this experiment establishes a LC/MS/MS method for confirming and quantitatively detecting various antibiotics in water meanwhile by optimizing the pretreatment method.

Experimental

Materials and Reagents

Chromatographic solvent: acetonitrile; experimental water: ultrapure water; Ammoniated methanol (V/V: 5%)
Formic acid aqueous solution (V/V: 0.1%)
Cleanert® LDC (PEP-2): 1 g/200 mL

Sample Preparation

Sample 500 mL of water; add 0.25 g of EDTA to it; adjust the pH value with hydrochloric acid to 4.0; store the mixed solution for further purification.

Activate the Cleanert® LDC (PEP-2) special columns for large-volume water samples (1 g/200 mL) with 9 mL of methanol, 5 mL of formic acid aqueous solution (V/V: 0.1%) in turn; load the aforesaid solution to be purified to the columns at the flow rate of 90 mL/min, leach the columns with 5 mL of water, and keep pumping them for 15 min; elute the columns with 20 mL of ammoniated methanol (V/V: 5%), and collect the eluent; blow the eluent with nitrogen to dry at 45 °C, and dissolve it with 1 mL of acetonitrile aqueous solution (V/V: 20%) to the specified volume; filter a proper amount of supernatant with a 0.22 μm syringe filter for the next LC/MS/MS detection.

Dilute the high-concentration pesticide-mixed standard solution with blank sample matrix solution to the matrix-mixed standard working solution (concentration: 0.01 μg/mL).

Instrumentation

HPLC Column: Unisol C18, 150mm × 2.1 mm, 5 μm, 100 Å
Mobile Phase: A: 0.1 % formic acid-Water; B: Acetonitrile
Injection volume: 10 μL
Column Temperature: 30 °C

Scanning mode: Positive ion scanning;
Ion source: ESI source;
ESI voltage: 5500 V;
Atomized gas pressure: 55 psi;
Curtain gas pressure: 20 psi;
Auxiliary gas pressure: 50 psi;
Ion source temperature: 650 °C;
Collecting mode: MRM.

Scanning mode: Negative ion scanning;
Ion source: ESI source;
ESI voltage: -3500 V;
Atomized gas pressure: 60 psi;
Curtain gas pressure: 15 psi;
Auxiliary gas pressure: 50 psi;
Ion source temperature: 600 °C;
Collecting mode: MRM.

Table 1 HPLC Gradient Elution Conditions

Time (min)	Flow Rate (mL/min)	A%	B%
1.00	0.30	90	10
3.00	0.30	40	60
5.00	0.30	5	95
7.00	0.30	5	95
8.00	0.30	90	10
20.00	0.30	90	10

Table 2 HPLC Gradient Elution Condition on Negative Ion Mode

Time (min)	Flow Rate (mL/min)	A%	B%
1.50	0.30	85	15
5.00	0.30	30	70
5.01	0.30	10	90
6.00	0.30	10	90
6.01	0.30	85	15
10.00	0.30	85	15

Table 3 MS Parameters of Antibiotics

Antibiotics	Q1	Q3	DP/V	CE/V
Erythrocin	734.6	158.1	81	49
		576.8	86	49
Josamycin	828.3	174.3	121	45
		109.3	126	45
Kitasamycin	772.4	215	116	41
		174.1	121	41
Oxolinic Acid	262	244	41	27
		216	41	41
Flumequine	262	244	41	27
		202	41	45
Nalidixic Acid	233	215	40	19
		187	40	35
Sulfapyridine	250	156	65	24
		184	65	24
Sulfachloropyridazine	285	156	50	23
		108	50	35
Sulfamethazine	265	156	60	25
		172	60	25
Sulfamethizole	271	156	50	20
		107	50	32
Sulfmethoxypyridazine	281	156	70	25
		215	60	25
Sulfametoxydiazine	281	156	70	25
		108	70	35
Florfenicol	356	336	-78	-23
		185	-78	-23

Results and Discussion

As is shown in Table 1, the standard addition recoveries of in-water antibiotics detected by LC/MS/MS method based on SPE technology are between 60% and 120%, and the variable coefficients of the antibiotics are less than 20%, conforming to the detection requirements. As is shown in Figure 1, 2, 3, 4, 5, 6, 7 and 8, the detection results show the good purification effect of the Cleanert® LDC (PEP-2) as well as the good peak shapes and stable retention time of the antibiotics detected with Unisol C18.

Table 4 Recoveries and Reproducibility of Antibiotics in Water Samples (0.02 μg/L Spiked Sample)

Antibiotics	Recoveries/%	CV/%	RT/min
Erythrocin	74.8	14.2	5.91
Josamycin	82.3	2.0	6.19
Kitasamycin	100.7	15.0	6.05
Oxolinic Acid	92.6	14.0	7.11
Flumequine	104.2	6.3	6.58
Nalidixic Acid	107.8	8.6	7.08
Sulfapyridine	94.9	10.7	5.65
Sulfachloropyridazine	75.5	0.3	6.31
Sulfamethazine	75.1	12.4	5.81
Sulfamethizole	95.5	14.1	5.98
Sulfmethoxypyridazine	80.6	7.9	6.01
Sulfametoxydiazine	68.3	4.6	6.19
Florfenicol	86.3	3.9	7.07

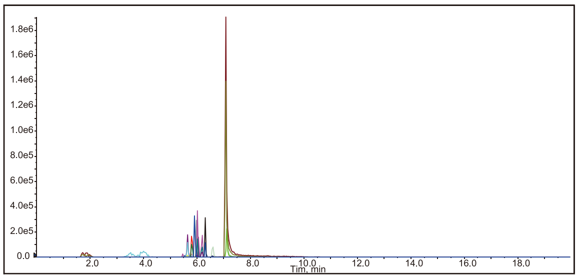


Figure 1 LC-MS/MS Chromatogram of 0.01 μg/mL Standard Solution Mixture in Positive Ion Mode

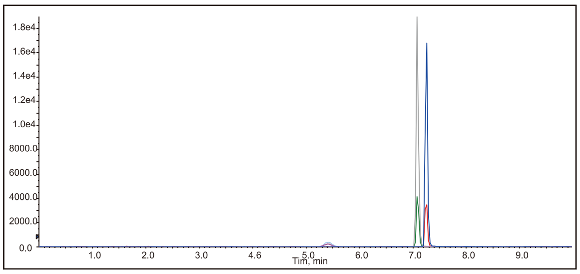


Figure 2 LC-MS/MS Chromatogram of 0.01 μg/mL Standard Solution Mixture in Negative Ion Mode

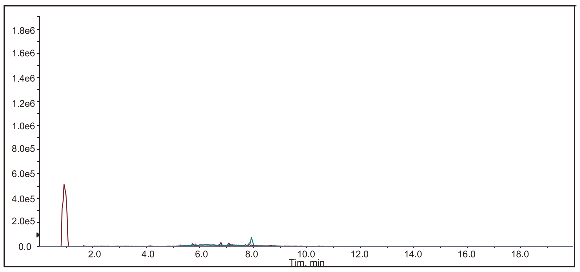


Figure 3 LC-MS/MS Chromatogram of Blank Sample in Positive Ion Mode

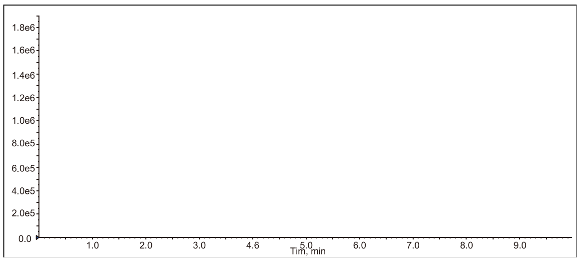


Figure 4 LC-MS/MS Chromatogram of Blank Sample in Negative Ion Mode

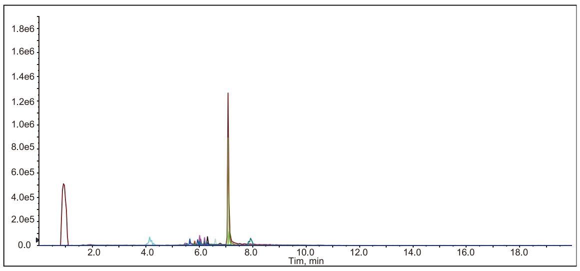


Figure 5 LC-MS/MS Chromatogram of 0.01 µg/mL Standard Solution Mixture in Water Matrix in Positive Ion Mode

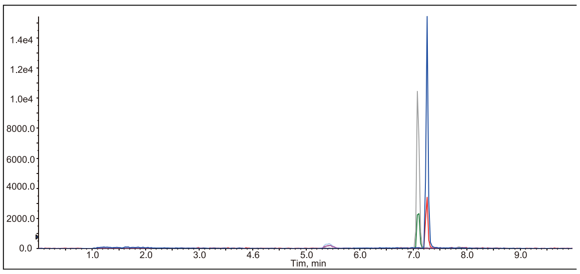


Figure 6 LC-MS/MS Chromatogram of 0.01 µg/mL Standard Solution Mixture in Water Matrix in Negative Ion Mode

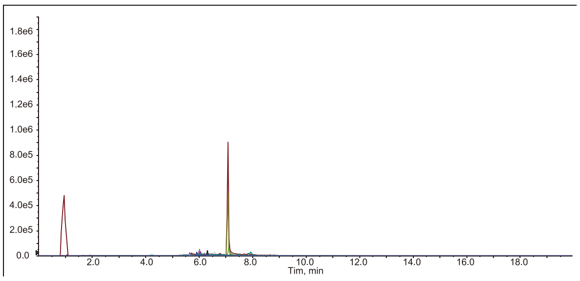


Figure 7 LC-MS/MS Chromatogram of 0.02 µg/L Spiked Water Sample in Positive Ion Mode

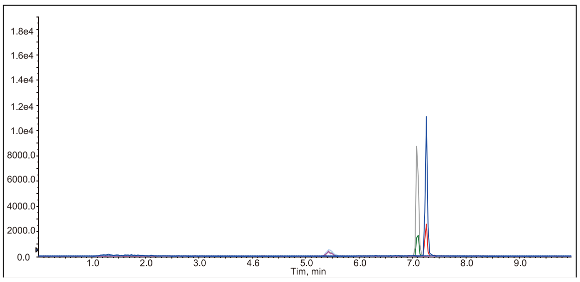


Figure 8 LC-MS/MS Chromatogram of 0.02 µg/L Spiked Water Sample in Negative Ion Mode

Conclusion

This experiment establishes a LC/MS/MS detection method for antibiotics in water and determines the contents of antibiotics in water on the basis of SPE technology. The sample added with standard solution (0.02 µg/L) is detected, and the resultant recoveries are between 60% and 120%, conforming to the requirement. The high stability of SPE method and good reproducibility of chromatographic columns show this LC/MS/MS detection method can be used for detecting the residues of antibiotics in water.

Ordering Information

Products	Specification	Cat.No.
Cleanert® LDC (PEP-2)	1 g/200 mL	LPE000100-2
Unisol C18	2.1 × 150 mm, 5 µm, 100 Å	UO950502-2
SPE-M08 Positive Pressure SPE Device	8 Positions	SPE-M08
Nitrogen blowing instrument	Maximal 15 sample	NV15-G
Guard cartridge holder	Suitable for 4.6×10 mm and 2.1×10 mm	SH-100
Direct-connected Guard Column	5 µm,100 Å, 2.1×10 mm	VA950102-0S
1.5 mL vials	Screw neck vials, 12 × 32 mm	AV1001-6
Caps and Septa	Screw neck cap, center hole; red silicone/white PTEE septa, slitted	AV2200-0
Syringe Filter (Nylon)	0.22 µm, diameter 13 mm, 200/pk	AS021320
Disposable Syringe	2 mL, 100/pk	LZSQ-2ML

Biological Applications

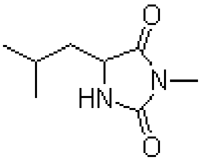
Extraction the biomarker of dimethylformamide in hemoglobin (Cleanert® MAS-B Plate, P/N: MS-B-0302W)

EYB10001

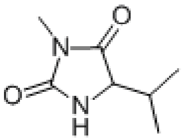
Introduction

Dimethylformamide (DMF) is an organic solvent widely used in chemical and pharmaceutical industries and organic synthesis laboratories. Occupational contact of DMF through respiratory and dermal might cause sister chromatic exchange and damage the cellular DNA of liver and kidney.

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. The treated samples were detected by LC-MS/MS without further concentration. Table 1 summarized the information of MIH and MVH.



MIH



MVH

Experimental

Materials and Methods

Solutions: Diluted the stock solution of MVH and MIH with methanol/water(1:1,v/v) to required concentrations.
MAS plates: Cleanert® MAS-B 96-well Plates (25mg/well) were from Bonna-Agela Technologies.

Sample Preparation

0.1g hemoglobin was weighed accurately and placed in 15mL tube. 0.25mL of 20μg/mL MIH and 4.75mL of hydrochloric acid/ acetic acid(1:1,v/v) was added into the tube and vortexed for 3min. Then the tube was heated in boiling water to degrade hemoglobin for 1h. 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 5mL by adding methanol/water(1:1,v/v). Then 200μL of the treated sample was transferred into Cleanert MAS-B 96-well Plate and mixed with 600μL 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

LC Instrument: Shimadzu LC-20A
HPLC Column: Unisil C18(2.1×50 mm, 3 μm, 150 Å)
Mobile Phase: Acetonitrile/0.1% formic acid-Water, 35/65(v/v)
Flow rate: 0.2 mL/min
Injection volume: 2 μL
Detection: MS (ABI4000+) – The parameters as showed in Table 2 and Table 3.
Ion source: ESI+
Scan mode: MRM

Table 1 MS parameters

Analytes	IS/V	TEM/ºC	GS1/ Pa	GS2/ Pa	CUR/ Pa
MVH and MIH	4500	300	65	40	15

Table 2 Precursor/Product Ions of MVH and MIH

Analytes	Q1	Q3	DP/V	CE/V
MVH	157.2	129.1	61	16
		71.9	66	23
		55.2	65	37
MIH	7171.1	143.1	62	18
		86	66	22
		69	62	29

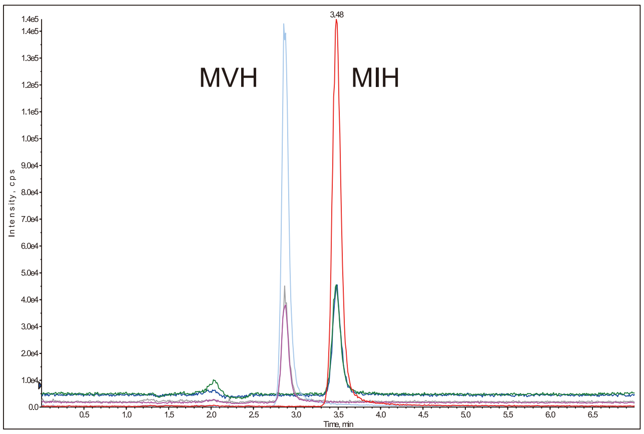


Fig 1. Chromatogram of MVH and MIH (250ng/mL)

Results and Discussion

LOD and Standard Curve

Prepared a series concentration of standard solution of MVH, added fixed concentration of MIH in each solution, detected the mixture under an optimized condition. Draw the curve by concentration ratio and peak area ratio of MVH divide to MIH. The linearity curve was obtained with expression of y = 1.034x + 0.037 (R_2=0.9998), where y stands for peak area ratio, x stands for concentration ratio.

Table 3. LOD of MVH and MIH standard

Analyte	LOD
MVH	1.0ng/mL
MIH	1.0ng/mL

Table 4 Response of MVH and MIH standard

Concentration		MVH/MIH concentration ratio	Peak area		MVH/MIH peak area ratio
MVH/ng/mL	MIH/ng/mL		MVH	MIH	
5	250	0.02	1.50E+04	5.70E+05	0.03
20	250	0.08	5.41E+04	5.27E+05	0.10
50	250	0.20	1.30E+05	4.90E+05	0.27
100	250	0.40	2.46E+05	5.29E+05	0.47
250	250	1.00	6.28E+05	5.68E+05	1.11
500	250	2.00	1.08E+06	5.16E+05	2.08

Computational Formula

The results were calculated from formula (1):

$$M = \frac{A_1 / A_1^{'}}{A_2 / A_2^{'}} \times \frac{M_1^{'}}{M_2^{'}} \times \frac{M_2}{M_S} P_s \quad \cdots\cdots\cdots\cdots\cdots\cdots (1)$$

where:

M—— Content of MVH in hemoglobin, mg/kg;
A1—— Peak area of MVH in practical sample;
A1′——Peak area of MIH in practical sample;
A2——Peak area of MVH in calibration solution;
A2′——Peak area of MIH in calibration solution;
M1′——Quality of MIH added into practical sample, mg;
M2′——Quality of MIH added into calibration solution, mg;
M2——Quality of MVH added into calibration solution, mg;
MS——Quality of practical sample, kg;
PS——Purity of MVH standard substance;

Recoveries and Precisions

Blank hemoglobin was treated and analyzed with the same procedure described above to ensure the sample was drug free. Standard solutions of MVH and MIH were added in to blank samples accurately. The recoveries of the spiked samples were listed in Table 6 range from 100.4% to 105.5% with RSD from 2.9% to 4.6%. The chromatogram of spiked hemoglobin was shown in Fig 2.

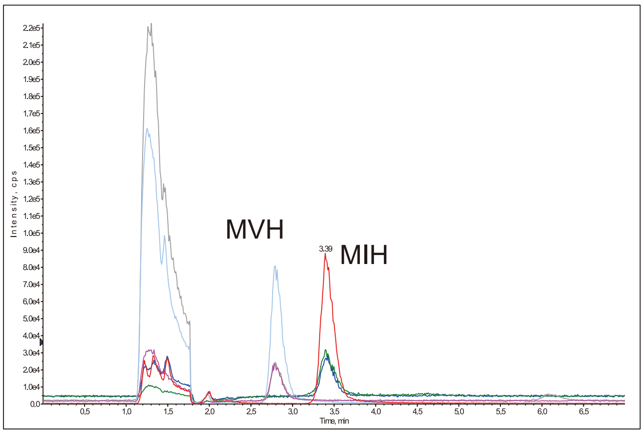


Fig 2. Chromatogram of hemoglobin spiked with 50mg/kg MVH

Table 5 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%

Monitoring of actual sample

Certain amount of hemoglobin was weighed as a test target and mixed with MIH standard solution accurately. Then the sample was processed with the method described above. After calculating according to formula (1), the content of MVH in the hemoglobin was 13 mg/kg.

Anion exchange mechanism of the Cleanert MAS-B plate was utilized to remove the degraded hemoglobin from the sample which present electronegative in the solution. The degraded hemoglobin was absorbed by anion exchange sorbent, while MVH and MIH were eluted directly from the sorbent due to their electropositive. Direct injection of the eluate without further concentration simplified the manipulation. The rapid clean-up procedure of the sample results in a short run of LC-MS/MS within 7min. supporting a high throughput application.

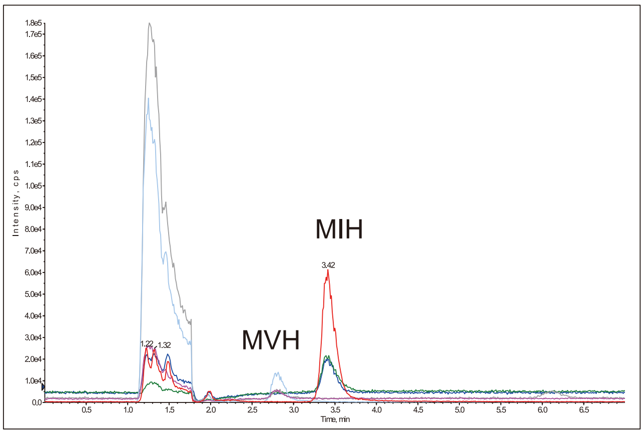


Fig 3. Chromatogram of actual hemoglobin

Conclusion

Cleanert® MAS-B 96-well Plate with 25 mg sorbent in each well 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 study provided a solution for monitoring cumulative exposure to DMF.

Ordering Information

Products	Specification	Cat.No.
Cleanert® MAS-B	30 mg / 2 mL / well	MS-B-0302W
96-well Collection plate	1.0 mL Squaral well	96SP1036
Cleanert® M96 Positive Pressure Device	Adapt to 96-well plate	SPE-M96
96-well Vacuum Manifold	Adapt to 96-well plate	VM96
Unisol C18	2.1×50 mm, 3 µm, 150 Å	UO930502-0

SPE Method for the Determination of Folic Acid and 5-Methyltetrahydrofolicacid from Serum (Cleanert® PAX Plate, P/N: AX0302-W))

EYB10003

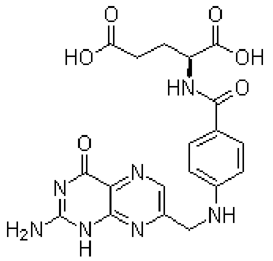


Figure 1 Structure of Folic Acid

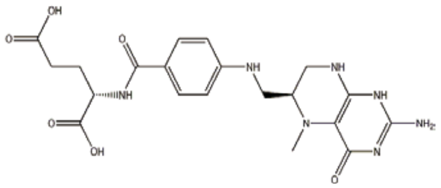


Figure 2 Structure of 5-Methyltetrahydrofolicacid

Experimental

Sample Pre-treatment

In order to reduce decomposition of folic acid, ascorbic acid and 2-mercaptoethanol was used in the experiment as antioxidant. The concentration of ascorbic acid and 2-mercaptoethanol were 100 μg/mL, respectively.

400 μL serum was diluted by 400 μL water containing antioxidant, vortexed by 2min. The mixture would be purified by Cleanert® PAX plate (30 mg/well).

SPE Procedure

Condition: 1 mL methanol

Equilibrium: 500 μL water, followed by 500 μL water containing antioxidant

Loading: 800 μL pre-treatment sample, eluted by gravity

Washing: 500 μL water containing 0.1% formic acid, and followed by 250 μL methanol. Cleanert® M96 positive pressure SPE device was used to dry the plate.

Elution: 500 μL methanol containing 2% formic acid, eluted by gravity.

Concentration and reconstitution: the eluate was concentrated to dryness at 37 ºC by Cleanert® V96 nitrogen evaporator. The residual was reconstituted by 100 μL water and analyzed by LC-MS/MS.

HPLC Conditions

Column: Unisol C18(2) (3.0x100 mm, 3 μm, 110 Å);
Flow rate: 500 μL/min;
Temperature: 30 ºC;
Injection: 10 μL;
Mobile Phase: A-0.1% formic acid in water;
B-0.1% formic acid Methanol

Table 1 HPLC gradient parameters

Time (min)	A%	B%
0	95	5
1	95	5
1.01	20	80
5	20	80
5.01	95	5
10	95	5

MS Conditions

Mode: Sciex 4000+, Scanning mode: Positive; Scan type: MRM

Table 2 Quantifier and qualifier MRM transitions and retention times for compounds

Compound	Retention time	Q1	Q3	CE/V
5-Methyltetrahydrofolicacid	3.54 min	460.3	313.2	16
			193.6	23
Folic Acid	3.73 min	442.4	295.1	37
			175.9	18

Results

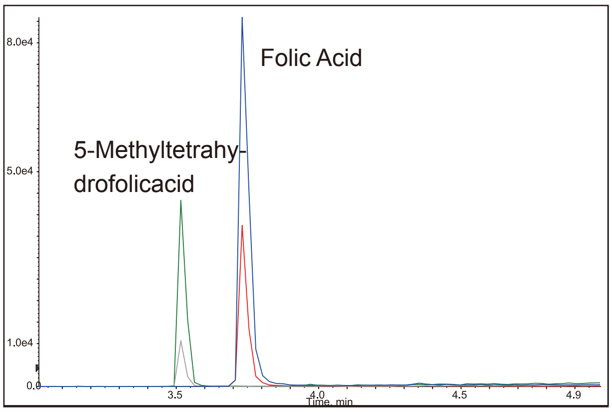


Figure 3: Standard solution

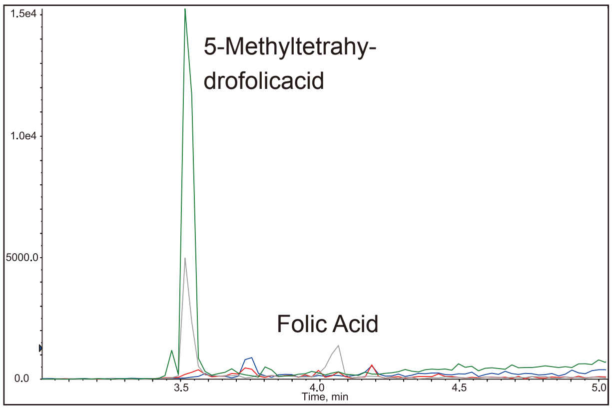


Figure 4: Blank serum

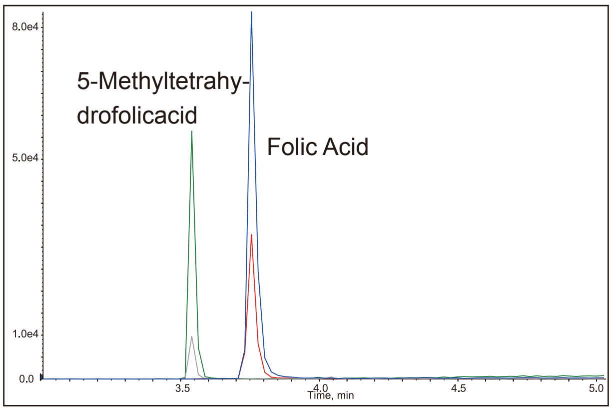


Figure 5: Spiked serum

Table 3 Recoveries of analytes in serum

Compounds	1 ng/mL		5 ng/mL		20 ng/mL	
	Average recovery (n=5)	RSD (n=5)	Average recovery (n=5)	RSD (n=5)	Average recovery (n=5)	RSD (n=5)
5-Methyltetrahy-drofolicacid	89.6%	8.9%	105.8%	5.4%	101.2%	3.3%
	91.5%	10.2%	99.4%	3.7%	95.8%	2.3%

Conclusion

This experiment use Cleanert® PAX extract folic acid and 5-Methyltetrahydrofolicacid from Serum. From 1ng/mL to 20 ng/mL,the recovery over 89%, RSD less than 11% at low concentration and 5% at high concentration. The folic acid and 5-Methyltetrahydrofolicacid LOQ is 0.05 ng/mL and 0.08 ng/mL, so could be used in DMPK and clinical research.

Ordering Information

Products	Specification	Cat.No.
Cleanert® PAX	30 mg / well	AX0302-W
96-well Collection plate	1.0 mL Squaral well	96SP1036-Y
Cleanert® M96 Positive Pressure Device	Adapt to 96-well plate	SPE-M96
Cleanert® V96 Evaporator	Adapt to 96-well plate	NV96-G
96-well Vacuum Manifold	Adapt to 96-well plate	VM96
Unisol C18(2)	3.0×100 mm, 3 μm, 110 Å	UO931003-2

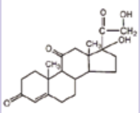
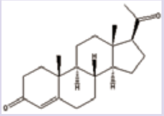
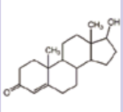
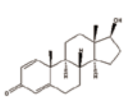
Cleanert® SLE for the Extraction of Steroid Hormones from Serum (Cleanert® SLE, P/N:HC2003Q-9)

EYB10010

Introduction

This method is a LC-MS/MS method for the determination of steroid hormones in serum.

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

Standard Solution

The standards were dissolved by methanol to get stock solutions at the concentration of 1 mg/mL.
Then stock solutions were diluted to required concentration by methanol.

Sample Preparation

This experiment employed Cleanert® SLE (200 mg / 3 mL) 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 1~2 mL/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 °C and reconstitute the residue by 200 μL of Acetonitrile:Water (3:7, v/v), and then analyzed by LC-MS/MS.

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 ºC
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

Table 1 MS parameters

Analyte	IS/V	TEM/ºC	GS1/ Pa	GS2/ Pa	CUR/ Pa
Boldenone, Testosterone and Progesterone	4500	550	40	40	25
Cortisone	-4500	500	35	40	20

Results and Discussion

Chromatogram

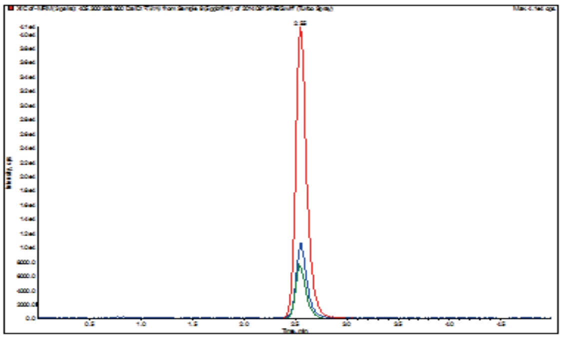


Figure 1 Chromatogram of Cortisone (5 ng/mL)

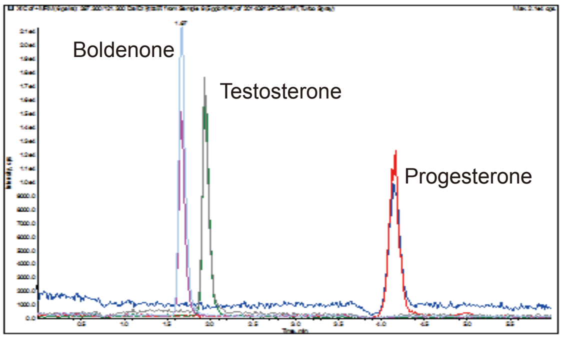


Figure 2 Chromatogram of boldenone, testosterone and progesterone (5 ng/mL)

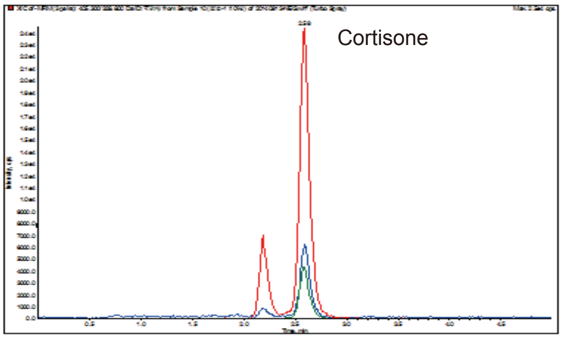


Figure 3 Chromatogram of actual serum sample (negative mode)

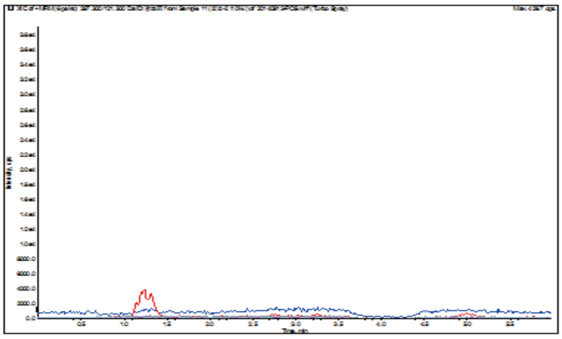


Figure 4 Chromatogram of actual serum sample (positive mode)

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%

Conclusion

It is a preliminary method for extracting steroid hormones from serum because the recovery data of progesterone and testosterone is a little unsatisfactory. According to our experience, approximate content of organic solvent, such as methanol, in the serum sample could benefit to increase recoveries, because methanol will help progesterone and testosterone dissolve in aqueous serum solution.

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 mL Square well	96SP2036-2
Cleanert® M96 Positive Pressure Device	adapt to 96well plate	SPE-M96
Cleanert® V96 N_2 Evaporator	adapt to 96well plate	NV96-G-S
1.5 mL vials	Screw neck vials, 12×32 mm	AV1001-6
1.5 mL vials caps	Screw neck cap, center hole; red silicone/ white PTFE septa, slitted	AV2200-0
Filtration membrane (PTFE)	φ13; 0.22 µm	AS041320-T
Disposable Syringe	2 mL, needless	ZSQ-2ML

The Determination of LCPs in Human Serum Using Cleanert® PPT Protein Precipitation Plates by LC-MS/MS (Cleanert® PPT, P/N: 96CD1025-M)

EYB10015

Introduction

Lyso-Phosphatidylcholines(LPCs), a kind of phospholipid, has multiple physiological functions and it is closely related with diabetes, atherosclerosis, lipid abnormality and other metabolic diseases and cardiovascular diseases. There is a significant change of LPCs in liver metabolism, liver diseases and hepatotoxicity. In recent years, LPCs has been involved in the research of liver diseases and chemical hepatoxoxicity model.

This application note describes the analysis of five LPCs in human serum using Cleanert® PPT protein precipitation plates with LC-MS/MS. With Cleanert PPT protein precipitation plate, centrifugation and supernatant transfer are eliminated, reducing the chance of sample loss and saving time. Extraction gained from Cleanert® PPT protein precipitation plate can be introduced into LC-MS/MS. Excellent method linearity is proved for each analyze, together with good accuracy and precision performances.

Experimental

Materials and Reagents

Cleanert® PPT protein precipitation plates, Cleanert® M96 Positive Pressure Device, Cleanert® V96 N2 Evaporator, Cleanert® 96-well Collection Plate, Durashell C18(L) (3.0×50 mm, 5 μm, 150 Å) were provided from Bonna-Agela Technologies; Standard samples of LPC 14:0, LPC 15:0, LPC 16:0, LPC 17:0, LPC 18:0 were purchased from Avanti; Reserpine (internal standard); Methanol; Formic acid; Water; Ammonium formate purchased from Honeywell; Normal saline(0.9%);

Sample Preparation

Due to the difficulty of obtaining substrates with no target compounds, human serum was replaced by normal saline in this methodological experiment. In order to maintain the biological integrity of LPCs in human blood, all the methanol used in experiment contain 0.1% 2,6-bis (1,1-dimethylethyl)-4-methylphenol (BHT).

In the calibration curve and reproducibility test, normal saline was used as the substrate. Add 5 μL standard solution into 20 μL normal saline, vortex and wait for further preparation. In spiking human serum test, serum is substrate. Add 5 μL standard solution into 20 μL serum, vortex well and wait for further preparation.

Transfer 150 μL methanol (including 100 ppb reserpine) to the Cleanert® PPT, then add the premixing sample, vortex, wait for 10 min and then collect the elute into a collector plate with Cleanert® M96 positive pressure device.

Set the temperature of Cleanert® V96 N2 Evaporator to 30 ºC and dry the extraction by nitrogen.

Reconstitute with 1 mL 80% methanol in water, vortex well. The sample was ready for LC-MS/MS analysis.

Recovery data

LC-MS/MS, API 4000+. SCIEX
Column: Durashell C18(L), 3.0 × 50 mm, 5 μm, 150 Å
Temperature: 30 °C
Flow rates: 800 μL/min
Injection: 5 μL
Mobile phase: A: 0.1% formic acid in water with 25 mM ammonium formate;
B: 0.1% formic acid in methanol
Ion source: ESI+
Scan mode: MRM

Table 1 Gradient

Time (min)	A%	B%
0	70	30
0.5	50	50
1	0	100
4	0	100
4.1	70	30
5	STOP	

Table 2 MS Parameters

Compounds	t_R/min	Q1	Q3	DP	CE
Reserpine	2	609.3	448.2	140	40
		609.3	195*	120	45
C14:0	2.46	468.4	285.3	110	40
		468.4	450.4*	120	27
C15:0	2.54	482.3	299.5	120	36
		482.3	464.2*	120	28
C16:0	2.63	496.4	478.2*	124	16
C17:0	2.72	510.4	327.2	120	30
		510.4	492.4*	120	20
C18:0	2.83	524.5	341.5	115	28
		524.5	506.2*	127	18

Note: *is the quantitative ion

Results and Discussion

Calibration curve and method sensitivity

Calibration was preformed using 5 μL standard working solutions with 20 μL normal saline to obtain six concentration lever (Table 3, S1-S7). Figure 3 shows the sensitivity of method.

Table 3 Concentration lever and sensitivity

	C14:0	C15:0	C16:0	C17:0	C18:0
S1/ppm	0.125	0.25	3.75	0.375	2.5
S2/ppm	0.25	0.5	7.5	0.75	5
S3/ppm	0.5	1	15	1.5	10
S4/ppm	1.25	2.5	37.5	3.75	25
S5/ppm	2.5	5	75	7.5	50
S6/ppm	3.75	7.5	112.5	11.25	75
S7/ppm	7.5	15	225	22.5	150
Calibration Equation	y=0.725x+0.00264	y=0.212x-0.0256	y=0.212x+0.268	y=0.366x+0.00653	y=0.31x+0.287
R2	0.9965	0.9913	0.9956	0.9938	0.9961
LOQ/ppm	0.027	0.12	0.32	0.059	0.072

Accuracy and Precision

Take normal saline as substrate, conduct two recovery experiments with two parallel samples (concentrations S3andS5). Table 4 shows the experimental results.

In order to inspect and study the accuracy and precision of this method, 8 parallel tests were conducted separately and sample concentrations were S3 and S5. S3 is corresponding to LQC and S5 to HQC. Take normal saline as substrate. Table 5 shows the experimental results.

Table 4 Recoveries of spiking samples in saline

	C14:0	C15:0	C16:0	C17:0	C18:0
Spiking samples in low concentration/ppm	1.25	2.5	37.5	3.75	25
Spiking samples in high concentration /ppm	3.75	7.5	113	11.3	75
Measured concentration of low concentration samples-1/ppm	1.29	2.28	38.1	3.31	24.3
Measured concentration of low concentration samples-2/ppm	1.44	2.85	43	4.09	28.1
Recoveries of low concentration samples-1	103.2%	91.2%	101.6%	88.3%	97.2%
Recoveries of low concentration samples-2	114.4%	114.0%	114.7%	109.1%	112.4%
Measured concentration of high concentration samples-1/ppm	3.44	6.77	102	9.83	69.9
Measured concentration of high concentration samples-2/ppm	3.35	6.53	103	9.94	69
Recoveries of high concentration samples-1	91.7%	90.3%	90.3%	87.0%	93.2%
Recoveries of high concentration samples-2	89.3%	87.1%	91.2%	88.0%	92.0%

Table 5 Accuracy and Precision of spiking samples in saline (n=8)

	C14:0	C15:0	C16:0	C17:0	C18:0
Recoveries of LQC	96.3%	99.9%	103.6%	96.6%	100.0%
CV	6.3%	6.4%	8.5%	7.1%	7.0%
Recoveries of HQC	96.8%	99.1%	97.5%	93.8%	97.7%
CV	11.3%	12.1%	12.3%	12.0%	11.3%

It can be seen from Table 4 and Table 5 that this method can be adopted to dispose and detect LPCs in normal saline. In order to further inspect its adaptability to serum samples, human serum was used in another recovery experiment and the result can be seen in Table 6. Because of the large concentration of LPCs in human serum, two human serum samples were detested to deduct endogenous LPCs. The equation of recovery of spike sample is:

$$\text{Recoveries of spiking samples} = \frac{C_A - C_M}{C_S} * 100\%$$

C_A—measured concentration of spiked samples
C_M—average concentration of human serum samples
C_S—spiked concentration

Table 6 Method performance of spiking samples in human serum

	C14:0	C15:0	C16:0	C17:0	C18:0
Spiking samples in low concentration/ppm	1.25	2.5	37.5	3.75	25
Spiking samples in high concentration /ppm	3.75	7.5	113	11.3	75
Spiking samples in human serum-1/ppm	0.827	2.46	107	2.92	47.3
Spiking samples in human serum-2/ppm	0.678	2.21	92	2.45	39.4
Measured concentration of low concentration samples-1/ppm	1.97	4.5	140	6.24	72
Measured concentration of low concentration samples-2/ppm	2.17	4.99	136	6.67	70
Recoveries of low concentration samples-1	97.4%	86.6%	108.0%	94.8%	114.6%
Recoveries of low concentration samples-2	113.4%	106.2%	97.3%	106.3%	106.6%
Measured concentration of high concentration samples-1/ppm	4.8	9.46	196	13.8	113
Measured concentration of high concentration samples-2/ppm	4.72	9.69	216	14.8	122
Recoveries of high concentration samples-1	107.9%	95.0%	85.4%	98.4%	92.9%
Recoveries of high concentration samples-2	105.3%	98.1%	103.1%	107.2%	104.9%

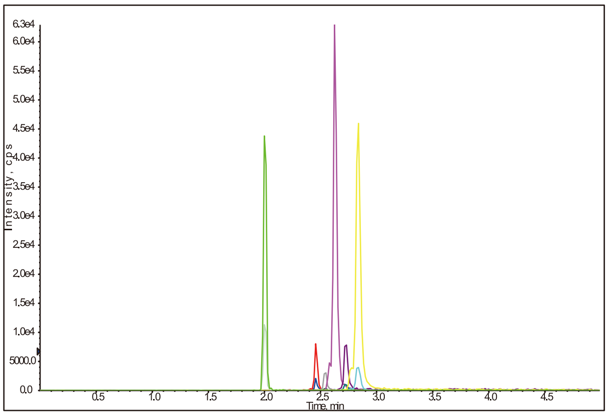


Figure 1 Chromatogram of standard working solution S2

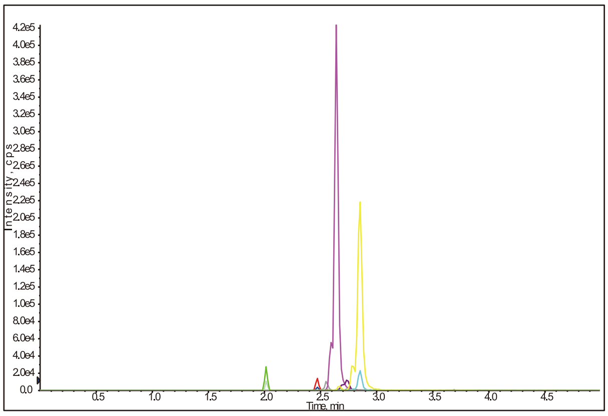


Figure 2 Chromatogram of spiking sample in human serum

Conclusion

This experiment purified the LPCs from normal saline and human serum by protein precipitation and detect by LC-MS/MS. The result shows that this method is simple and stable, easy and fast to detect LPCs in human serum. It is suitable for clinical study.

Ordering Information

Products	Specification	Cat.No.
Durashell C18(L)	3.0 × 50 mm, 5 μm, 150 Å	DC950503-L
Cleanert® PPT	96-well plates, 1 mL Square well	96CD1025-M
Cleanert® V96 N_2 Evaporator	Adapt to 96-well plate	NV96-G-S
Cleanert® M96 Positive Pressure Device	Adapt to 96-well plate	SPE-M96
96-well Collection Plate	2.0 mL/well, adapt to 96-well plate	96SP1036-Y

The Determination of 25-hydroxyvitamin D in Serum Using Cleanert® PEP Micro Plate by LC/MS/MS (Cleanert® PEP Micro Plate, P/N: PE00501-MW)

EYB10016

Introduction

25-hydroxyvitamin D (25-OH VD), including 25-hydroxyvitamin D2 (25-OH VD2) and 25-hydroxyvitamin D3 (25-OH VD3), is one of the main metabolic forms of vitamin D. It is considered as testing marker of vitamin D for its long-life and biological stability. The determination of vitamin D in human body has attracted more attentions from researchers of pharmaceutical and clinical field. LC-MS/MS is regarded as the "Gold Standard" of evaluating the nutritional status of vitamin D due to the high accuracy and reproducibility. In this application, an effective sample preparation procedure was developed for the extracting of 25-OH VD in serum using Cleanert® PEP Micro Plate by LC-MS/MS.

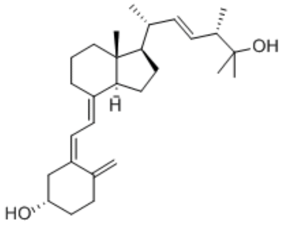


Figure 1 Structure of 25-OH VD2

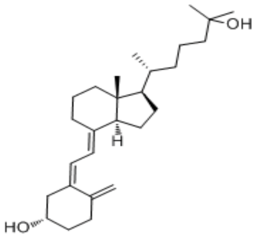


Figure 2 Structure of 25-OH VD3

Experimental

Materials and Reagents

Cleanert® PEP Micro Plate (5 mg/well), Cleanert® PPT protein precipitation plate, SPE-M96 Positive Pressure Device, Cleanert® 96-well collection plate, Unisol C18 (2.1×50 mm, 3 μm) were purchased from Bonna-Agela Technologies; 25-OH VD2, 25-OH VD3, d6-25-OH VD3 were purchased from Sigma-Aldrich; Calibration samples were purchased from RECIPE;

Sample Preparation

Transfer 25 μL zinc sulfate solution (0.2 M) and 200 μL methanol/acetonitrile (1/1) containing internal standard into the Cleanert® PPT Micro Plate, add 100 μL serum, vortex and wait for 5 min. Put Cleanert® PPT and collection plate on the Cleanert® M96 orderly, adjust pressure to 4 psi, collect the extract and wait for further SPE preparation.
Cleanert® PEP Micro Plate (5 mg/ well) used for further purification. The steps are as follow:
Condition: 200 μL methanol, 200 μL water of 60% methanol;
Loading: Add 200 μL solvent from Cleanert® PPT;
Washing: Add 200 μL 5% methanol in water, 200 μL 60% methanol in water.
Elution: Add 100 μL methanol/ isopropanol (95/5). Collect eluent and add 40 μL water in it. Shake it and analyze by LC-MS/MS.

Instrumentation

Detector: LCMS/MS, QTRAP 5500. SCIEX
Column: Unisol C18, 2.1×50 mm, 3 μm
Temperature: 40 ºC
Injection: 20 μL
Mobile phase: A: 0.02% formic acid-water;
B: 0.02% formic acid-methanol
Ion source: ESI+
Scan mode: MRM

Table 1 Gradient

Time (min)	Flow Rate	A%	B%
0	800 μL/min	30	70
0.2	800 μL/min	30	70
1.5	800 μL/min	0	100
2.3	800 μL/min	0	100
2.31	800 μL/min	30	70
3		STOP	

Table 2 MS Parameters

Compounds	t_R/min	Q1	Q3	DP	CE
25-OH VD2	1.62	413.3	337.3	80	15
		413.3	355.4	80	14
25-OH VD3	1.59	401.3	257.3	80	20
		401.3	365.2	80	16
d6-25-OH VD3	1.59	407.3	371.4	80	19

Results and Discussion

Linear Range and Sensitivity

Replace blood sample with 6% BSA solution to make a standard curve which contains 6 points. The 25-OH VD2 concentrations were 1, 2, 5, 10, 20 and 40 ng/mL and the 25-OH VD3 concentrations were 5, 10, 25, 50, 100 and 200 ng/mL. Table 3 shows the curve equation of the extracts.

Table 3 Linear Range and Sensitivity

Compounds	25-OH VD2	25-OH VD3
Regression Equation	y=0.00852x+0.000384	y=0.0111x+0.00697
R2	0.9962	0.9990
LOD(ng/mL)	1	0.67

Recoveries of spiking sample

Human serum was used to inspect the feasibility of this method. Serum samples of 10 patients were mixed due to the existence of determinated in human serum. The measured concentrations of 25- OH VD2 and 25-OH VD3 in the mixed serum were 1.26 ng/mL and 36.6 ng/mL respectively and these data were used to deduct background in computing the recovery. Table 4 shows the experiment results.

Table 4 Recoveries of spiked serum samples

	25-OH VD2			25-OH VD3		
	Spiked concentration /ng/mL	Measured concentration /ng/mL	Recoveries /%	Spiked concentration /ng/mL	Measured concentration /ng/mL	Recoveries /%
Spiked sample 1 in low concentration	2	2.8	77.00%	10	47.4	108.30%
Spiked sample 2 in low concentration	2	2.9	82.00%	10	46	94.30%
Spiked sample 1 in high concentration	10	12.2	109.40%	50	85.5	97.90%
Spiked sample 2 in high concentration	10	12.5	112.40%	50	88.8	104.50%

Notes: The value of low concentration 25-OH VD2 sample was equivalent to that of the background of mixed serum, resulting in large error and low recovery rate.

Test Result of RECIPE

Calibration and QC samples, bought from RECIPE, were used to test the accuracy of this method, the analytical results are as follows:

Table 5 Accuracy of RECIPE test

	25-OH VD2			25-OH VD3		
	Label concentration /ng/mL	Measured concentration /ng/ mL	Accuracy /%	Label concentration /ng/mL	Measured concentration /ng/ mL	Accuracy /%
level-1	2.5	2.46	98.4	0.97	0.97	100
level-2	8.2	8.57	105	9.35	9.3	99.4
level-3	24.8	25.8	104	27.9	28.4	102
level-4	68.5	63.6	92.8	77.3	76.3	98.8
QC-level 1	16.3	15.1	92.6	20.5	23	112
QC-level 2	36.6	37.8	103.3	44.3	48	108.3

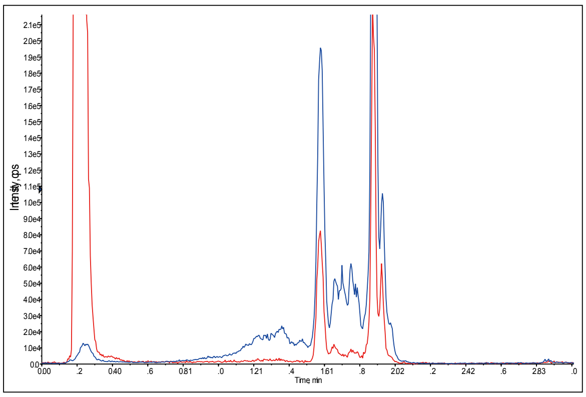


Figure 3 Chromatography of 25 ng/mL 25-OH VD$_3$ in serum

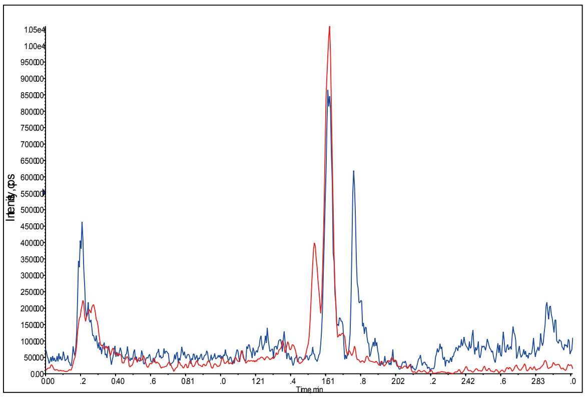


Figure 4 Chromatography of 5 ng/mL 25-OH VD$_2$ in serum

Conclusion

This experiment developed a quick method to purify the 25-OH VD2 and 25-OH VD3 from serum. First use the Cleanert PPT to precipitate protein of serum, then transfer the solvent into Cleanert PEP MicroPlate and add only 100μL solvent to eluent. The method should be saving time by no need for further concentration. So this method can be used in clinical study for detection of 25-OH VD in serum.

Ordering Information

Products	Specification	Cat.No.
Cleanert® PEP MicroPlate	5 mg/1 mL/Well	PE00501-MW
Cleanert® PPT	2.2 mL Square well	96CD2025-Q
Unisol C18	2.1 × 50mm, 3 μm	UO930502-0
Cleanert® M96 Positive Pressure Device	Adapt to 96-well plate	SPE-M96
96-well Collection Plate	1.0mL, 8×12, round well and round bottom	96SP1036-Y
96-well Mat	8×12, silica, round well, piecible	96GP2036-M

Speciality Applications

Banned azo dyes in textiles (Cleanert® SLE Azo Dyes Extraction Column, P/N: GB/T17592-2006)

EAE10100

Reduce the textiles in citrate buffer solution by sodiumdithionate to obtain forbidden aromatic amines that possibly exist. Extract the aromatic amines by proper liquild-liquid partition cartridge. After concentration, dilute to volume with proper organic solvent for determination by GC-MS. If necessary, choose one or more other methods to confirm the existence of isomers. HPLC/DAD or GC/MS is employed for quantification.

Materials

(1) Cleanert® SLE extraction cartridge
  20 cm×2.5 cm (i.d.) polypropylene cartridge, packed with 20 g of diatomite.
(2) Citrate buffer (0.06 mol/L, pH = 6.0)
  Dissolve 12.526 g of citric acid and 6.320 g of sodium hydroxide in water and dilute to 1000 mL.
(3) Sodiumdithionate solution
  200 mg/mL sodiumdithionate in water, fresh prepared with solid sodiumdithionate ($Na_2S_2O_4 \geq 85\%$) before use.

Experimental

Sample Preparation

Cut representative sample into small pieces of 5 mm × 5 mm and mix. Transfer 1.0 (accurate to 0.01 g) of sample into reactor and add 16 mL of citrate buffer at 70 ± 2°C. Seal the reactor and shake up until all samples are soaked in liquid. Put the reactor in water bath at 70 ± 2°C for 30 min to soak the textiles thoroughly. Add 3.0 mL of sodiumdithionate solution, seal and shake up. After another 30 min in water bath, cool the reactor to room temperature in 2 min.

Extraction and Concentration

(1) Extraction:
  Press the sample with a glass rod into the reactor and transfer the liquid into diatomite extraction cartridge. Allow to adsorb for 15 min. Elute the cartridge with ether four times (20 mL×4). For each time, combine the ether and eluate, and load onto the cartridge. Control the flow rate. Collect the eluate in a round-bottom flask.
(2) Concentration:
  Evaporate the eluate to 1 mL by rotary evaporator at 35 °C and dry under a slow stream of nitrogen.

GC-MS Analysis

Capillary Column: DA-5MS, 30 m×0.25 mm×0.25 μm, or a corresponsive one; Injection Temperature: 250°C;
Column Temperature: 50°C (0.5 min) 20°C/min 150°C (8 min) 20°C/min 230°C (20 min) 20°C/min 260°C (5 min)
MS Interface Temperature: 270°C; MS Scan Range: 35~350 amu; Injection Mode: splitless; Carrier Gas: He(≥99.999%);
Flow Rate: 1.0 mL/min; Injection Volume: 1 μL; Ionization Source: EI; Ionization voltage: 70 eV.

Ordering Information

Products	Specification	Cat.No.
Cleanert® SLE	14.5 g/60 mL	GB/T17592-2006
DA-5MS	30 m×0.25 mm×0.25 μm	1525-3002

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