Application of QuEChERS and Solid Phase Extraction in Food Safety

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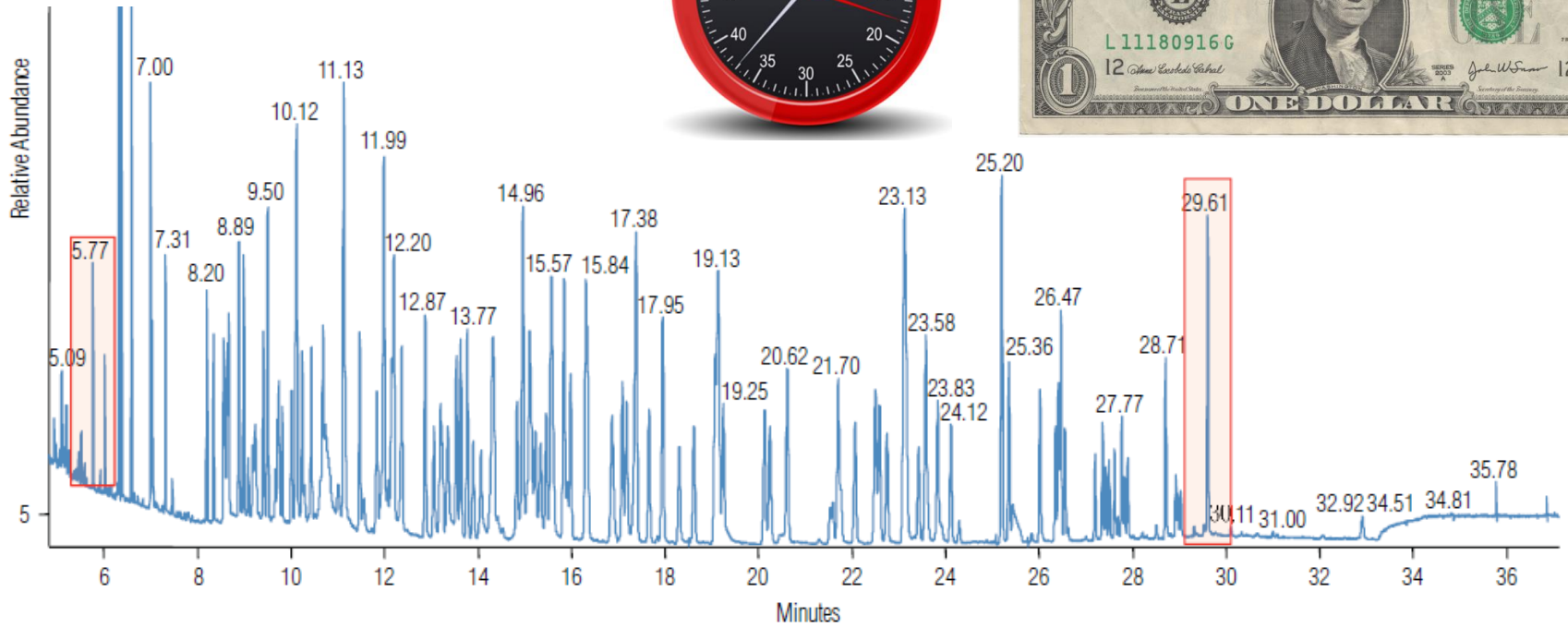
Outline

- Analytical Challenges
- Meeting the Challenge
- QuEChERS applications
  - Multi residues pesticides in agriculture food
  - Multiple Mycotoxins in Grain
- SPE applications
  - Multiple veterinary drugs screening in animal products
  - Hormone residues in animal-derived food

Analytical Challenges

- Diverse and complex food matrices
- Increasingly large number of target compounds
- Low limits of detection
- High throughput (Efficient)
- Cost effective



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How do we over come the Challenges?

- Sample preparation
  - Removal of suppressants
  - Reduced matrix effects
- High chromatographic resolution
  - Accurate quantification
  - Accurate qualification
  - Speed in analysis
- Selectivity of detection
  - Accurate quantification
  - Accurate qualification
  - Speed in analysis
- Reproducible products

Sample preparation options

Commonly employed techniques:

- Filtration
- Conventional SPE – silica, polymeric, Hypercarb
  - Targeted extraction
- QuEChERS
  - Multi residue extraction
- Liquid/Liquid Extraction

Why Use SPE for Food Analysis ?

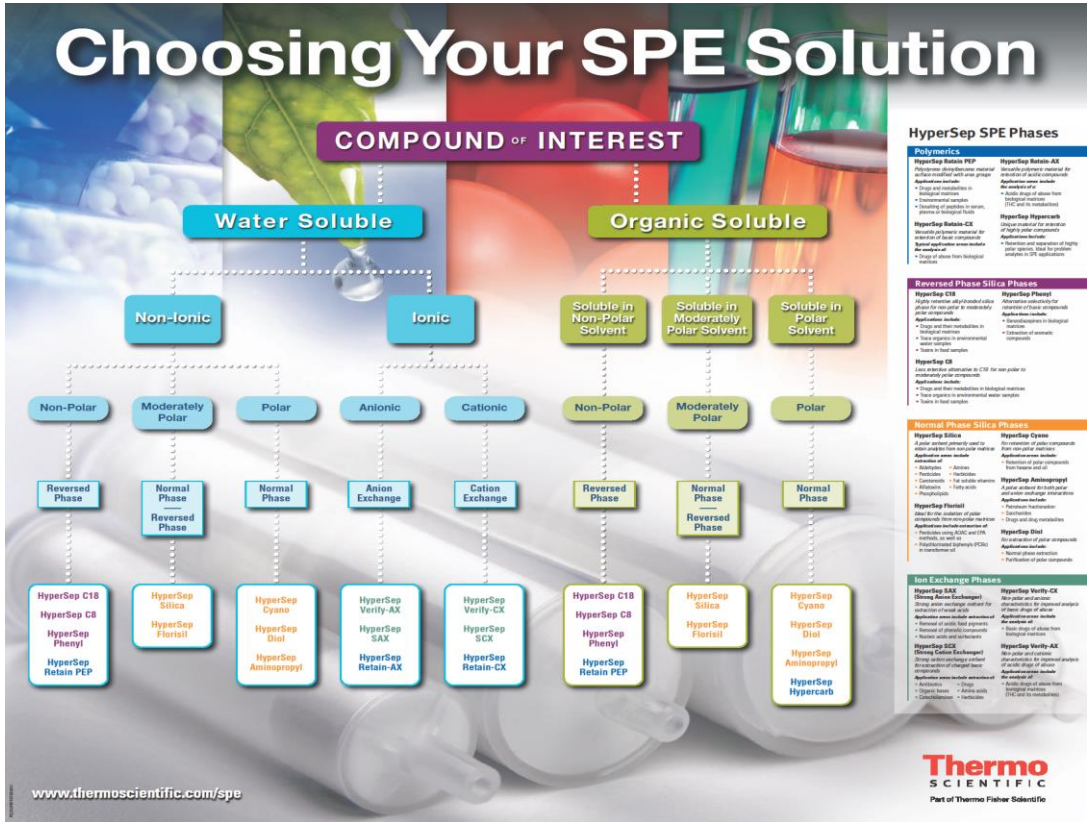
- Sample Enrichment
  - To achieve sub ppb detection limits, usually the sample must be concentrated 10-100 times
    - using only liquid extraction (LE), as much as **100 mL** of solvent must be evaporated
    - using SPE to concentrate the extract, only **a few mL** of solvent must be evaporated

- Sample Cleanup
  - Significant cleanup of food matrix can often be accomplished using SPE
  - Multiple SPE cartridges can often be used for optimum cleanup

SPE - Considerations

The choice of SPE mechanism (RP, NP, IEX, MM) depends upon;

- Physicochemical differences between the analyte and matrix
- Analyte solubility
- Analyte polarity (LogP/LogD)
- Analyte charge state (pKa analyte/pH of solution)



SPE Strategy

- Strategy 1: Retention, cleanup, elution, most effective cleanup and enrichment for individual compound class analysis

- Strategy 2: Pass-through cleanup, more effective cleanup for multiresidue analysis

- Strategy 3 : Dispersive cleanup, ex. QuEChERS, acceptable cleanup for multiresidue screening
  1. Combine sorbent, sample matrix and solvent into a vessel
  2. Sample is filtered or centrifuged. **Matrix interferences are retained by sorbent**
  3. Filtrate or supernatant is collected for analysis **Analytes are in the filtrate or supernatant**

What is QuEChERS (dispersive SPE)?

- QuEChERS or dispersive SPE is a sample preparation method which is typically employed for the extraction of pesticides from food matrices
- QuEChERS stands for:
  - **Qu**ick
  - **E**asy
  - **Ch**eap
  - **E**ffective
  - **R**obust
  - **S**afe
- Typical formats are:
  - Sorbent pre packed in 50, 15 and 2mL centrifuge tubes
  - Sorbent pre-packed in mylar pouches
- Different sorbent configurations are designed to target specific food matrices

Sample Homogenisation Before Extraction

- Unavoidable steps in the analysis
- Prerequisite to obtain representative sub-samples
- Critical importance

Laboratory Sample

Analytical Sample

Test portion

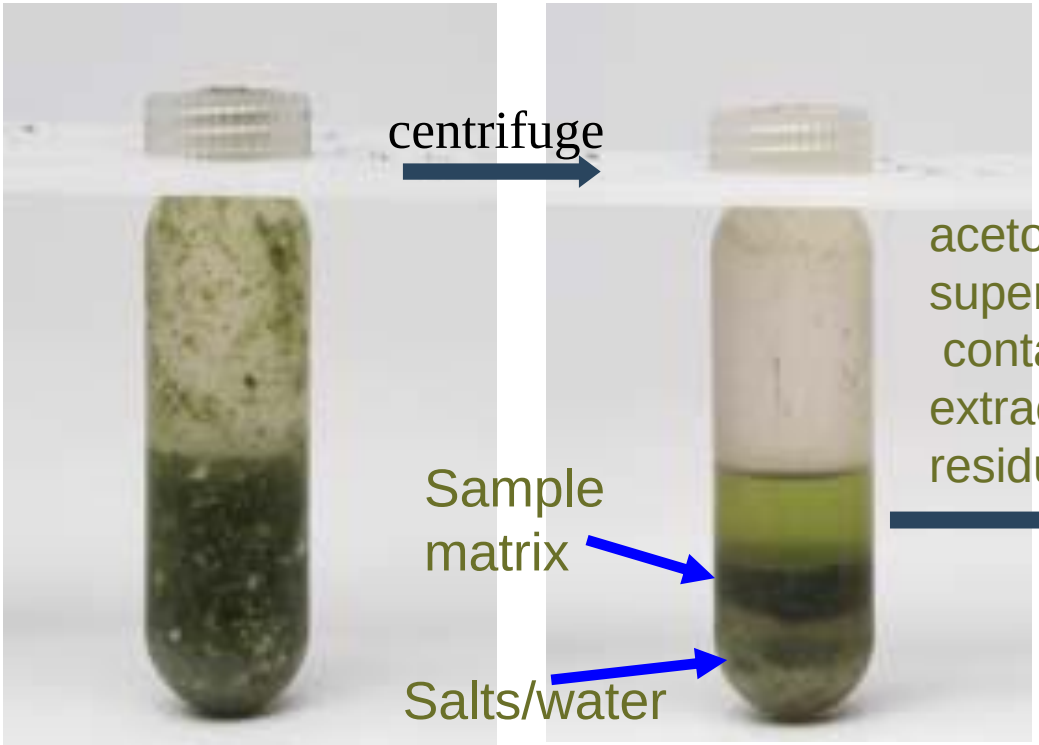
How does QuEChERS work?

QuEChERS is typically a 4 step process:

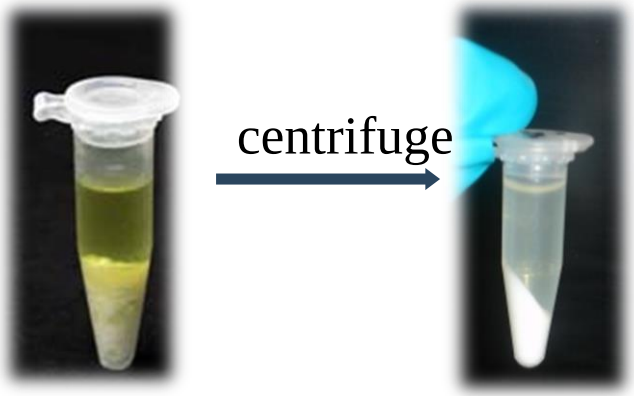
- Stage 1: Select the appropriate product based on the matrix type
- Stage 2: Extraction (liquid/liquid) - 50mL centrifuge tube
- Stage 3: Sample clean-up to remove interferences that can interfere with subsequent analysis - 2 and 15mL centrifuge tube
- Stage 4: Analysis - typically performed via GCMS (LCMS can also be present)

What is QuEChERS? *Extraction & Clean-up in a Tube*

1) Extraction



2) Dispersive SPE



Note: Add sample to the tube, then solvent, then sorbent then mix, to avoid agglomeration

QuEChERS Method Variations

Method	Description
Original QuEChERS Method – introduced in 2003	Uses Sodium Chloride to enhance extraction
Dispersive AOAC 2007.01 Method	Uses Sodium Acetate as a buffer replacing Sodium Chloride
Dual Phase Variation	Uses PSA & GCB to remove high levels of chlorophyll and plant sterols
European Version	Similar to AOAC method – uses sodium chloride, sodium citrate dihydrate and disodium citrate sesquihydrate

Original QuEChERS Method:

Stage 1: Selecting the Method

1. Weigh 15 g of homogenised (hydrated at least 80%) sample in a 50 mL centrifuge tube
2. Add 15 mL acetonitrile (or 1:1 acetone/hexane, ethyl acetate) and IS
3. Shake briefly
4. Add 4 g anhydrous magnesium sulfate and 1g sodium chloride
5. Shake by hand for 1 minute
6. Centrifuge at 5,000 rpm for 5 min
7. Transfer a portion of supernatent to a QuEChERS clean up tube
8. Shake for 30 sec
9. Centrifuge for 1 min at 6,000 rpm
10. Transfer 0.5 mL aliquot for analysis

Stage 1: Selecting the Method

1. Weigh 15 g of homogenised (hydrated at least 80%) sample in a 50 mL centrifuge tube
2. Add 15 mL 1% acetic acid in acetonitrile (or 1:1 acetone/hexane, ethyl acetate) and IS
3. Shake briefly
4. Add 6 g anhydrous magnesium sulfate and 1.5 g anhydrous sodium acetate
5. Shake by hand for 1 min
6. Centrifuge at 3,700 rpm for 5 min
7. Transfer a portion of supernatent to a QuEChERS clean up tube
8. Shake for 30 sec
9. Centrifuge for 1 min at 3,700 rpm
10. Transfer 0.5 mL aliquot for analysis and add TPP and either formic acid or toluene / magnesium sulfate

Stage 1: Selecting the Method

- Using sodium acetate as a buffer protects base sensitive compounds
  - Folpet
  - Dichlofluanid
  - Chlorthanonil
  - Dicofol
  - Captan
  - Tolyfluanid

AOAC Method –Disadvantages

- The presence of acetic acid
  - PSA will absorb acetic acid
  - Less sample clean-up
  - Higher baseline

- Only use this method if looking at specific compounds

Schenck Method – for Polar Aromatic Compounds:

Stage 1: Selecting the Method

Using dual layer PSA/GCB cartridge

- Extraction phase as AOAC method
- Pre-rinse the SPE cartridge with 5 mL of toluene
- Add an aliquot of the supernatent to the cartridge
- Start collection
- Elute with 6-12 mL of 3:1 acetone:toluene
- Concentrate for GCMS analysis –or-
- Concentrate to near dryness and reconstitute in mobile phase for LC analysis

Stage 1: Selecting the Method

1. Weigh 15 g of homogenised (hydrated at least 80%) sample in a 50 mL centrifuge tube
2. Add 15 mL acetonitrile (or 1:1 acetone/hexane, ethyl acetate) and IS
3. Shake briefly
4. Add 6 g anhydrous magnesium sulfate, 1.5 g sodium chloride, 1.5 g sodium citrate tribasic dihydrate, 0.75 g sodium citrate dibasic
5. Shake by hand for 1 min
6. Centrifuge at 5,000 rpm for 5 min
7. Transfer a portion of supernatent to a QuEChERS clean up tube
8. Shake for 30 sec
9. Centrifuge for 1 min at 6,000 rpm
10. Transfer 0.5 mL aliquot for analysis

Stage 2: Select the Right Product

Matrix Type	Examples	Sorbent Requirements for Clean-Up
General Matrices	• Apples • Cucumber • Melon 	$MgSO_4$, PSA Removal of excess water organic acids, fatty acids, sugars
Fatty Matrices	• Milk • Cereals • Fish 	$MgSO_4$, PSA, C18 Additional removal of lipids & sterols
Pigmented Matrices	• Lettuce • Carrot • Wine 	$MgSO_4$, PSA, C18, GCB Additional removal of pigments & sterols
High Pigmented Matrices	• Spinach • Red Peppers 	$MgSO_4$, PSA, C18, GCB, Chlorofiltr™ Additional removal of chlorophyll

Stage 2: Select the Right Product

Material	Purpose
Magnesium Sulphate	Removal of excess water
PSA (Primary / Secondary Amine)	Removal of organic acids, fatty acids, sugars
C18	Removal of lipids & sterols
GCB (Graphatized Carbon Black)	Removal of pigments & sterols
Chlorofiltr™	Removal of chlorophyll

Application – Pesticides in Cucumber

Application Note 20549

QuEChERS Dispersive Solid Phase Extraction for the GC-MS Analysis of Pesticides in Cucumber

Anita I Khan, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Key Words
QuEChERS, pesticide residue analysis, cucumber, food safety

Abstract
QuEChERS dispersive SPE is a simple, fast and quantitative sample preparation method. This application demonstrates the effectiveness of this technique in the GC/MS analysis of pesticides in cucumber, using a Thermo Scientific TraceGOLD TG-5MS GC column for analysis.

The recoveries for the spiked pesticides in cucumber matrix at 50 ng/g were between 75.2 to 119.6% with relative standard deviations ranging from 2.1 to 8.9% using the QuEChERS method described in EN15662.

Introduction

QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) is a dispersive Solid Phase Extraction (SPE) technique for extracting multi-residue pesticides from fruits and vegetables. The advantages of this methodology are speed, ease of execution, minimal solvent requirement and cost. The QuEChERS methodology was developed by Anastassiades et al[1] and has become widely used in food safety analyses.

The method is:

- **Quick** – high sample throughput, typically 8 samples can be prepared in under 30 min
- **Easy** – it requires less handling of extracts than other techniques i.e. fewer steps are required
- **Cheap** – less sorbent material is needed and less time is required to process samples compared to other techniques
- **Effective** – the simple technique gives high and accurate recovery levels for a range of different compound types.
- **Rugged** – the method can detect a large number of pesticides including charged and polar pesticides
- **Safe** – unlike other techniques, it does not require the use of chlorinated solvents. Extraction is typically carried out using acetonitrile, which is both GC and LC compatible.

The sample preparation approach described in the European EN15662 QuEChERS procedure[2] was used for extracting pesticides from cucumber. This is a two stage process: sample extraction, followed by dispersive SPE.

In the sample extraction stage, the food sample is homogenized to increase the available surface area of the sample to provide optimal extraction efficiencies. The homogenized sample is placed in the extraction tube containing magnesium sulfate and salts (sodium chloride, sodium citrate tribasic dihydrate, sodium citrate dibasic sesquihydrate). Magnesium sulfate ensures that, upon addition of acetonitrile, a phase separation is induced between water and organic solvent with the pesticides of interest being extracted into the organic phase. When acetonitrile is poured into the extraction tube containing the homogenized sample,

- QuEChERS sample preparation
- Analysis using 5% diphenyl / 95% dimethylpolysiloxane GC phase

Sample Preparation

The methodology described in this application note is for the preparation of calibration standards and sample spike (Figure 1).

Extraction Stage	Dispersive SPE Clean-up
(1) Add 10 g of homogenized cucumber in 50 mL FEP extraction tube. Shaken thoroughly	(5) Transfer 11 mL of acetonitrile extract to QuEChERS extraction tube P/N 60105-227.
(2) Add pesticide standard Sample spike: Add 10 mL of 50 ng/mL pesticide standard Add in acetontrile. Blank: No pesticide standard added, 10 mL of actonitrile added	(6) Shake vigorously for 5 minutes and centrifuge for 6 minutes at 3500 rpm
(3) Add the content of the QuEChERS metalized pouch into the centrifuge tube PN 60105-337 very slowly and under ice bath	(7) Transfer 1 mL of sample extract to a GC vial Calibration standards: Add 10 µL of 1, 2.5, 5, 10, 25, 50, 100 µg/mL pesticide standard mix into 990 µL of sample extract.
(4) Shake vigorously for 5 minutes and centrifuge for 6 minutes at 3500 rpm	(8) Add 10 µL of 100 µg/mL internal standard (triphenylphosphine) to 1 mL of sample extract.

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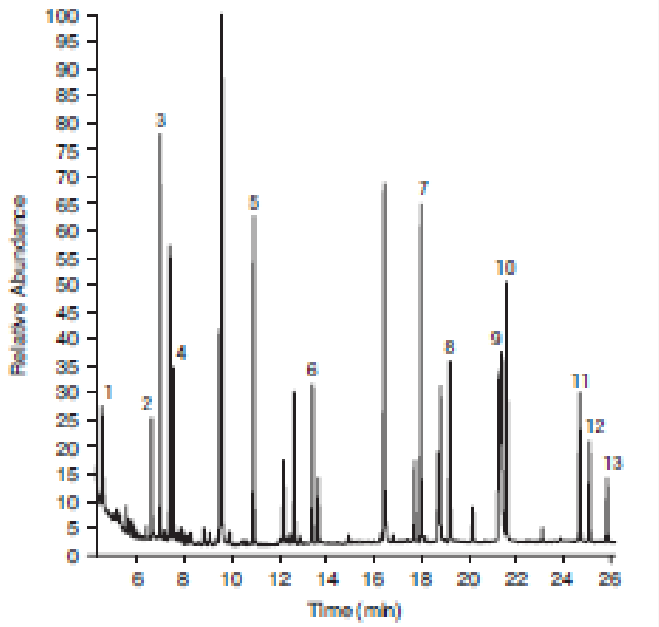
Application – Pesticides in Cucumber



Pesticides	t_R (min)	Linearity	Nominal concentration ng/g	Measured concentration (n=6) ng/g.	Average % Recovery (n=6)	Recovery %RSD (n=6)
1. Dichlobenil	4.52	0.9988	50	58.7	117.4	2.1
2. Tribromoanisol	6.60	0.9990	50	54.3	108.5	6.0
3. Sulfotep	6.95	0.9984	50	59.8	119.6	2.3
4. Hexachlorobenzene	7.49	0.9983	50	55.2	110.4	2.8
5. Parathion	10.90	0.9979	50	53.0	106.0	5.9
6. Triphenylphosphine (IS)	13.41	-	-	-	-	-
7. EPN	17.90	0.9985	50	46.1	92.1	6.7
8. Azinphos methyl	19.20	0.9984	50	37.6	75.2	4.9
9. Permethrin isomer a	21.38	0.9987	50	49.8	99.5	8.9
10. Permethrin isomer b	21.58	0.9985	50	50.9	101.9	4.8
11. Fenvalerate isomer a	24.60	0.9985	50	47.7	95.4	8.9
12. Fenvalerate isomer b	25.02	0.9973	50	50.6	101.2	7.2
13. Deltamethrin	25.84	0.9949	50	51.7	103.3	6.8

- TraceGOLD TG-5MS GC base line resolution 12 pesticides
- Linear range of 25-1000ng/g (R^2 0.99)
- QuEChERS method:
  - Recoveries >75%
  - Reproducibility <9%RSD.

Application – Pesticides in Grapes

Application Note: ANGSC PESTGRAPES 0709

QuEChERS Dispersive Solid Phase Extraction for the GC/MS Analysis of Pesticides in Grapes

Anila Khan, Luisa Pereira, Stephen Aspey, Rob Bunn, Ruth Lewis, Thermo Fisher Scientific, Runcorn, UK

Key Words
- QuEChERS
- Food Safety
- Pesticides Residue Analysis
- TRACE TR-5MS
- TRACEGuard

Introduction

QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) is a dispersive Solid Phase Extraction (SPE) technique for extracting multi-residues of pesticides from fruits and vegetables. The advantages of this methodology are speed, ease of execution, minimal solvent requirement and cost to perform when compared with conventional solid phase extraction techniques.

The QuEChERS methodology was developed by Anastassiades *et al* and has become widely used in food safety analyses.[1] The method is:

- Quick – high sample throughput, typically 8 samples can be prepared in just under 30 min
- Easy – it requires less handling of extracts than other techniques and no laborious steps are involved
- Cheap – less sorbent material is needed and less time is need to process samples compared to other techniques
- Effective – the simple technique gives high and accurate recovery levels for a range of different compound types e.g polar pesticides, pH dependent compounds
- Rugged – the method can detect a large number of pesticides including pH dependent and polar pesticides
- Safe – unlike other techniques, it does not require any chlorinated solvents. Extraction is typically carried out using acetonitrile, which is both GC and LC amenable

The QuEChERS procedure is usually a two stage process: sample extraction, followed by dispersive SPE. In the sample extraction stage, the food sample is homogenized to maximize the available surface area of the sample for better extraction efficiencies. The homogenized sample is placed in the extraction tube containing magnesium sulphate and sodium acetate. Magnesium Sulfate ensures that upon addition of acetonitrile, a phase separation is induced between water and organic solvent with the pesticides of interest being extracted into the organic phase. When acetonitrile is poured into the extraction tube containing the homogenized sample, an exothermic reaction occurs between the magnesium sulphate and water, which can lead to low recoveries of the pesticides. This effect can be reduced by adding the salt and sample to the extraction tube while this is immersed in an ice bath or by weighing the sample into a FEP tube and then adding the solvent and salts slowly. The tube is then capped, shaken vigorously and centrifuged. The second stage of the QuEChERS method uses dispersive SPE, which involves transferring a portion of the acetonitrile extract to a clean-up tube containing a combination of sorbents for removal of unwanted sample components. This may be followed by solvent exchange to improve compatibility of samples to GC analysis, and additional sample clean-up to reduce matrix effects and therefore improve method robustness.

Internal standards are used to minimize errors that might be introduced in the different steps of the QuEChERS method, as well as compensate for GC injection variability. Furthermore, adding analyte protectants such as sorbitol can be useful for labile pesticides at intermediate pH, which can be prone to decomposition in the GC injector port.

The pesticides analyzed are a mixture of organophosphate, organochlorine, pyretheroid, benzenoid, triazole and dicarboximide compounds. Lehotay reviewed the LC and GC analyses of pesticides in produce and the type of pesticide that is likely to be found in each matrix.[2] The requirements for pesticide residue analysis in fruit and vegetables are established by organizations such as World Health Organization, Japanese Food Chemical Research Foundation, EEC Directives, and the US-EPA.[3,4,5,6] These organizations establish which pesticides need to be determined in different produce and the Method Regulatory Limits (MRLs). The pesticides determined in this study are all listed by the four regulatory organizations and all have minimum MRLs of 50 ng/g (ppb). The recoveries of the pesticides in grapes are based on this value.

Goal

To demonstrate QuEChERS dispersive SPE as a simple, fast and quantitative sample preparation method for the GC/MS analysis of pesticides in grapes.

Additionally, demonstrate the suitability of the Thermo Scientific TRACE TR5-MS analytical column combined with the Thermo Scientific TRACEGuard guard column for pesticide analysis.

Experimental

Sample Preparation

Reagents

Green grapes obtained from the local supermarket
Acetonitrile HPLC grade *(Thermo Fisher P/N A\0626\17)*
Hexane GC grade *(Thermo Fisher P/N H\0355\17)*
Acetone GC grade *(Thermo Fisher P/N A\0600\17)*
Glacial acetic acid HPLC grade *(Thermo Fisher P/N A\0406\PB15)*

Pesticide (peak number, name)	Rt (min)	Linearity (R^2)	Average % recovery (n=3)	Recovery % RSD
1. Chlorpyrifos methyl	14.92	0.9961	76	14.5
2. Metalaxyl	15.40	0.9908	100	7.9
3. Malathion	15.94	0.9989	103	19.0
4. Chlorpyrifos	16.12	0.9919	77	21.5
5. Penconazole	17.23	0.9978	94	7.2
6. Procymidon	17.45	0.9968	106	4.0
7. Dicofol	20.21	0.9964	110	9.9
8. Permethrin Isomer a	21.42	0.9980	102	3.9
9. Permethrin Isomer b	21.55	0.9965		

organophosphate,organochlorine, pyretheroid, benzenoid, triazole recoveries of greater than 76% & and RSD's less than 11%

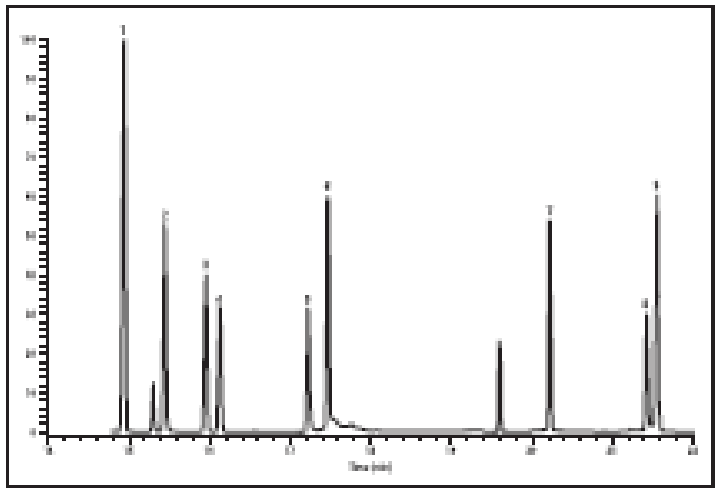


Figure 2: TIC for the GC/MS analysis of grapes spiked with 1 ng/µL of each pesticide

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Step	Description	Capacity (mL)	Cat. No.	Quantity
Original Method				
Extraction	4000mg magnesium sulfate, 1000mg sodium chloride	50	**60105-211**	250 Pack
Clean-Up	150mg magnesium sulfate, 50mg PSA, 50mg C18	2	**60105-204**	100 Pack
	150mg magnesium sulfate, 50mg PSA, 50mg C18, 50mg GCB	2	**60105-223**	100 Pack
	1200mg magnesium sulfate, 400mg PSA, 400mg C18	15	**60105-225**	50 Pack
AOAC 2007.1				
Extraction	6000g magnesium sulfate, 400mg PSA, 400mg C18, 400mg GCB	50	**60105-210**	250 Pack
Clean-Up	150mg magnesium sulfate, 50mg PSA	2	**60105-203**	100 Pack
	150mg magnesium sulfate, 50mg PSA, 50mg C18	2	**60105-204**	100 Pack
	150mg magnesium sulfate, 50mg PSA, 50mg C18, 50mg GCB	2	**60105-223**	100 Pack
	900mg magnesium sulfate, 300mg PSA, 150mg GCB	15	**60105-205**	50 Pack
	900mg magnesium sulfate, 300mg PSA, 150mg C18	15	**60105-206**	50 Pack
	1200mg magnesium sulfate, 400mg PSA	15	**60105-224**	50 Pack
	1200mg magnesium sulfate, 400mg PSA, 400mg C18	15	**60105-225**	50 Pack
	1200mg magnesium sulfate, 400mg PSA, 400mg C18, 400mg GCB	15	**60105-226**	50 Pack
European EN15662				
Extraction	6000g magnesium sulfate, 1500mg sodium chloride, 1500mg sodium citrate tribasic dihydrate, 750mg sodium citrate dibasic sesquihydrate	50	**60105-212**	250 Pack
	4000mg magnesium sulfate, 1000mg sodium chloride, 1000mg sodium citrate tribasic dihydrate, 500mg sodium citrate dibasic sesquihydrate	50	**60105-216**	250 Pack
Clean-Up	150mg magnesium sulfate, 25mg PSA	2	**60105-219**	100 Pack
	150mg magnesium sulfate, 25mg PSA, 2.5mg GCB	2	**60105-221**	100 Pack
	150mg magnesium sulfate, 25mg PSA, 7.5mg GCB	2	**60105-222**	100 Pack
	900mg magnesium sulfate, 150mg PSA	15	**60105-215**	50 Pack
	900mg magnesium sulfate, 150mg PSA, 45mg GCB	15	**60105-217**	50 Pack
	900mg magnesium sulfate, 150mg PSA, 15mg GCB	15	**60105-218**	50 Pack
	900mg magnesium sulfate, 150mg PSA, 150mg C18	15	**60105-227**	50 Pack
Dual Phase Method				
	200mg GCB on top, 400mg PSA on bottom, separated by a frit	6	**60105-207**	30 Pack
	250mg GCB on top, 500mg PSA on bottom, separated by a frit	6	**60105-208**	30 Pack
	500mg GCB on top, 500mg PSA on bottom, separated by a frit	6	**60105-209**	30 Pack

HyperSep Dispersive SPE Multipacks

Description	Cat. No.	Quantity
4000mg anhydrous magnesium sulfate, 1000mg sodium chloride	**60105-332**	50 Pack
4000mg anhydrous magnesium sulfate, 1000mg sodium chloride, 500mg sodium citrate dibasic sesquihydrate, 1000mg sodium citrate tribasic	**60105-333**	50 Pack
4000mg anhydrous magnesium sulfate, 1000mg sodium acetate	**60105-334**	50 Pack
6000mg anhydrous magnesium sulfate, 1500mg sodium acetate	**60105-335**	50 Pack
6000mg anhydrous magnesium sulfate, 1500mg sodium chloride	**60105-336**	50 Pack
6000mg anhydrous magnesium sulfate, 1500mg magnesium sulfate, 1500mg sodium citrate dihydrate, 750mg disodium citrate sesquihydrate	**60105-337**	50 Pack
8000mg anhydrous magnesium sulfate, 2000mg of sodium chloride	**60105-338**	50 Pack
8000mg anhydrous magnesium sulfate, 3500mg of sodium chloride	**60105-339**	50 Pack

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Multiple Mycotoxins in Grain Using QuEChERS

Application Note 21121

Determination of Multiple Mycotoxins in Grain Using a QuEChERS Sample Preparation Approach and LC-MS/MS Detection

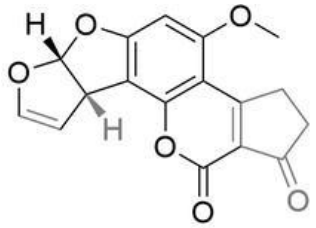
Jon Bardsley, Mike Oliver, Thermo Fisher Scientific, Runcorn, UK

17 Mycotoxins in Grain on Accucore aQ

Key Words
Mycotoxins, food, HyperSep, QuEChERS, dispersive SPE, Accucore aQ, TSQ Vantage

Goal
To demonstrate a fast, easy, and cost-effective approach for the determination of 16 mycotoxin residues in grain-based food using QuEChERS sample preparation with a Thermo Scientific™ Accucore™ aQ HPLC column and a Thermo Scientific™ TSQ™ Vantage™ triple quadrupole mass spectrometer for HPLC separation.

Introduction
Mycotoxins are toxic secondary metabolites that are naturally produced by several species of fungi on agricultural products, particularly grain-based products. Mycotoxins are chemically stable and cannot be destroyed during food processing and heat treatment. Therefore, they may occur in the field, in raw materials during storage, and in processed foods. To date, more than 300 mycotoxins, possessing varying degrees of toxicity, have been identified, although only a relatively few of these are widely accepted as presenting a significant food or animal feed safety risk.[1] Studies have shown that limited, final extracts may still contain large amounts of matrix components that can negatively affect the detection system.



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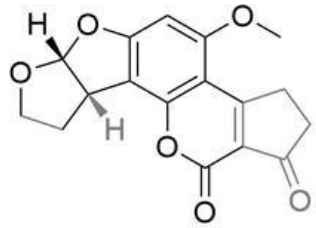

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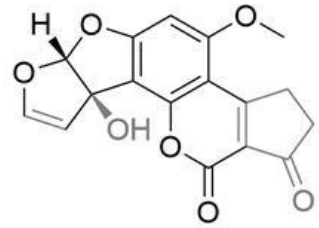

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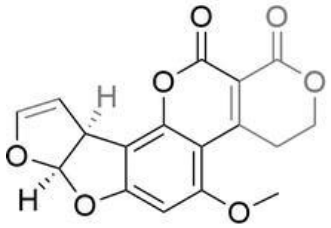

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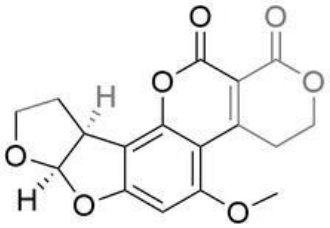

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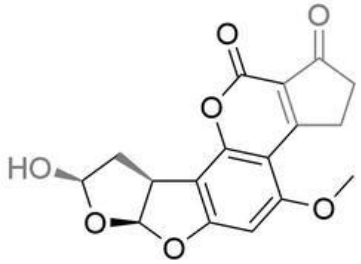

AFB_{2a}

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Multiple Mycotoxins in Grain Using QuEChERS

- **Sample extraction**

1. Place 5 g of sample into a 50 mL centrifuge tube.
2. Add 10 mL water.
3. Vortex briefly and allow to hydrate for at least 15 minutes.
4. Add 250 µL of 1 µg/mL internal standard. Add 10 mL acetonitrile containing 2% formic acid.
5. Shake/vortex samples for 15 minutes to extract mycotoxins.
6. Add the contents of a HyperSep Dispersive SPE Mylar Pouch (P/N 60105-340) containing the extraction salts.
7. Immediately shake for 1 minute.
8. Centrifuge for 5 min at ≥ 3000 *g*.

- **Sample cleanup**

1. Transfer 1 mL of supernatant to a dSPE tube (HyperSep Dispersive SPE Clean-up Product **P/N 60105-204).** 150mg anhydrous magnesium sulfate, 50mg PSA, 50mg C18
2. Vortex for 30 seconds.
3. Centrifuge for 5 min at ≥ 3000 *g*.
4. Transfer 500 µL of purified supernatant to a 5 mL test tube.
5. Evaporate the acetonitrile extract to dryness and reconstitute with 500 µL methanol / water (50:50, v/v) for better chromatographic performance.
6. Filter the extract, using a 0.2 µm syringe filter (P/N F2513-2), directly into an autosampler vial (P/N 60180-600).

Multiple Mycotoxins in Grain Using QuEChERS

For improved sensitivity at low ppb concentrations, the dSPE step can be scaled-up by following the steps below:

1. Transfer 8 mL of supernatant to a 15 mL HyperSepDispersive SPE Clean-up Product (1200 mg anhydrousMgSO4, 400 mg PSA, and 400 mg C18, 15 mL centrifuge tube, P/N 60105-204).
2. Vortex for 30 seconds.
3. Centrifuge for 5 minutes at ≥ 3000 g.
4. Transfer 5 mL of supernatant to a 5 mL test tube.
5. Evaporate the sample to dryness at 40–50 ° C under a gentle stream of nitrogen.
6. Reconstitute sample in 1 mL methanol / water (50:50, v/v).

Multiple Mycotoxins in Grain Using QuEChERS

Instrumentation	Thermo Scientific™ Dionex™ UltiMate™ 3000
Column	Accucore aQ, 100 × 2.1 mm, 2.6 µm (P/N 17326-102130)
Guard column	Accucore aQ, 10 × 2.1 mm, 2.6 µm (P/N 17326-012105)
Run time	17 min (including 4 min equilibration)
Temperature	45 °C
Injection volume	5 µL
Autosampler temperature	10 °C
Wash solvent	Methanol / water (1:1, v/v)
Flow rate	400 µL/min
Waste divert	Mobile phase was diverted to waste from 0–1.5 and 13–17 min to reduce ion source contamination
Mobile phase A	10 mM ammonium formate in water
Mobile phase B	Methanol

LC gradient.

Time (min)	A (%)	B (%)
0.0	100	0
1.0	75	25
4.0	75	25
5.0	60	40
8.0	60	40
8.5	40	60
9.5	40	60
10.0	0	100
13.0	0	100
13.2	100	0
17.0	100	0

Multiple Mycotoxins in Grain Using QuEChERS

Accuracy and precision data for the 16 mycotoxins fortified at two concentrations.

	20 µg/kg		100 µg/kg	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Nivalenol	71.4	11.2	67.2	6.5
Deoxynivalenol	106.7	4.1	97.0	2.8
3-Acetyldeoxynivalenol	100.4	3.9	97.2	1.9
Fusarenon X	96.3	3.9	96.2	3.8
Neosolaniol	100.5	3.3	99.4	2.0
Diacetoxyscirpenol	102.6	2.8	99.0	2.3
Altemariol	94.8	4.9	85.9	5.4
β-zearalanol	94.5	9.2	92.7	4.6
α-zearalanol	93.9	10.5	89.0	3.5
Zearalenone	92.4	9.4	87.6	4.5
Ochratoxin A	93.8	3.0	94.7	3.8
T-2 toxin	96.2	4.5	94.2	2.8
Aflatoxin B1	97.0	2.7	91.7	5.4
Aflatoxin B2	97.4	2.9	91.4	4.8
Aflatoxin G1	95.0	3.3	92.0	4.1
Aflatoxin G2	95.5	3.1	93.9	2.7

Matrix-matched calibration curves of the 16 mycotoxins were prepared at concentrations of 5, 12.5, 25, 50, and 100 ng/mL and were found to give linear responses over the entire concentration range with correlation coefficients (R2) typically greater than 0.995.The signal-to-noise ratio (S/N) at the lowest calibration level (5 ng/mL; 10 ng/g) was found to be >10 for all 16 compounds. Therefore, the LOQ was estimated to be ≤10 ng/g in this study.

Animal-Derived Food multiple vet. drug residue analysis

Pork,
Chiken,
liver, duck

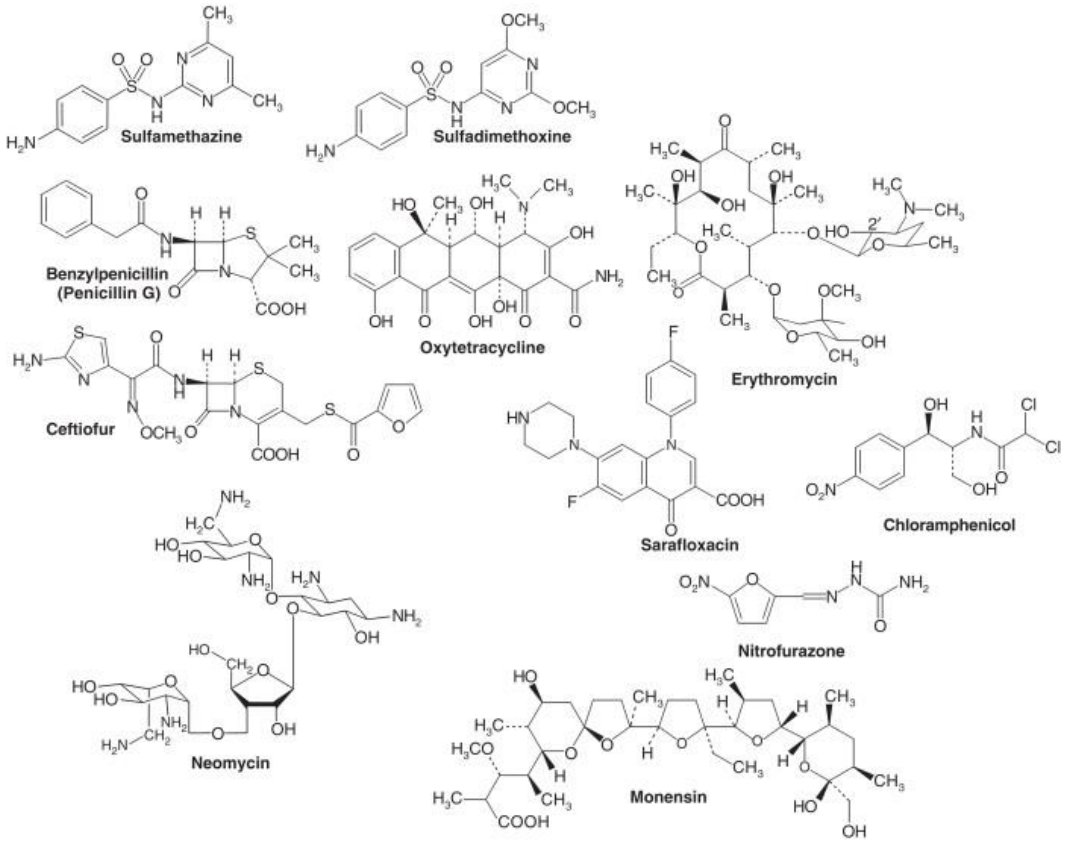
Homogenized

Homogenization for 1min

Ultrasonic extraction 15 min

SPE clean up

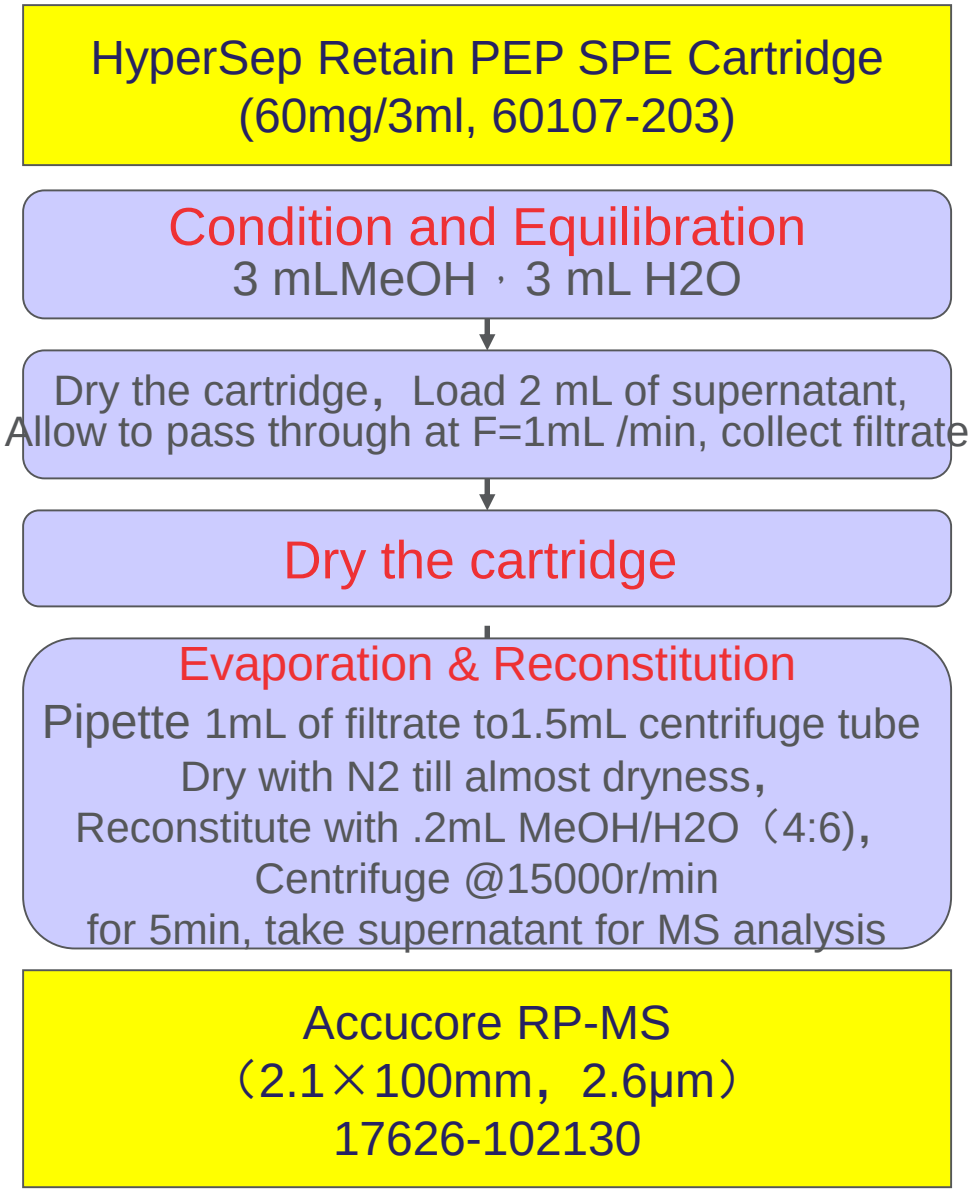
MS analysis



HyperSep Retain PEP pass through application for multiple vet. drug residue analysis

Accurately weigh 2.5g sample into a 50mL centrifuge tube, first add 1mL water to mix, then add 9mL containing 0.5% formic acid-acetonitrile, vortex to disperse and mix; Hold homogenizer for 1~2min

If homogenizer is not available, it is recomemended To shake for 20 min instead. Ultrasonic extraction at 40 C for 15 min, centrifugation at 4 C for 12000rpm/min for 5 min. The supernatant is to be purified by SPE.



SPE pass through application for multiple vet. drug residue analysis

- Column: **Accucore RP-MS (2.1 x 100 mm, 2.6 μm)**
- Positive mode :
  - mobile phase A: water (0.1% formic acid);
  - mobile phase B: acetonitrile (0.1% A) Acid);
- Negative mode :
  - mobile phase A: water (0.03% ammonia);
  - mobile phase B: acetonitrile (0.03% ammonia)
- Flow rate: 0.3 mL/min;
- Injection volume: 5 μL;
- Column temperature: 30 ° C.
- The mobile phase elution gradient is shown in Table

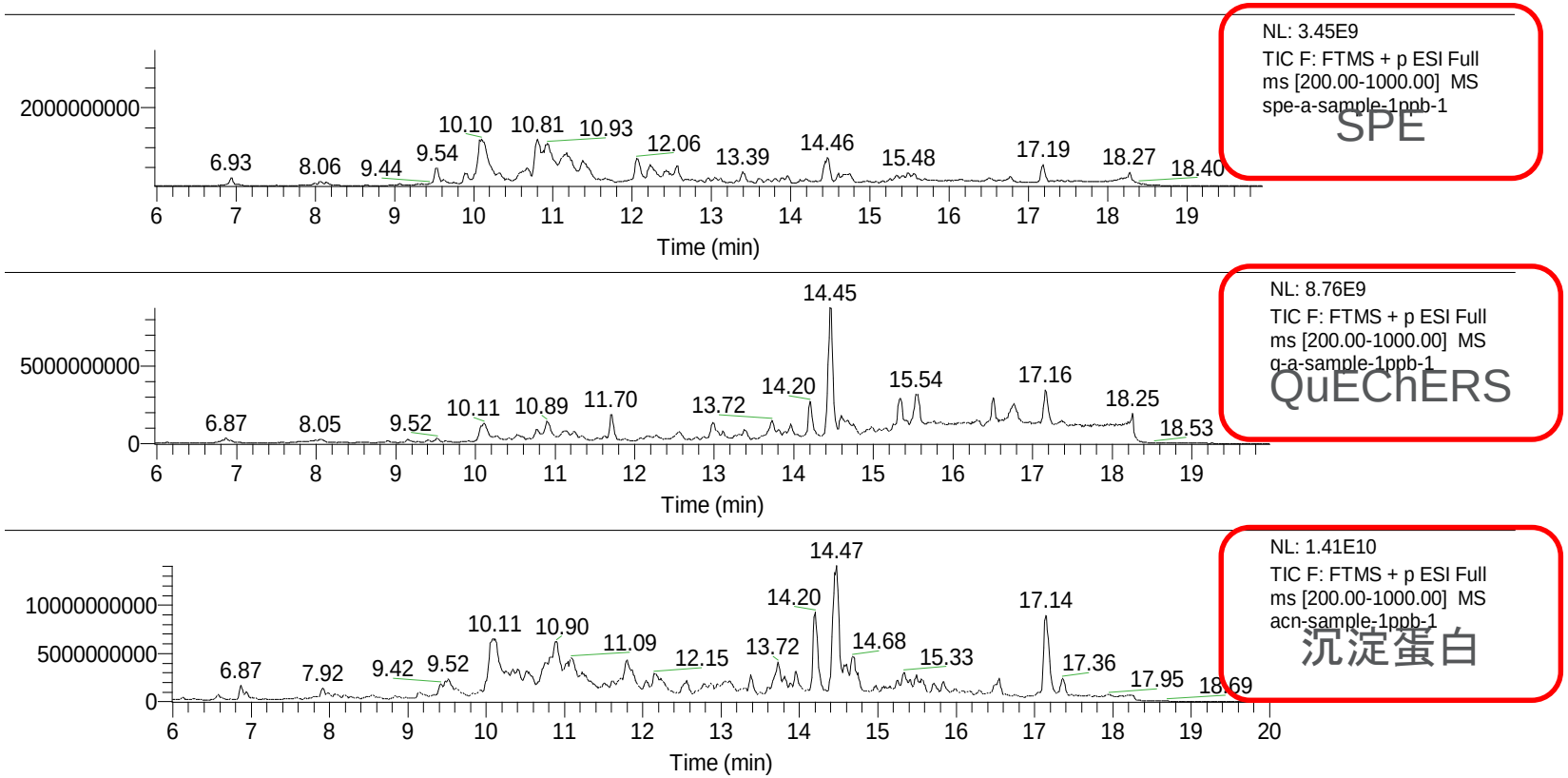
时间 /min	A	B	流速：μL/min
0.00	95	5	300
15.0	5	95	300
17.0	5	95	300
17.1	95	5	300
20.0	95	5	300

参数名称	设置
喷雾电压	3500/3000v (+/-)
雾化温度	400℃
离子传输管温度	350℃
鞘气	40
辅助气	5
反吹气	1
Full Scan Resolation	70000
Full Scan Mass Range	100–1000m/z
ddMS2 Resolation	17500
MS Isolation	2.0 m/z

- Mass spectrometry conditions: ESI source, unlike triple quadrupole, Orbitrap high-resolution mass spectrometry does not require optimization of mass spectrometry conditions and collision energy for each compound, so the time spent on method development is greatly reduced. The ion source parameters and mass spectrometry scan parameters are shown in Table

SPE pass through application for multiple vet. drug residue analysis

Comparison of the clean up effects of the three sample prep methods



The data indicating that the sample background and lipid interferences purified by the HyperSep Retain-PEP SPE column are obvious.

HyperSep Retain-PEP can effectively remove most of the fat and phospholipids, and the use of pass through strategy sample purification method can greatly improve sample processing.

SPE pass through application for multiple vet. drug residue analysis

对猪肉、猪肝、鸡肉、鸭肉等基质中添加了194种常见兽药，加标浓度为1.0μg/Kg和5.0μk/Kg的回收率做了统计，

回收率基本在50-120%之间，以下列出了部分化合物的回收率数据结果。

英文名称	中文名称	Add 1ppb		Add 5ppb	
		测定值	Recovery(%)	测定值	Recovery(%)
17-Methyltestosterone	甲基睾酮	0.68	68%	3	60%
2-Aminoflubendazole	2-氨基氟苯咪唑	0.74	74%	4.2	84%
4-EPICHLORTETRACYCLINE HYDROCHLORIDE	差向金霉素	0.62	62%	3.3	66%

第 7 页

The results showed that the addition concentration was 1.0 μg/Kg, and the detection rate of all compounds in the matrix was >84%; for addition 5.0 μg/Kg and the detection rate of all compounds in the matrix was >92%. The compound recovery is between 50-120%.

Sample prep for hormone residues in Animal-Derived food

Weigh 5 g sample，add 3 mL of 10 % sodium bicarbonate and 10 mL ethyl acetae，Homogenized 30 s. Shake 10 min（4℃), centrifuge @6000 r/min 10 min, transfer upper organic layer to ashigatabin. Repeat 10 mL Ethyl Acetate extraction again. combine upper organic layer and evaporate to dryness under 40℃，and reconstituted with 30%MeOH and dilute to 5mL

Thermo HyperSep C_{18}
500mg,3mL,60108-304

Condition and Equilibrium
3 mLMeOH，3 mLWater

Load sample
1mL/min

Wash
2 mL 30% MeOH

Elute
5 mL MeOH

Evaporate and reconstitution
1mL 50%Water/ACN

Thermo Hypersil Gold C_{18}
2.1x150mm,5μm, 25005-152130

Sample prep for hormone residues in animal-derived food

- LC Conditions
- Column：Thermo Hypersil Gold (150 × 2.1 mm,5 μm)
- Gradient see Table 1，A: 0.1%FA B:MeOH
- Flow Rate: 250 μL/min
- Injection Volumn:10 μL

- **MS Conditions：**
- ESI: Positive mode
- SRM: Scan mode，
- Spray volt：4500V；
- Ion transfer temp ：350 ℃；

表1 流动相梯度洗脱条件

Time（min）	A （%）	B （%）
0	90	10
5	10	90
12	10	90
12.1	90	10
14	90	10

表2 11种合成类固醇类激素的SRM质谱条件

	药物名称	母离子	碎片离子（碰撞能量 V）
1	睾酮（Testosterone）	289.2	97.2（24），109.2（26），253.2（16）
2	甲基睾酮（Methyl testosterone）	303.2	97.2（30），109.1（28），285.1（17）
3	黄体酮（Progesterone）	315.2	97.2（22），109.1（27）
4	群勃龙（Trenbolone）	271	199（26），253（20）
5	勃地龙（Boldenone）	287.2	121（25），135（15），269（12）
6	诺龙（Nandrolone）	275.2	109（28），257（15）
7	美雄酮（Methandienone）	301	121（27），149（15），283（12）
8	司坦唑醇（Stanozolol）	329.2	81（42），121（36），95（38）
9	丙酸诺龙（Nandrolone propionate）	331.1	239（17），257（14）
10	丙酸睾酮（Testosterone propionate）	345.2	97.1（29），109.1（28）
11	苯丙酸诺龙（Nandrolone phenylpropionate）	407.2	105.2（29），257.1（17）

Sample prep for hormone residues in Animal-Derived food

LC/MS/MS色谱图

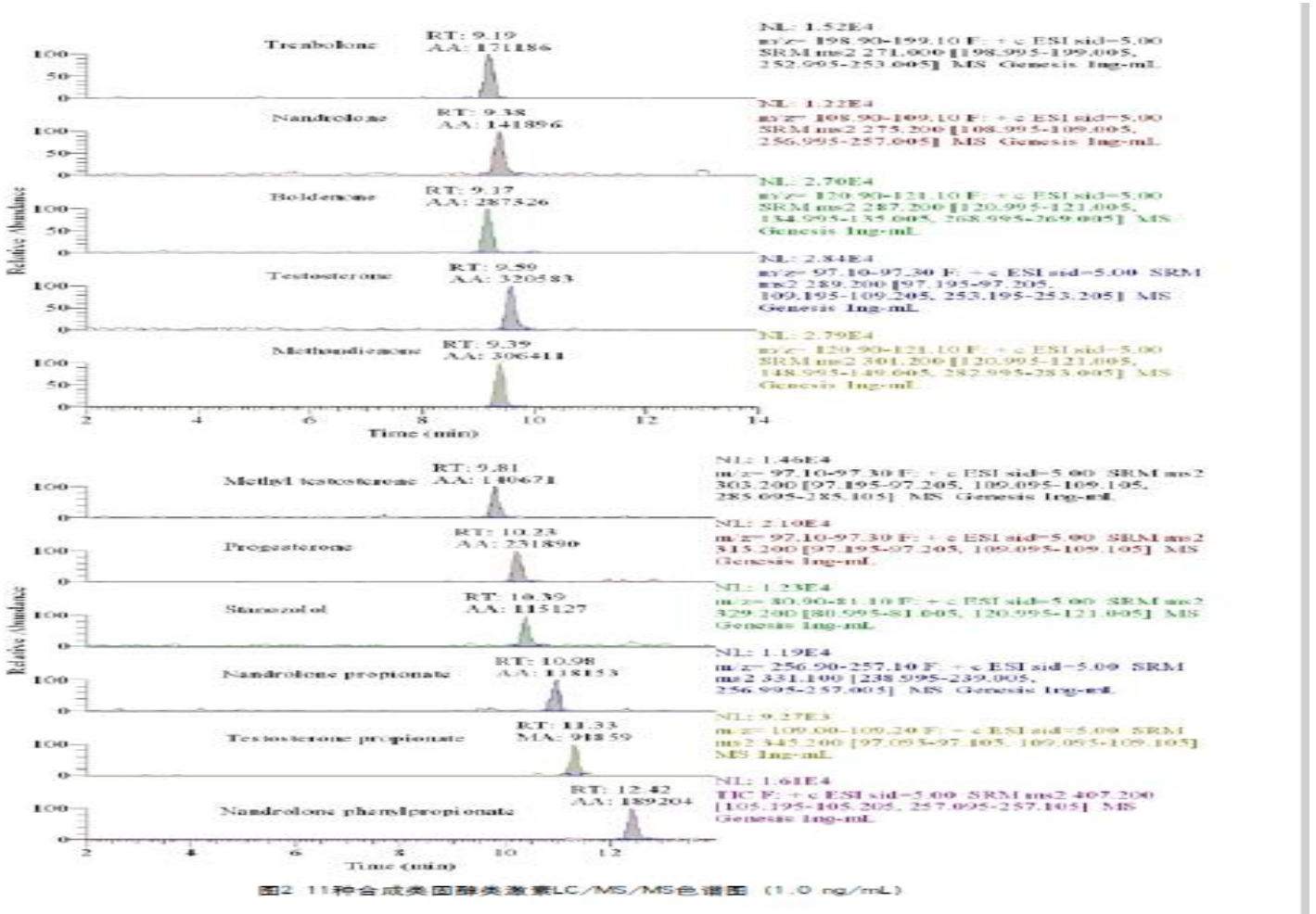


图2 11种合成类固醇类激素LC/MS/MS色谱图 (1.0 ng/mL)

This method can be applied to the extraction of synthetic steroid hormone in pork, beef, mutton, chicken，egg, milk etc. animal-derived foods . The recovery is in the range of 50 - 105 %.

Thermo Scientific Food Communities: Resources

- View application notes, on-demand webinars, product information, and many more resources on our Pesticides and Food Communities Libraries:

www.thermoscientific.com/pesticides www.thermoscientific.com/foodandbeverage