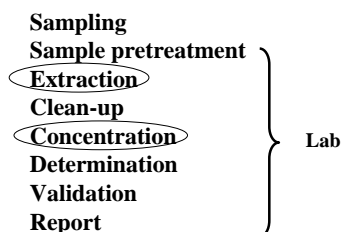


Extraction and Concentration of Pesticide Residue

- Prof. Fengmao Liu
- 62731978
- lfm2000@cau.edu.cn

Pesticide Residue analytical procedures



Procedures prior to analysis

the **bottleneck** of analytical methodologies as it constitutes the principal source of error and remains as one of the most time-consuming steps.

Sampling, field sample preparation, lab sample preparation, Extraction, Clean-up, Concentration.

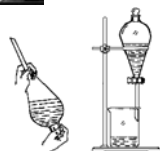


Sample extraction

Transfer PR to solvent from commodity sample.

solid-liquid extraction: Solvent extraction from solid sample

liquid-liquid extraction



Water sample
Liquid-liquid extraction, LLE
Solid Phase Extraction, SPE



Air sample

Cold-extraction

squeezing extraction of PR from the absorbent immersed in solvent.

easy (procedure), simple (equipment), fast, only fit to the squeezable absorbent of PUF

Soxhlet extraction

with boiling solvent
recommended by EPA

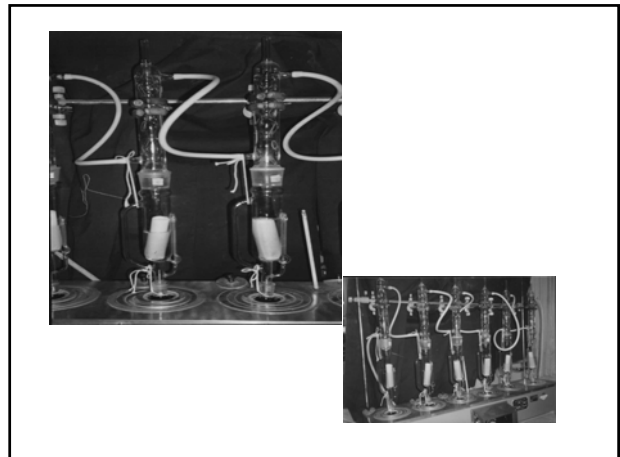
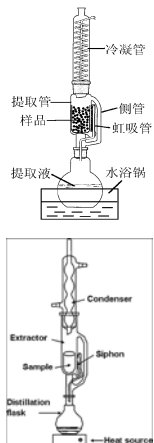


Soxhlet extraction: Soxhlet extractor

The sample is placed in a thimble-holder that is gradually filled with condensed fresh extractant (term used to refer to the solvent used for extraction) from a distillation flask.

When the liquid reaches the overflow level, a siphon aspirates the solute from the thimble-holder and unloads it back into the distillation flask, thus carrying the extracted analytes into the bulk liquid.

This operation is repeated until the complete extraction.



PR Extraction

Soil sample

- Solvent immersing extraction
- Oscillation extraction
- Ultrasonic extraction

振荡浸出法

- Immersing the homogenized sample in extraction solvent
- Oscillating the sample
- Sucking filtration of samples
- Concentrate the eluent.



Oscillating extraction



Sucking filtration

PR Extraction

Crop or animal tissue

- Rinse
- Oscillating
- Blending or homogenizing
- Soxhlet extraction
- Digestion



PR Extraction

Digestion method

Acid digestion
Alkali digestion
Enzymatic digestion

Microwave assisted digestion

the homogenized fish sample is treated by 20mL mixture of 4:1 glacial acetic acid and perchloric acid, seal the sample and put it in microwave oven.

Extraction solvent

1. Inertness.

No chemical reaction with the solvent, no effect to the equipment.

2. Purity.

Testing method

300~500mL of solvent is concentrated with K-D Apparatus to 3~5mL.

No interferences peak in GC-ECD with 2 microliter injection.

Purify the reagent

Hexane: reflux distillation 20min with the present of NaOH, then redistilled, discarded the first and the last 10%.

Acetone: reflux distillation with the present of potassium permanganate until the purple color stable, dry it with K_2CO_3 , filtration and re-distillation, collect the portion of 56°C.

Acetonitrile: collect the distillation portion from 81~82°C with the present of 1ml H_3PO_4 , 30g P_2O_5 in 4 L acetonitrile.

Ethyl acetate: reflux distillation for 4h with the present of 100mL acetic anhydride and 10 droplets of concentrated H_2SO_4 in 1 L ethyl acetate. Dry it with K_2CO_3 , filtration and re-distillation, collect the portion of 77°C.

P44

Extraction solvent

3. Polarity “相似相溶原理”

Low polarity of OCl is easily extractable with low polarity of solvent, such as hexane.

More polar OP or phenoxy acid herbicide, with polar solvent, such as CH_2Cl_2 , $CHCl_3$, acetone.

Mixture of solvents often have better result.

4. Commodity-based:

Water sample, extracted by solvent or absorbent.

Soil, the mixture of water and solvent

High water content sample, water immiscible solvent

High fat content sample, low polarity solvent

Extraction solvent

5. Boiling point

Proper range 45~80°C
too low, easy to evaporate
too high, difficult to concentrate, especially to thermo labile pesticides.

6. Toxicity: acetonitrile, methanol has better effect than acetone for few interferences. However, their toxicity...

7. Cost: price of the solvent

8. Analytical target range: single residue or multi residue

Solvent used

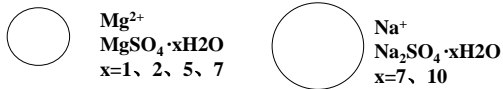
LgKow (-3~7)

acephate	-0.89	methamidophos	-0.8
atrazine	2.5	malathion	2.75
chlorpyrifos	4.7	endosulfan	4.79
permethrin	6.1	cypermethrin	6.6

mixture of solvent

acetone acetonitrile ethyl acetate

Water removal from the extract



原子(离子)半径(pm)周期表

Shaking extraction with solvent
Soxhlet extraction

LLE
SPE

Alternative Extraction techniques

		Page
SFE	supercritical fluid extraction	77
ASE	accelerated solvent extraction	80
MAE	microwave assisted extraction	83
SPME	solid phase micro-extraction	87
LPME	liquid phase micro-extraction	91
MSPD	matrix solid phase dispersion	98
DSPE	dispersive solid phase extraction	101
Low-Temperature Extraction		102
CPE	cloud point extraction	110

Homework title?

Supercritical Fluid Extraction

Utilizes the combination of gas or liquid properties of the supercritical fluid (SF).

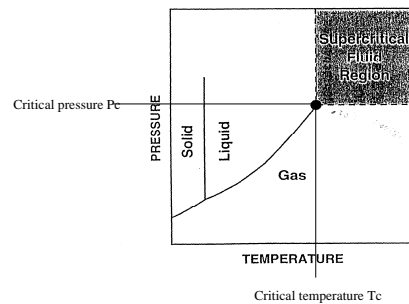
SF - dense gases

SF is any substance at a T and P above its critical point.

It can diffuse through solids like a gas, and dissolve materials like a liquid.

Near to the critical point, minor change in P or T can cause large change in density, allowing many properties of a supercritical fluid to be "fine-tuned".

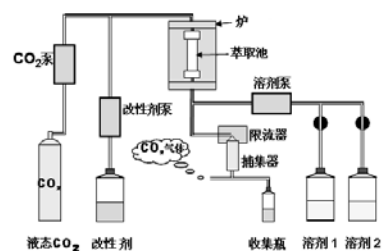
Supercritical Fluid, SF



Supercritical Fluid, SF

	Gas	SF		Liquid
	P= 1atm, T=15-30°C	P = Pc, T = Tc	P = 4Pc, T = Tc	P= 1atm, T=15-30°C
Density (g/cm ³)	0.0006-0.002	0.2 - 0.5	0.4 - 0.9	0.6 - 1.6
Viscosity (10 ⁻⁴ g/cm*s)	1-3	1-3	3-9	20-300
coefficient of diffusion (cm ² /s)	0.1-0.4	0.7×10 ⁻³	0.2 × 10 ⁻³	(0.2 - 2) × 10 ⁻³

Supercritical Fluid Extraction



Ideal extraction solvent, SF

1. Solvent Strength of SF - density

Solubility of SF for macromolecule compounds increases with increasing density (that is with increasing pressure).

Solvent Strength of SF decreases with the decreasing pressure. When the pressure decreases to atmospheric pressure, the SF loses the dissolving power and the extracted compounds settle out.

Ideal extraction solvent, SF

2. Higher diffusion coefficient of SF than liquid solvent

It could diffuse into the matrix faster, and the dissolved pesticides diffuse faster out of the matrix into the solvent. In other words, pesticides could be extracted from the matrix faster.

3. Lower viscosity than liquid solvent

It could penetrate into porous solid materials more effectively than liquid solvents and, consequently, it may render much faster mass transfer resulting in faster extractions.

4. Unnecessary to concentrate for the use of CO₂ or N₂O.

Ideal extraction solvent, SF

5. To improve the solubility, by adding some cosolvents, or called entrainers, modifiers

Although scCO₂ has bipolar and good dissolving capacity, cosolvents should be added to compete active sites with analytes when SF is used to extract polar compounds. The addition of cosolvents could increase the dissolving capacity and enhance the extraction efficiency. In addition, cosolvents could improve the selectivity of the extraction.

Methanol、ethanol、isopropanol, <10%

Supercritical Fluid, CO₂

scCO₂

Easy to obtain the critical condition

sc CO₂ (T_c=31.1℃, P_c=7.38MPa)

Low price, low toxicity、non-ignitibility、low boiling point, released to environment after extraction at normal pressure
Only fit to the non polar or low polar compound.

Advantage of SFE:

Volume of organic solvent was reduced

Time of extraction was reduced.

Disadvantage, expensive equipment

Accelerated Solvent Extraction

Pressurized Fluid Extraction (PFE)

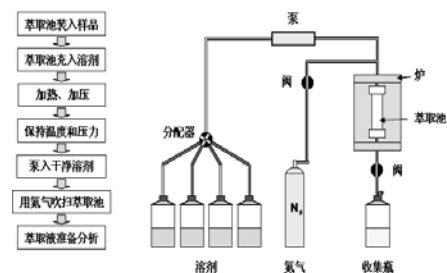
ASE is a technique for the extraction of solid and semisolid sample matrices using common solvents at elevated temperatures and pressures

High T (175℃—200℃) High P (102—136atm)

ASE operates at temperatures above the normal boiling point of most solvents, using pressure to keep the solvents in liquid form during the extraction process.

Typically, ASE methods are completed in 15–25 min, while consuming only 15–50 mL of solvent.

Accelerated solvent extraction



Accelerated Solvent Extraction

Benefits:

Extractions for sample sizes 1–100 g in few minutes
Dramatic solvent reduction
Wide range of applications
Fit to acidic and alkaline matrices
Approved for use by the U.S. EPA

High cost of equipment

Decomposition of thermo labile pesticides

Co-extract of interferences

Microwave Assisted extraction

Irradiation at a frequency about 2500MHz at 1000-1200W

Microwave could heat the sample quickly and accelerate the molecular motion. Its high penetrating power enables microwave energy penetrate inside the sample and radiate the whole sample quickly other than over heating the sample surface. Molecular motion in sample enables the sufficient action between the solvent and analytes, which accelerate the extraction processing.

Extraction time reduced-25min

Several samples can be extracted simultaneously

Domestic microwave ovens can be used (open style)

Solid Phase Micro Extraction

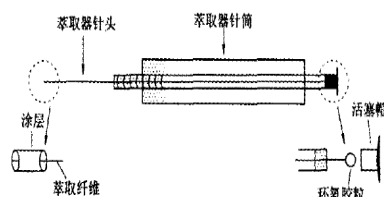
SPME, is a sample preparation technique used both in the laboratory and on-site. Developed in the early 1990s
Simple, inexpensive, without solvents.

SPME involves the use of a fibre coated with an extracting phase, that can be a liquid (polymer) or a solid (sorbent), which extracts different kinds of analytes (including both volatile and non-volatile) from different kinds of media, that can be in liquid or gas phase.

The quantity of analyte extracted by the fibre is proportional to its concentration in the sample as long as equilibrium is reached or, in case of short time pre-equilibrium, with help of convection or agitation.

After extraction, the SPME fibre is transferred to the injection port of separating instruments, such as a GC, where desorption of the analyte takes place and analysis is carried out.

SPME



Fiber Coating

SPME fibers are commercially available

- PDMS—nonpolar
- PA—polar

some fiber coatings based on solid sorbents such as

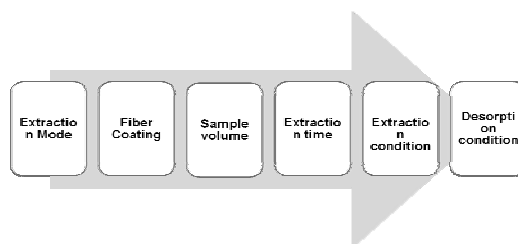
- PDMS-DVB
- PDMS-CAR
- CW-DVB
- CW-TRP

Bonding mode: bonded、non-bonded、partially cross-linked

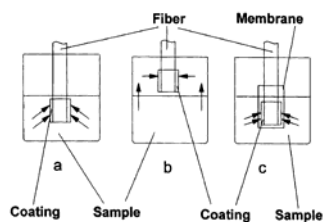
Polar comparison:

CAR - TPR > PDMS - DVB > PA > PDMS > PDMS (交联)

How to Develop SPME Method

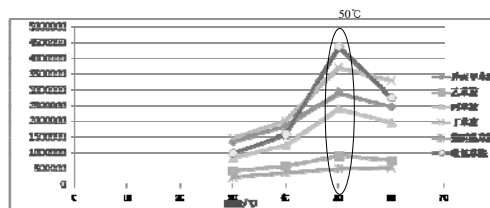


Solid Phase Micro Extraction



SPME mode:
a Dir-SPME
b HS-SPME
c membrane-protected SPME

Extraction efficiency with T



Liquid-Phase Micro-Extraction

LPME

is a miniaturized implementation of conventional LLE in which only microliters of solvents are used instead of several hundred milliliters in LLE. It is quick, inexpensive and can be automated.

Example: Aqueous solution of target pesticides was shaken and transferred into a vial containing a stirring bar. After 0.5mL of chloroform was added to the solution, the vial was sealed and the magnetic stirrer was turned on at 200rpm for 20min. (The extraction procedure could repeat for more than one time to obtain high extraction efficiency). The extract solvent (chloroform) was collected and volatilized to dryness under gentle N₂ stream. The extractant was then dissolved with 100ul methanol for determination.

Solvent, stirring speed, extraction solvent, time, salt

Liquid-Phase Micro-Extraction

农药学报 2012, 14(5): 461-474
Chinese Journal of Pesticide Science

<http://www.nyxb.com.cn>

• 专论与综述 •

液相微萃取技术在农药残留分析中的应用研究进展

王素利^{1,2}, 杨素萍¹, 刘丰茂^{*2}, 薛佳莹², 尤祥伟²

Single Drop Micro Extraction

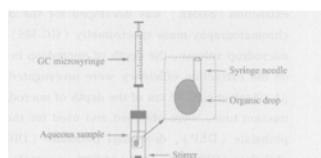


图 1 微滴液相萃取装置图

An organic solvent (10uL, extraction solvent) was drawn into a microsyringe. After immersing the needle under the sample solution, the extraction solvent was depressed to the tip and suspended in the sample solution. The magnetic stirrer was turned on at 1000rpm for 20min, and then the suspended extraction solvent was drawn back for instrument analysis.

Solvent, volume, immersing time, immersing depth

Dispersive Solid Phase Extraction

Dispersive SPE (DSPE), often referred to as the "QuEChERS" (Quick, Easy, Cheap, Effective, Rugged, and Safe) method

Popular in the area of multi-residue pesticide analysis.

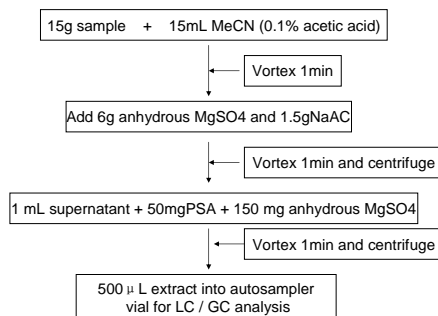
DSPE procedure

Samples are **extracted firstly** with an aqueous miscible solvent (e.g., acetonitrile) in the presence of high amounts of salts (e.g., NaCl and MgSO_4) and/or buffering agents (e.g. citrate) to induce liquid phase separation and stabilize acid and base labile pesticides.

Upon shaking and centrifugation, an aliquot of the organic phase is subjected to further clean up using SPE. **Unlike traditional methods using SPE tubes**, in dispersive SPE, clean up is facilitated by mixing bulk amounts of SPE (e.g., Supelclean PSA, ENVI-Carb, and/or Discovery DSC-18) with the extract.

After sample clean up, the mixture is **centrifuged** and the resulting supernatant can either be analyzed directly or can be subjected to minor further treatment before analysis.

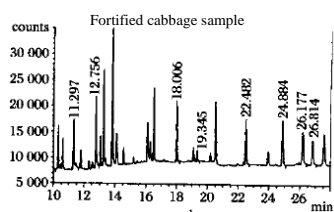
QuEChERS



GC-NPD

Homogenized sample was mixed with 40mL ethyl acetate, 15g Na_2SO_4 , 5g NaCl, 1g florisil, 0.05-0.1g activated carbon

CH_3CN was alternative



DSPE method

QuEChERS reported in 2003

DSPE

The proportion of florisil:

Different proportion florisil (0%、2.5%、5%、10%、20%) was added into 10g sample.

Result shows that good recovery was obtained at 10% of florisil addition.

The proportion of active carbon

Different proportion of active carbon (0.2%, 0.5%, 1.0%, 2.0%, 5%, 10%) was added into 10g sample.

Results:

0.2%: less pigment was adsorbed.

0.5%~1.0%: good recovery and better absorbing effect

>5%: Recovery decreased was more than 24.4%

农药与环境安全国际会议论文集, 中国农业大学出版社, 北京, 2003, 250-253

农药与环境安全国际会议论文集

研究简报

分散固相萃取 气相色谱 质谱方法快速净化测定枸杞中 12 种农药残留

李 莉, 江树人, 潘灿平, 周志强, 钱传范, 刘丰茂

Homogenized sample 5g + 10mL CH_3CN

Centrifugation

The supernatant was added with 125mg PSA and 300mg MgSO_4

Centrifugation

Concentrate the supernatant, inject to GC-MS

QuEChERS was used to PRs in *Lycium barbarum*



Less procedure than QuEChERS
For less water content in *Lycium barbarum*

RESIDUES AND TRACE ELEMENTS

LEI ET AL. JOURNAL OF AGRICULTURE INTERNATIONAL VOL. 36, NO. 1, 2007 275

Determination of Organophosphorus Pesticides in *Lycium barbarum* by Gas Chromatography with Flame Photometric Detection

LI Li, FENGQIAN LIU, CHENXIAN QIAN, SHIYEN BANG, ZHIBANG ZHANG, and CANGYONG PAN

Homogenized sample 5g + 10mL ethyl acetate (0.01g activated carbon)

Centrifugation

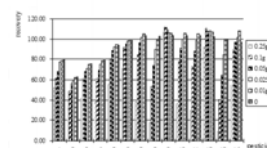
The supernatant (5mL)

Concentrate the supernatant, inject to GC-FPD

QuEChERS was used to 14 OPs in *Lycium barbarum*



Less procedure



药物分析杂志 Chin J Pharm Anal 2006, 26(6)

中药材柴胡中 8 种有机磷农药残留测定^{*}
王素利^{1,3}, 刘丰茂¹, 陈建民², 薛健^{2**}



DSPE

Homogenized sample 4g + 50mL CH₃CN + 0.2 activated carbon

Filtration

Concentration and inject to GC-FPD.

Less procedure than QuEChERS
For less water content in bupleurum chinense

Original Paper

Use of graphitic carbon black and primary secondary amine for determination of 17 organophosphorus pesticide residues in spinach

20g spinach sample + 50mL ACN

A 10 mL aliquot was transferred to a 100 mL flask

The residue was dissolved in 1 mL ethyl acetate or acetone. The extract was transferred to a 2 mL centrifuge tube for cleanup.


Activated carbon, PSA and GCB were used before analysis.

The upper layer was transferred into an autosampler vial for GC-MS analysis.

shaken for 30 min, then the extract was filtrated into a 100 mL measuring cylinder and shaken vigorously for 1 min.

evaporated to near dryness by rotary vacuum (evaporation at 38°C).

DSPE



❖MWCNTs as r-DSPE cleanup material for cabbage, spinach, grape, orange

Multi-walled carbon nanotubes in alternative matrix solid phase extraction materials in pesticide multi-residue analysis with QuEChERS method

❖Without ❖ r-DSPE ❖ r-DSPE
❖ r-DSPE (PSA) (MWCNTs)

❖J. Chromatogr. A 1225 (2012) 17–25. IF=4.531






ORIGINAL PAPER

Application of matrix solid-phase dispersion and liquid chromatography–mass spectrometry to fungicide residue analysis in fruits and vegetables

Matrix Solid Phase Dispersion

A representative sample (250 g) was chopped and homogenized

0.5 g portion sample

0.5 g of C18-bonded silica

This mixture was transferred

10 ml ethyl acetate at 1ml/min

LC–MS of 15 fungicide in F+V

SAMPLE

BLEND WITH PESTLE

TRANSFER

SOLID SUPPORT

BLENDED SAMPLE

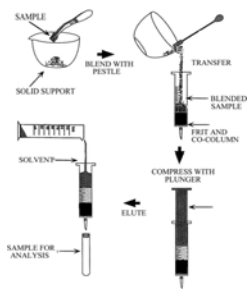
FIT AND CO-COLUMN

SOLVENT

COMPRESS WITH PLUNGER

ELUTE

SAMPLE FOR ANALYSIS



Factors in MSPD

- the effect of average particle size diameter. (40–100 μ m)
- the character of the bonded-phase.
- the best ratio of sample to solid support material. (1 to 4)
- the optimum choice of elution solvents and the sequence of their application to a column.
- the elution volume. It has been observed that for an 8 ml elution of a 2 g MSPD column blended with 0.5 g of sample that target analytes usually elute in the first 4 ml, approximately one column volume.
- the effect of the sample matrix itself.

Low-temperature extraction

Principle:

the lipids and wax in the sample could be precipitated in acetone solution at low temperature (–70°C), while the pesticide residue still left in acetone, cleanup by filtration.

Not easy to reach the condition of –70°C (adding dry-ice is a way)

Low-temperature extraction

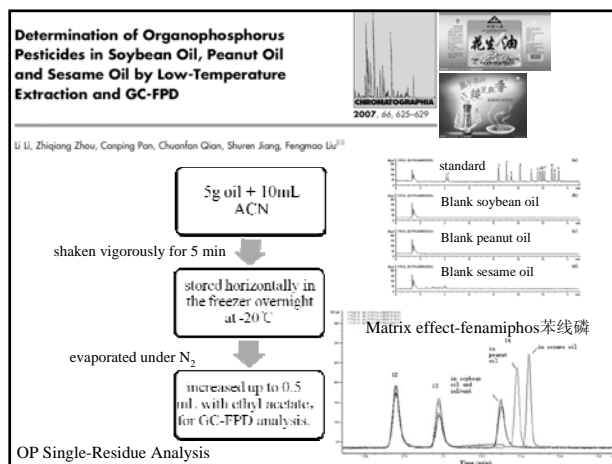
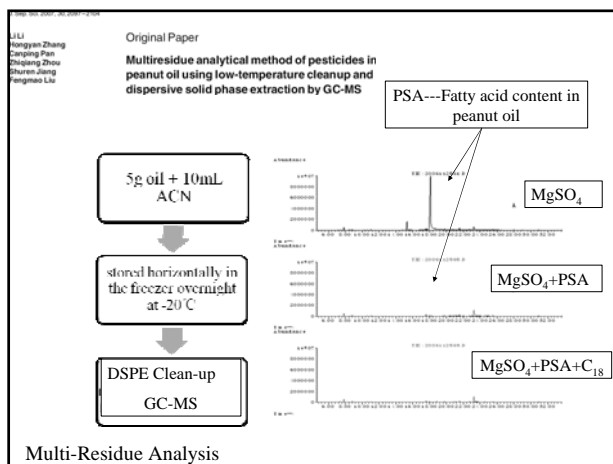
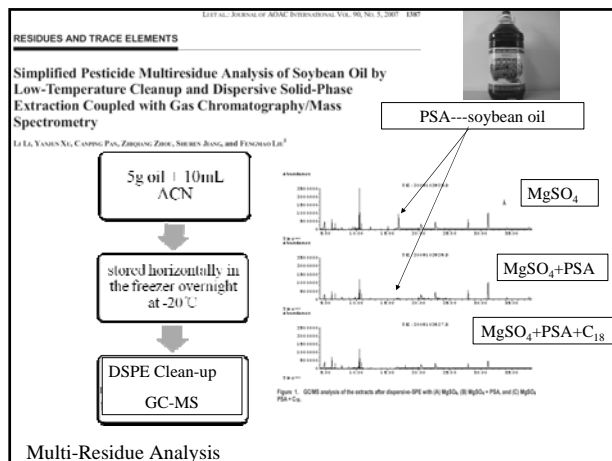
Application of the condition at -20°C

OP in soybean oil, peanut oil, sesame oil
5g sample,
add 10mL acetonitrile,
shake.
overnight under -20°C condition
transfer 1mL of the supernatant, acetonitrile phase
concentrate
change the solvent to ethyl acetate
inject.

Determination of Organophosphorus Pesticides in Soybean Oil, Peanut Oil and Sesame Oil by Low-Temperature Extraction and GC-FPD



Li Li, Zhiqiang Zhou, Cuiying Pan, Chuanfan Qian, Shuren Jiang, Fengmao Liu^{1,2}



Cloud Point Extraction

Cloud point of a nonionic surfactant

is the temperature where the mixture starts to phase separate and two phases appear, thus becoming cloudy.

This behavior is characteristic of nonionic surfactants containing polyoxyethylene chains, which exhibit reverse solubility versus temperature behavior in water and therefore "cloud out" at some point as the temperature is raised.

农药学报 2009, 11(2): 139-140
Chinese Journal of Pesticide Science

· 专论与综述 ·

浊点萃取法在农药残留分析中的应用

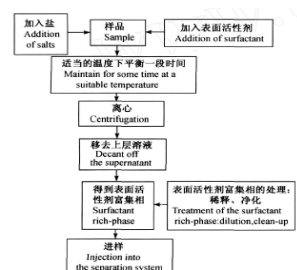
马立利¹, 张冬梅², 陈 毅², 李 莉², 王素利^{1,2}, 刘丰庆¹

CPE

When T increased, the two layers occurred.

PR in the layer of surfactant.
Polar interferences in water layer

Difficult analysis in GC (viscosity),
HPLC?



Triton X-114

CPE

For nonionic surfactant, phase separation happened when T increases to above critical point.

P113

Continuous Liquid-Liquid Extraction



Sample Concentration

Gas stream concentrator

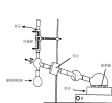
Kuderna-Danish (KD) evaporators

[illegible]

K-D浓缩器

A. K-D瓶; B. Snyder 柱, 是一种带有 2~3 个球的分馏柱; C. 刻度试管; D. 冷凝管; E. 抽气接头; F. 接收瓶。

Rotating film (vacuum) evaporators



- do not fill the distillation flask for more than **1/2 of its volume**;
- **firmly fix the distillation flask** to the evaporator by means of a clamp;
- **slowly** start rotation so that an even distribution of the liquid over the inner wall of the flask is obtained; do not let the flask turn at a higher speed than necessary;

Rotating film (vacuum) evaporators

- gradually lower the distillation flask into the water bath until evaporation starts; keep the boiling process well **under control** at all times (if this is not the case, lift the flask from the water bath and lower the temperature of the water);
- lift the distillation flask from the water bath as soon as the volume of the remaining liquid is approx.2-3 ml; **do not evaporate to complete dryness**;
- slowly **release the vacuum**;

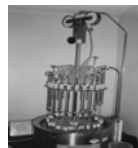
Rotating film (vacuum) evaporators

When only small amounts of co-extractives are present in the extract to be concentrated, and when the pesticides to be determined are volatile, **losses during the evaporation procedure** can occur. This can be circumvented by adding an inert "**holder**", such as *n*-hexadecane. A useful quantity is 2 ml of a 0.2% solution in *n*-hexane in a 500 ml evaporation flask.

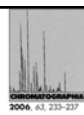
Nitrogen Evaporators

Nitrogen stream is used to evaporate the solvent.

Solvent which has less volume and higher volatility.
Pesticide which has lower vapor pressure.



Multi-Residue Analysis of Some Polar Pesticides in Water Samples with SPE and LC-MS-MS



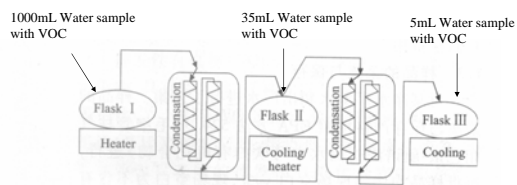
Pengmao Liu^{1,2,3}, G. Bischoff¹, W. Peckeme², Wenna Xu³, A. Kofel³

Higher vapor pressure and lower solubility could lead to higher Henry's constant and higher loss in the evaporation step.

Henry's constant =f (vapor pressure/ solubility)

Table 7. Recovery (%) of concentration step and their physico-chemical properties

Pesticide	Acephate	Carbofuran	Dimethoate	Isoprotruron	Methamidophos
Repeat 1(%)	118.4	47.8	16.7	114.2	29.7
Repeat 2(%)	95.3	37.3	10.0	94.1	48.0
Vapor pressure (25°C) (mPa)	0.226	0.072	0.75	0.008	4.7
Solubility in water	790g L ⁻¹	320mg L ⁻¹	23.8 g L ⁻¹	65mg L ⁻¹	>200g L ⁻¹
Henry's Constant (Pa m ³ mol ⁻¹) 20 °C	1.0 × 10 ⁻⁸	4.66 × 10 ⁻⁹	1.2 × 10 ⁻⁶	1.46 × 10 ⁻⁷	<1.6 × 10 ⁻⁶



Vapor dynamic headspace enrichment device

水蒸气顶空富集装置原理见图1。烧瓶I中加样品1 L,烧瓶II及接收瓶量中分别加超纯水1 mL作为吸收剂。样品经加热及水蒸气蒸馏,致使VOCs连同水蒸气同时释放出来。经二次冷凝,回收冷凝液至烧瓶量,待冷凝液至35 mL停止收集。收集到的35 mL冷凝液再次进行与前述类似操作。最后在接收瓶III中收集冷凝液至5 mL,密封,待恢复至室温后进行P&T-GC-MS分析。整个流程冷凝温度控制在0~1 °C范围内,烧瓶II和接收瓶量处以冰盐浴冷却回收液。

Fit to VOC

Questions

1. PR extraction method
2. How to select proper solvent?
3. Concentration method