TUNTWIN

EW-1

www.isa-lyon.fr

Workshop/Summer school

Sample preparation

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06/03/2023

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under the grant N° *952306*

Sample preparation, a key step in physico-chemical analysis: extraction, pre-concentration, purification

Solid matrices

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Sommaire

1. Complexity of solid matrices

2. Strategies for solid matrices
   - Solid-liquid extraction
   - Ultrasonic assisted extraction
   - Salt assisted extraction (QuEChERS)
   - Pressurised fluid extraction
   - Extraction-purification coupling

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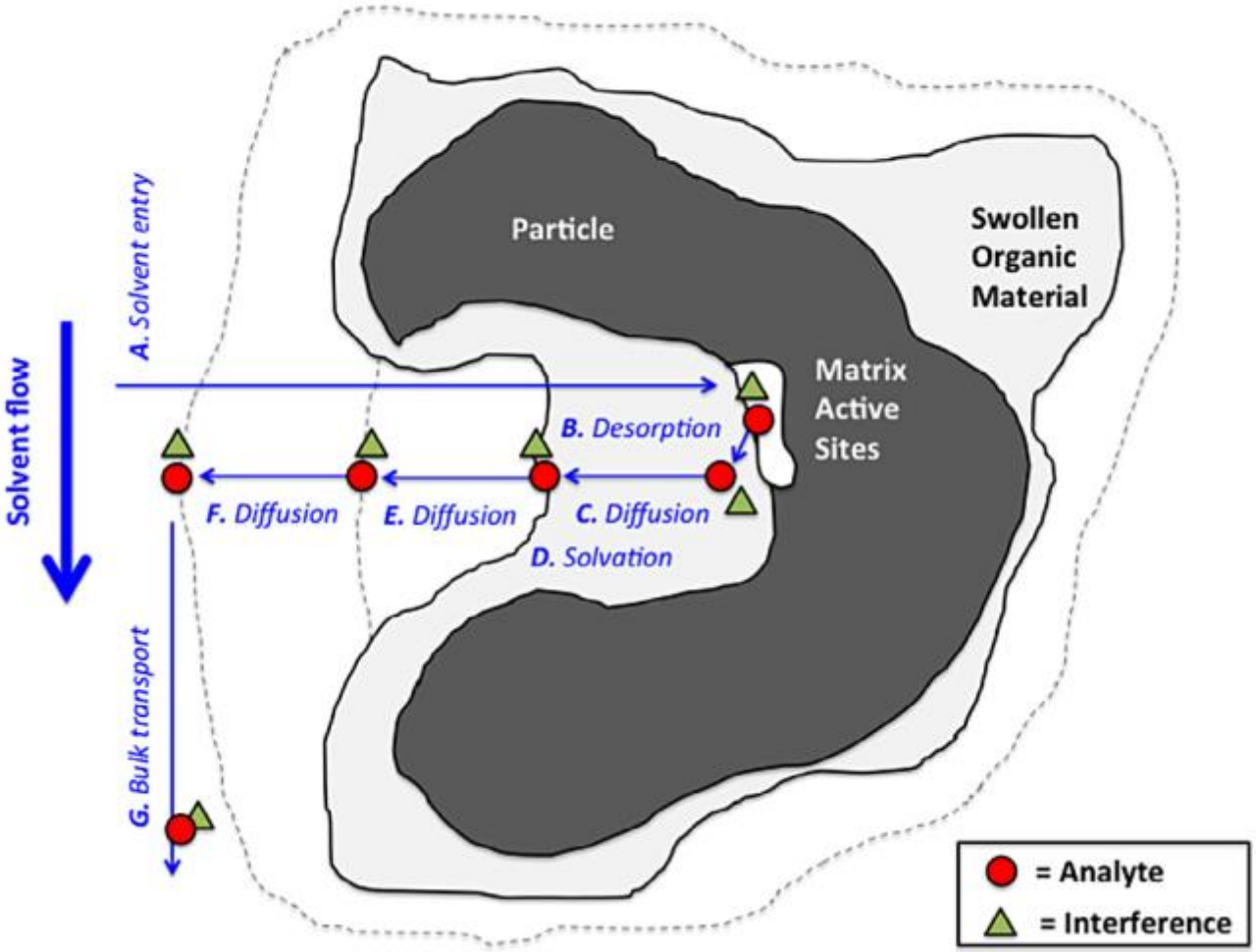
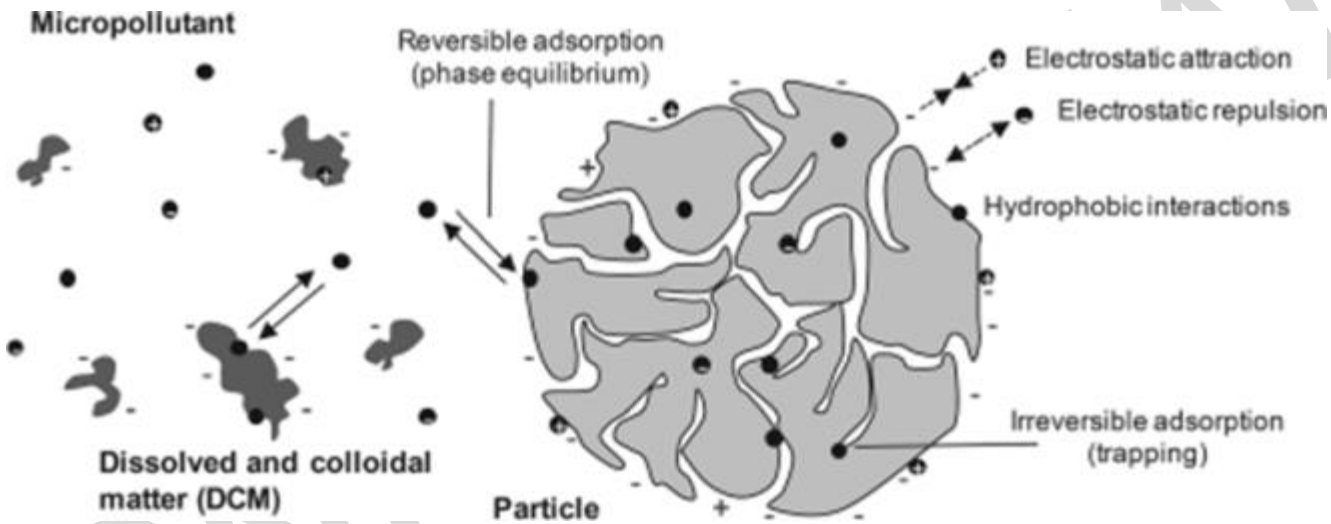
Introduction

Complexity of solid matrices

B. Subedi et al./Trends in Analytical Chemistry 68 (2015) 119–132



Complexity of solid matrices



Development of strategies before analysis

Extraction of compounds
from the matrix

↓

Trace concentration

↓

Removal of interferents

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Development of strategies before analysis

Matrix properties

- **Physical**
  - Hardness
  - Homogeneity
  - Granulometry
- **Chemical**
  - Moisture content
  - Organic ? Mineral ?
  - Macromolecules: proteins, lipids…
  - pH
  - Soils, sediments: cationic exchange capacity (CEC)

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Solid Matrix

Pre-treatment

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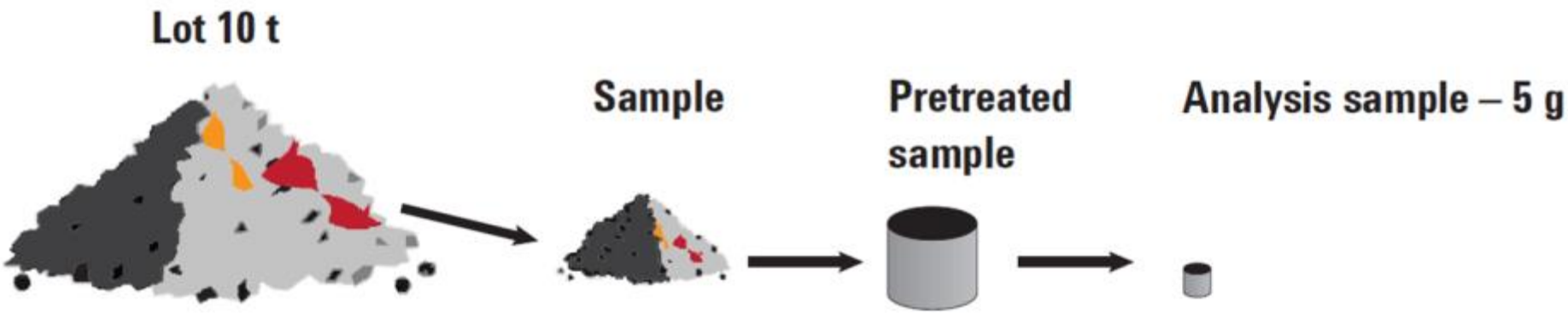
Pre-treatment



- Comminution
- drying
- sieving

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Pre-treatment

Comminution

- **Technique used depends on**
  - Hardness of the matrix
  - Granulometry of the matrix
  - Desired grain size
  - Sample quantity available
  - Material available in the laboratory

- **Be careful with**
  - Temperature
  - Material used (abrasion, contamination)

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Pre-treatment

Comminution

- knife grinder
- Ball grinder
- Mortar grinder
- Cryogrinder
- grinding + extraction

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Pre-treatment

Drying

- Ambiant temperature
- Oven
- Lyophilisation
- Drying agent: sodium/magnesium sulfate

Sieving

- Different granulometry

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Solid matrix

Conventional techniques

Solid Liquid Extraction

Solid Liquid Extraction : Definition

Solvent extraction method from solid samples.

It allows the separation of two or more constituents of a mixture by taking advantage of their differences in affinity between the solid and the extraction liquid

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Solid Liquid Extraction

Optimisation

- Solvent selection
- particle size

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Solid Liquid Extraction

Solvent selection = key step

- depends on the chemical nature of the analyte and the matrix
- must ensure good solubility of the analyte in the selected solvent
- depends on the diffusion coefficient of the solvent in the matrix
- depends on the subsequent analysis

Important factors :

- the diffusion coefficient of the solvent
- particle size
- ratio matrix mass / solvent volume

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Solid Liquid Extraction

Optimisation

- **Solvent selection**
- **Particule size**
- **Energy input:**
  - Stirring
  - Temperature
  - Pressure

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Solid Liquid Extraction

Optimisation

- **Solvent selection**
- **Particule size**
- **Energy input :**
  - Stirring:
    - manual
    - mechanical
    - Vortex
    - High frequency waves: ultrasound, microwave

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Mechanical stirring : Genogrinder

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Oscillating agitator

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Vortex

YU Sheng-Xiong et al. / Chinese Journal of Analytical Chemistry, 2015, 43(9): 1389–1394

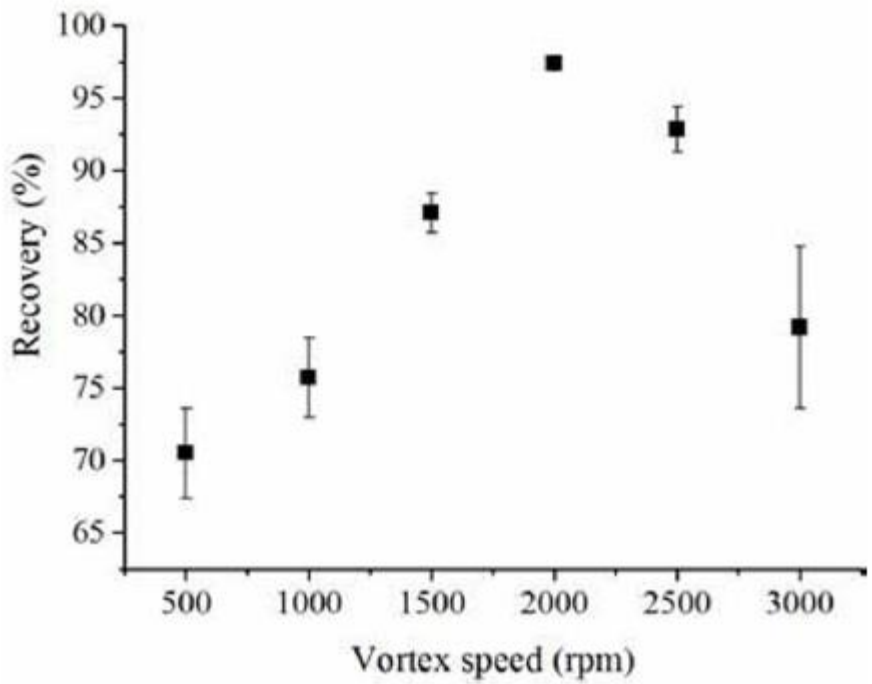

Fig.1 Effect of vortex speed on recovery of pentachlorophenol

Extraction and determination of PCP from soils

Solid Liquid Extraction

Optimisation

- **Solvent selection**
- **Particule size**
- **Energy input:**
  - Stirring
  - High frequency waves: ultrasound, microwave

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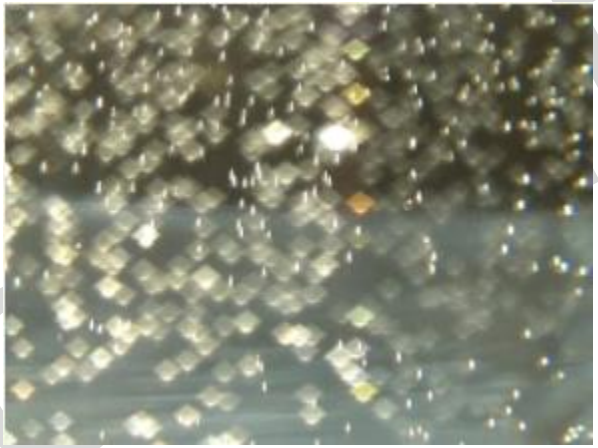
Ultrasons

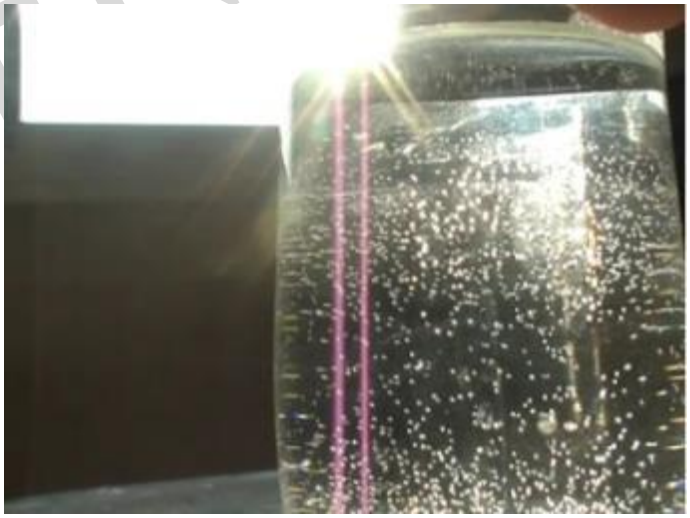
Extraction by ultrasound (bath or probe)

Waves characterized by a minimum frequency of 20 kilohertz

Rapid movement of the solvent allows solubilization of the analytes

Eroded solid particles -> better extraction





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Ultrasons

Extraction by ultrasound (bath or probe)

Waves characterized by a minimum frequency of 20 kilohertz

Rapid movement of the solvent allows solubilization of the analytes

Eroded solid particles -> better extraction

Beware of the temperature!!!

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Ultrasons

Extraction by ultrasound (bath or probe)

Eroded solid particles -> better extraction

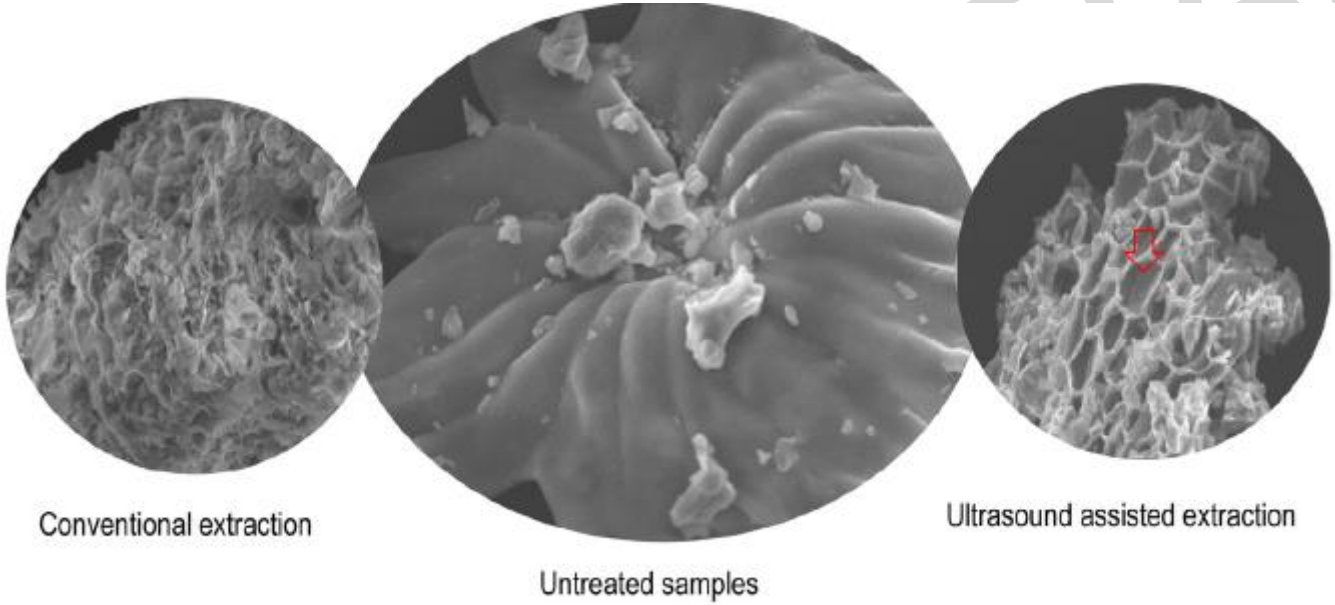

Fig. 1. SEM images of olive leaves after conventional solvent and ultrasound-assisted extraction. {Modified from [18]}.

Tiwari et al. (2015)

Ultrasons

Extraction by ultrasound (bath or probe)

Waves characterized by a minimum frequency of 20 kilohertz

Rapid movement of the solvent allows solubilization of the analytes

Eroded solid particles -> better extraction

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Ultrasons

Example: Amino acids in grapes

C. Carrera et al./Ultrasonics Sonochemistry 22 (2015) 499–505

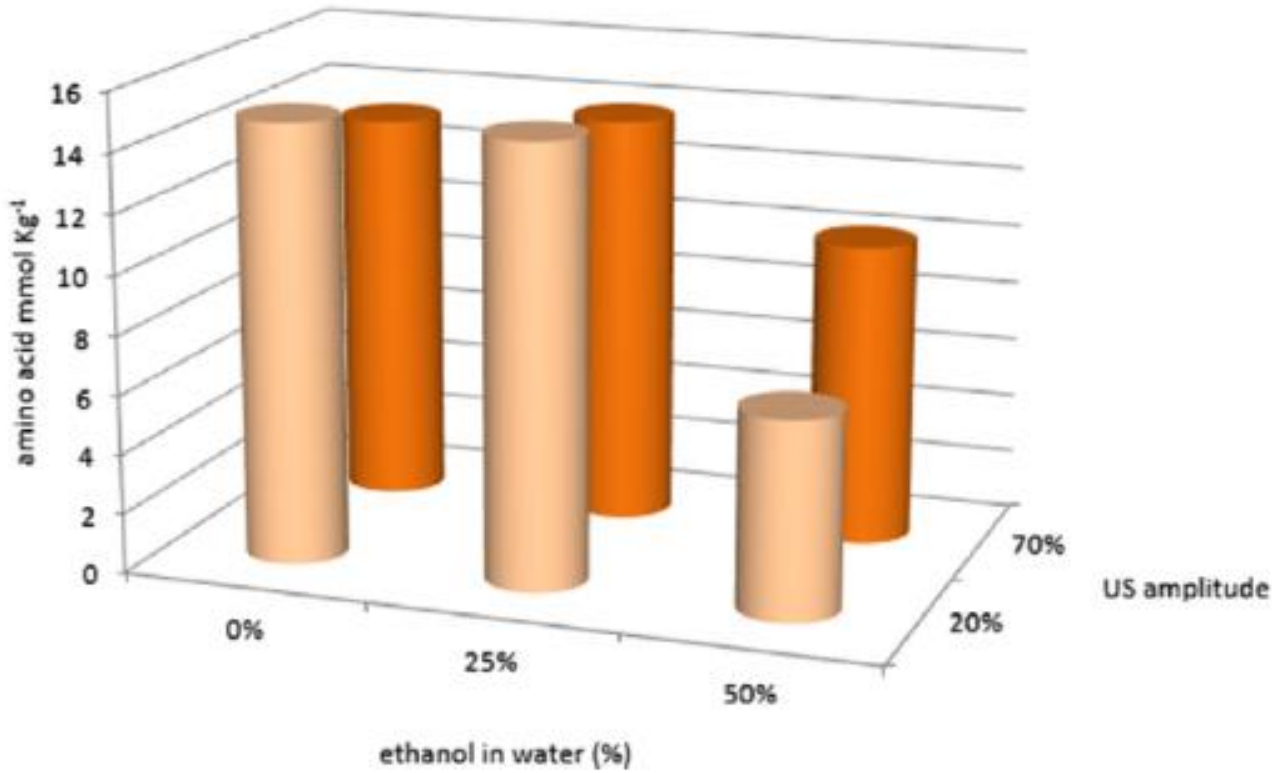

Fig. 2. Average amino acid recoveries for extracts obtained with specific solvent compositions and ultrasound amplitudes.

Ultrasons

Benefits

- Fast extraction
- Reduced solvent volume
- Investment

Disadvantages

- Not automatable
- Temperature control

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Extraction solide liquide

Optimisation

- **Solvent selection**
- **Particule size**
- **Energy input:**
  - Stirring
  - High frequency waves: ultrasound, microwave
  - Extraction assisted by salts

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QuEchERS

QuEChERS (Quick, Easy, Cheap, Efficient, Rugged and Safe)

History

2003: method developed for the analysis of pesticides in fruits and vegetables

2003 -2005: tested for the extraction of more than 300 pesticides

2009: standardized at the European level

AFNOR standard NF EN 15662

Anastassiades et al. (2003)

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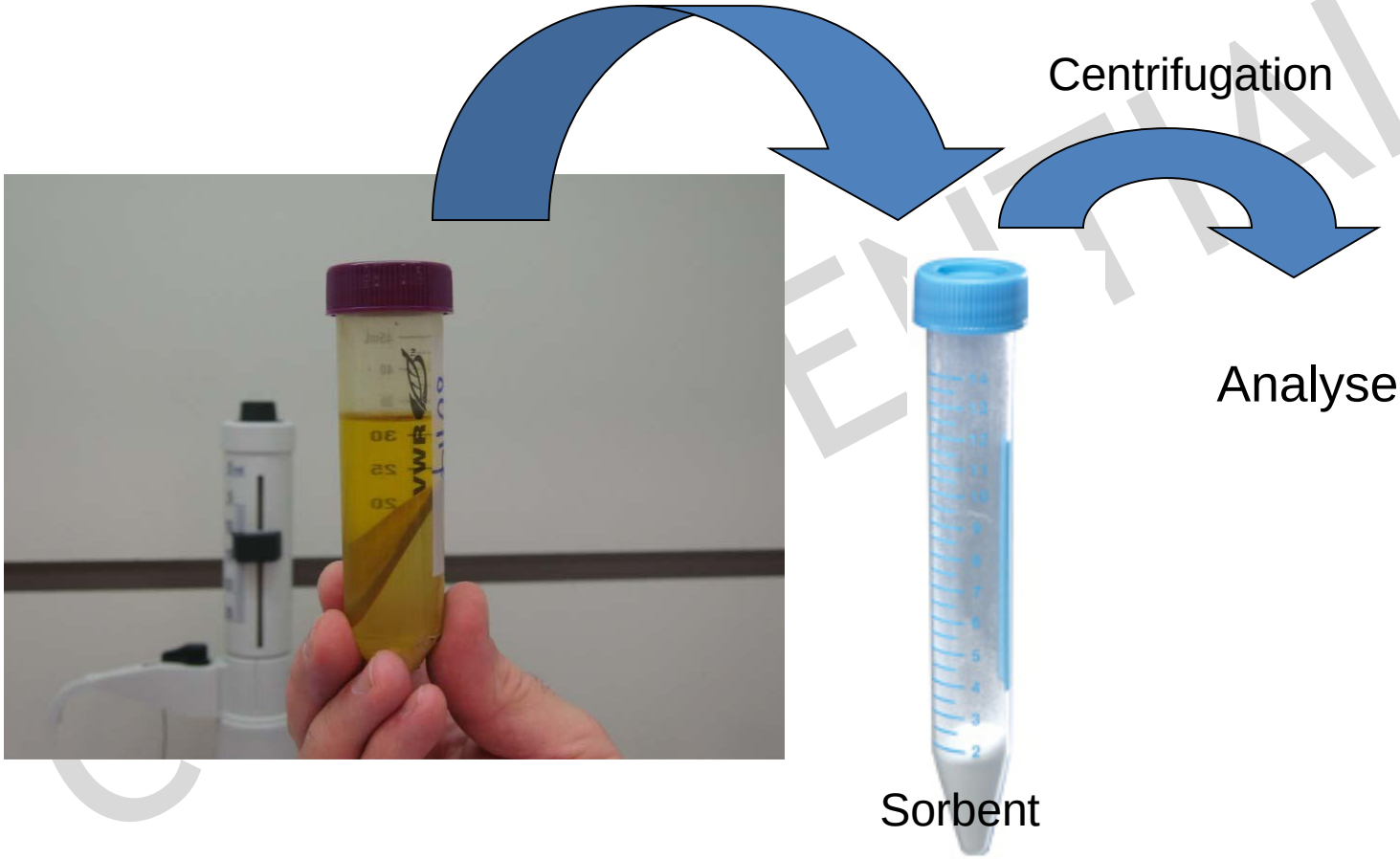
QuEchERS

Principle



QuEChERS: evolution

2003 Anastassiades *et al.*	2005 Lehotay *et al.*	2007 Anastassiades *et al.*
Original	**AOAC 2007.01**	**CEN 15662**
10-15 g sub sample ↓ 10-15 mL **MeCN** ↓ shake 0.4 g/mL anh.$MgSO_4$ 0.1 g/mL NaCl ↓ shake ↓ centrifuge 150 mg/mL anh.$MgSO_4$ 25 mg/mL PSA ↓ shake & centrifuge	10-15 g sub sample ↓ 10-15 mL **1% HOAc in MeCN** ↓ shake 0.4 g/mL anh.$MgSO_4$ 0.1 g/mL **NaOAc** ↓ shake ↓ centrifuge 150 mg/mL anh.$MgSO_4$ 50 mg/mL PSA ↓ shake & centrifuge	10-15 g sub sample ↓ 10-15 mL **MeCN** ↓ shake 0.4 g/mL anh.$MgSO_4$ 0.1 g/mL NaCl 0.1g/mL **$Na_3Cit{\bullet}2H_2O$** 0.05 g/mL **$Na_2Cit{\bullet}1.5H_2O$** ↓ shake ↓ centrifuge 150 mg/mL anh.$MgSO_4$ 25 mg/mL PSA ↓ shake & centrifuge
Option: **+ 50 mg C_{18} & 7.5 mg GCB**	**Option:** **+ 50 mg C_{18} & 2.5-7.5 mg GCB**	

Option: Scale-Up & Conc. in Toluene

Slide Devised by Urairat Koesukwiwat

« Modified » QuEChERS

- Addition of a polar solvent

Automated analysis of polar pesticides

Corn and Soy samples were pre-treated using the **QuPPe** "**Qu**ick, **P**olar **Pe**sticides Method" (www.qppe.com)

Weigh 5g of food subsample

↓

Add 10 mL of Water and soak for 10 min

↓

Add 10 mL of acidified methanol (1% formic acid).

↓

Shake vigorously and Centrifuge

↓

Filter extract (top) layer

↓

After filtration, transfer to vial to MPS for automated derivatization, extraction and analysis

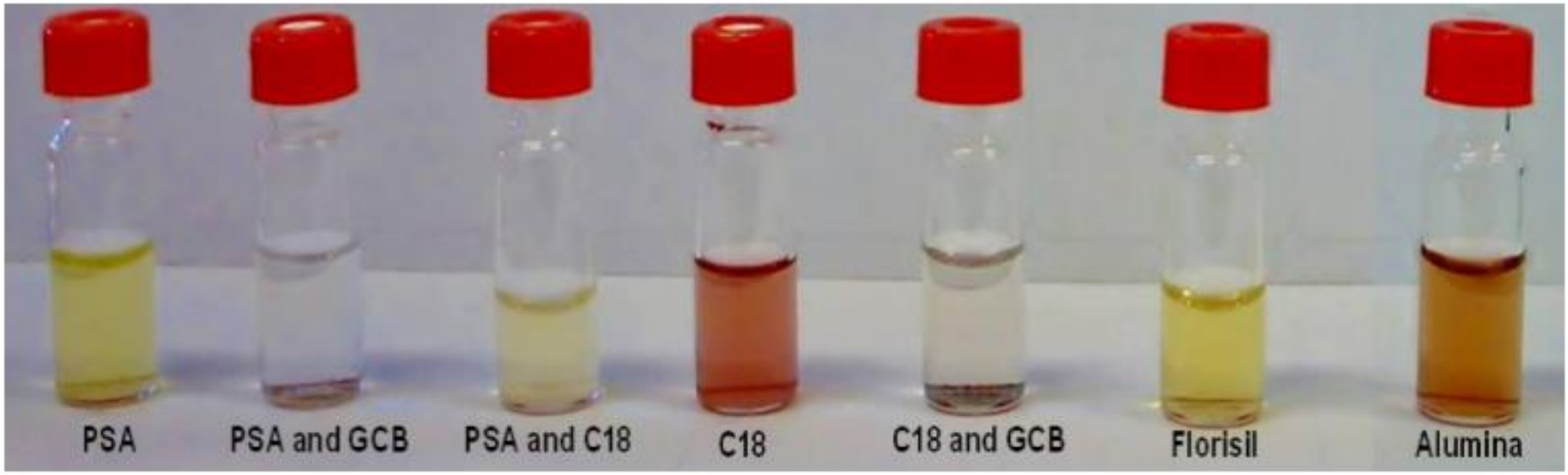
→ **Workflow Automation for LC/MS/MS Polar Pesticide Screening**

QuEChERS: dispersive SPE

effect of increasing amounts of graphitized carbon black on the decoloration of spinach extracts

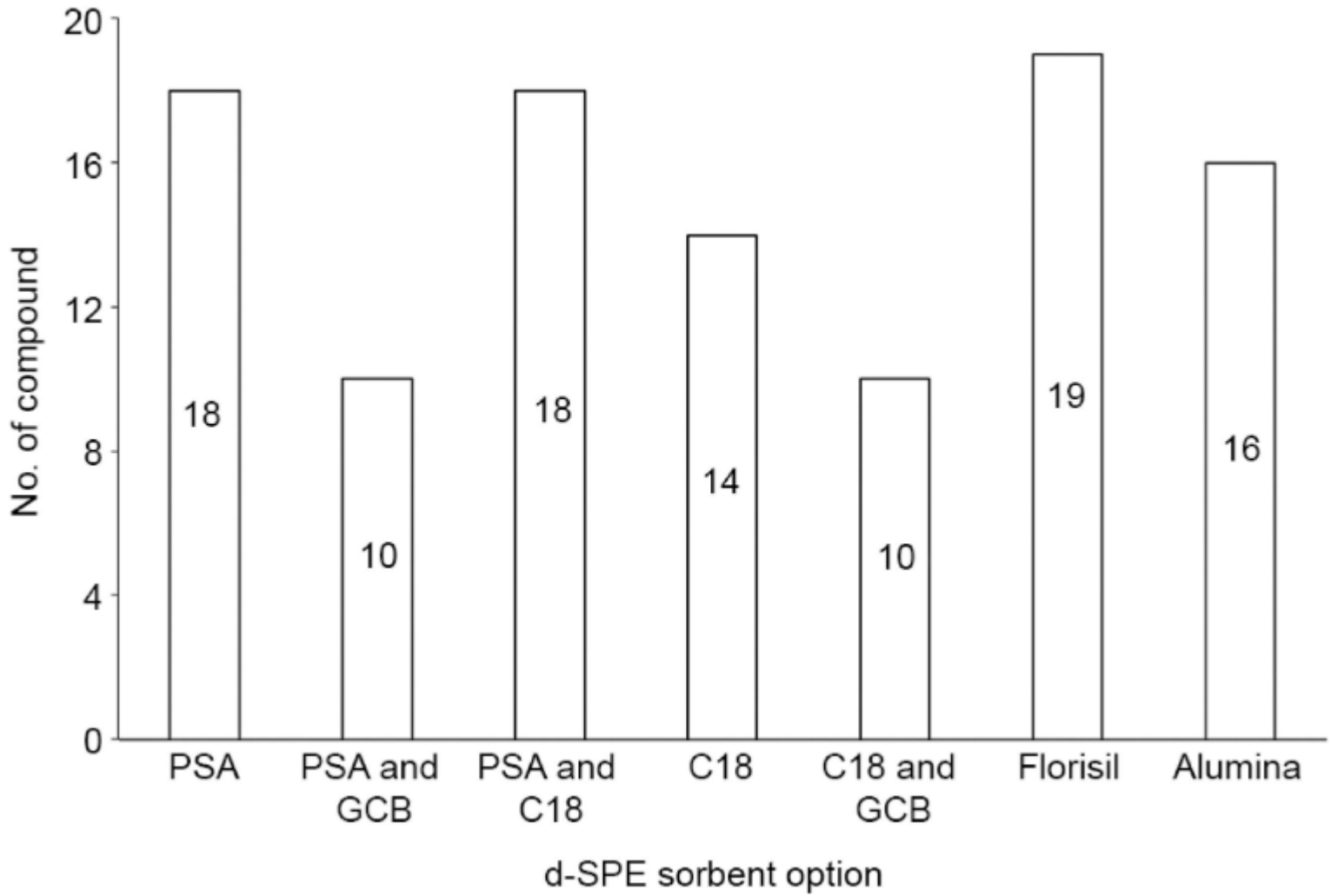
no cleaning	PSA only	PSA + 2,5 mg GCB	PSA + 7,5 mg GCB	PSA + 12,5 mg GCB	PSA + 25 mg GCB	PSA + 50 mg GCB

QuEChERS: dispersive SPE



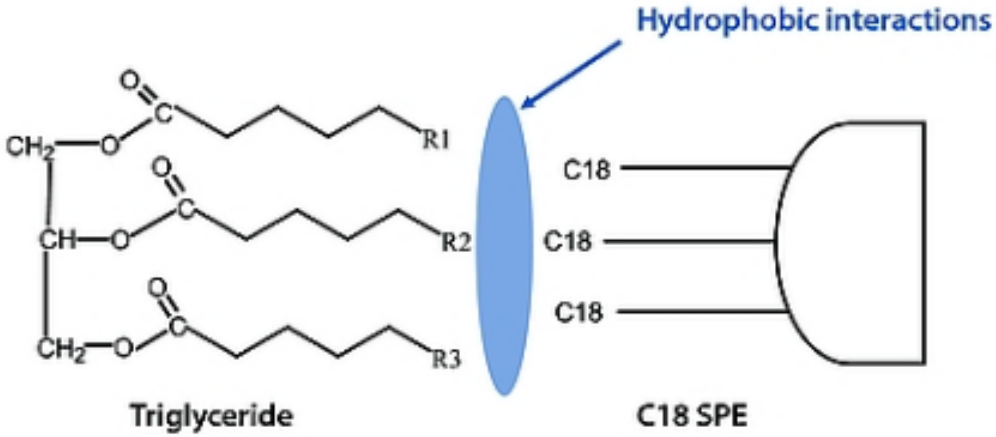
Volpatto et al.(2016)
Pesticides dans raisins
(GC-MS)

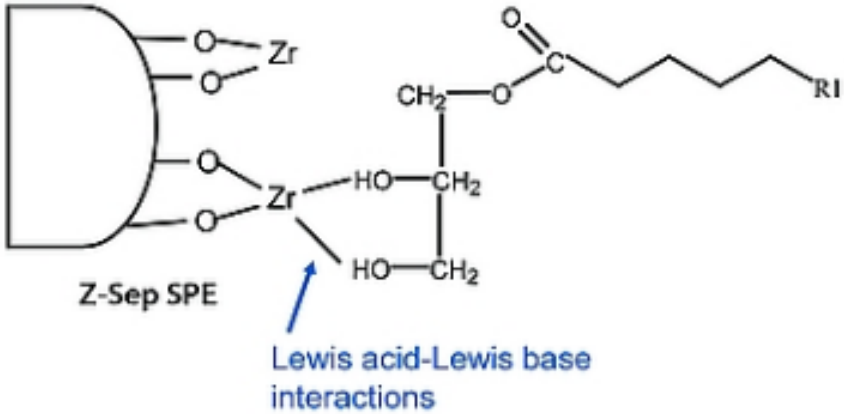
QuEChERS: dispersive SPE



QuEChERS: dispersive SPE

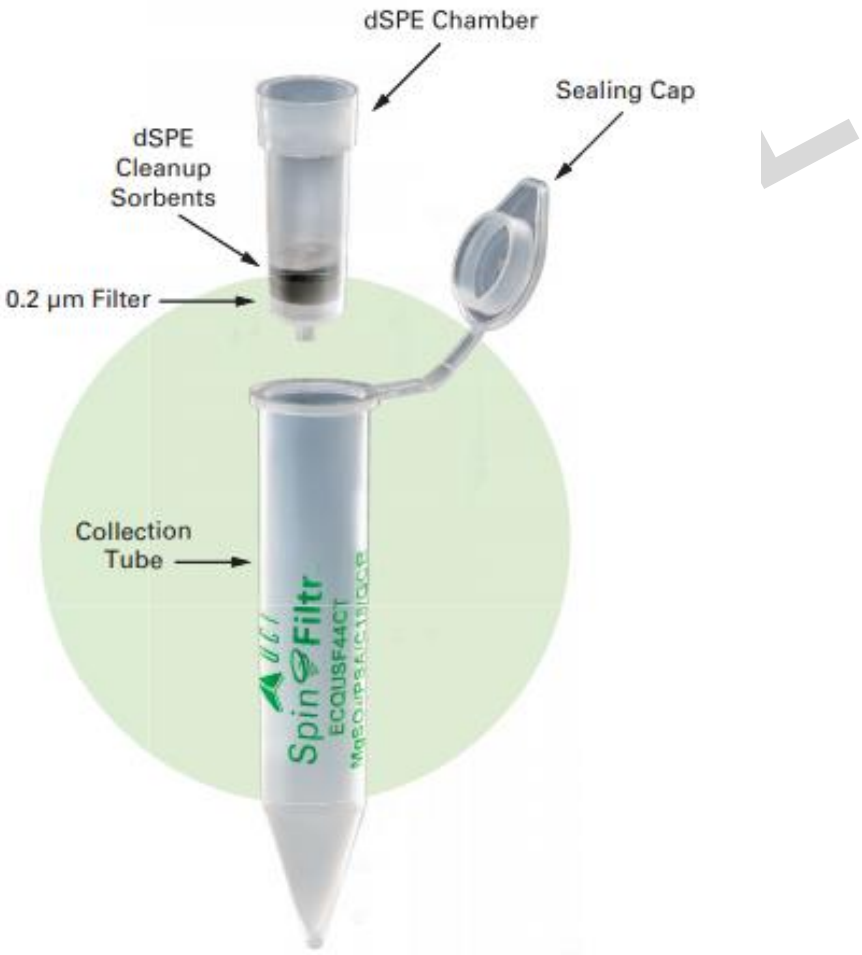
Retention Mechanism for Fats on Z-Sep/C18 and Z-Sep+

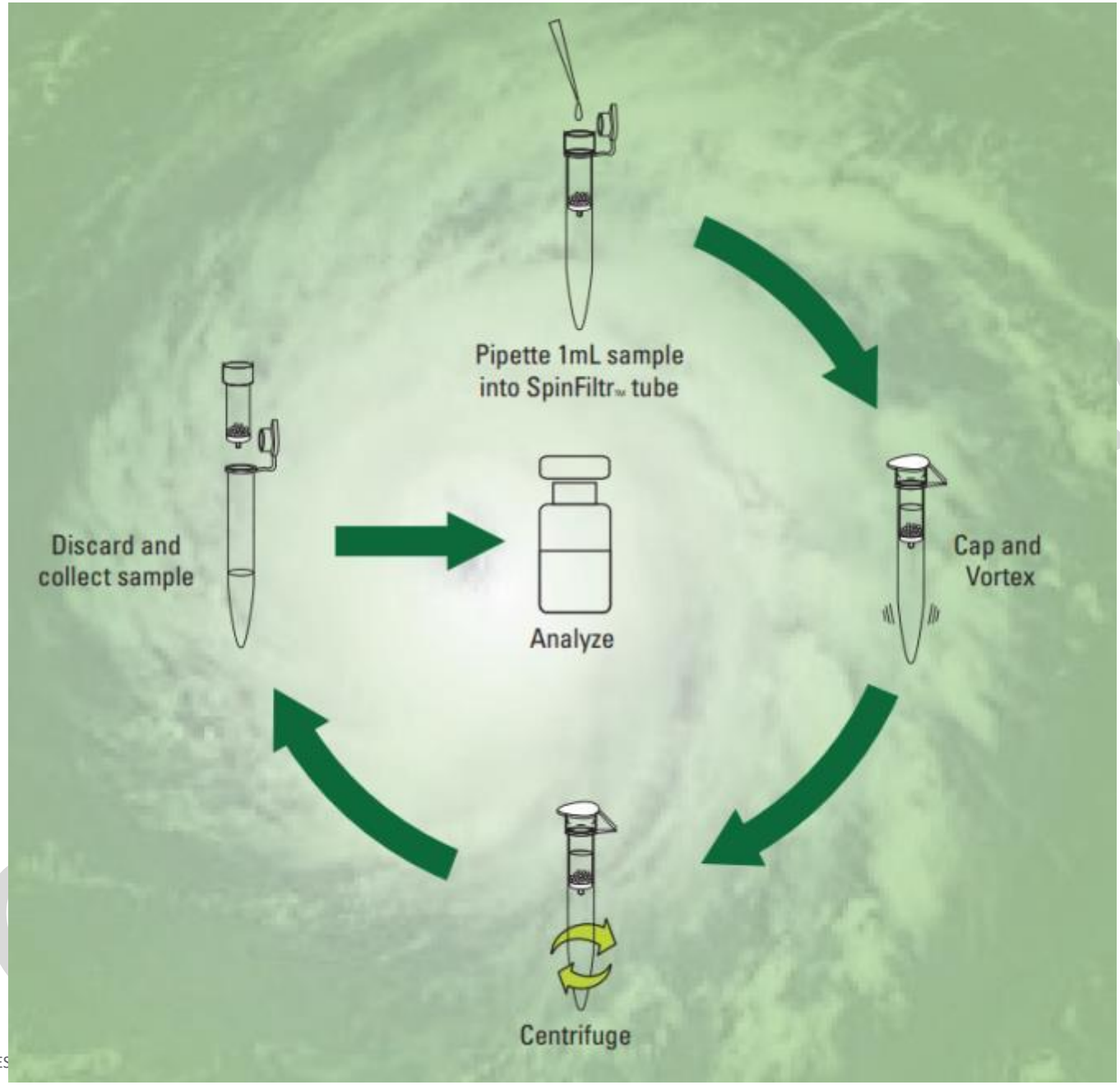




QuEChERS: SpinFiltr

Product Number	Contents
ECQUSF14CT	150 mg $MgSO_4$ 50 mg PSA
ECQUSF24CT	150 mg $MgSO_4$ 50 mg PSA 50 mg C18
ECQUSF34CT	150 mg $MgSO_4$ 25 mg PSA 2.5 mg GCB
ECQUSF44CT	150 mg $MgSO_4$ 50 mg PSA 50 mg C18 7.5 mg GCB
ECQUSF54CT	150 mg $MgSO_4$ 50 mg PSA 50 mg C18 50 mg Chlorofiltr®
ECQUSF64CT	150 mg $MgSO_4$ 50 mg C18 50 mg Chlorofiltr®




Pipette 1mL sample into SpinFiltr™ tube
Cap and Vortex
Centrifuge
Analyze
Discard and collect sample

QuEChERS/d-SPE Applications

Application	Publications
Pesticides	≈ 425
Veterinary drugs	≈ 50
Environmental (*e.g.* PAHs)	≈ 35
Mycotoxins	≈ 15
Natural Products (*e.g.* alkaloids)	≈ 15
Reviews	≈ 15
Other	≈ 15

(*e.g.* BPA, acrylamide, PFOS/PFOA, nerve agents, phthalates, dyes/ink, sildenafil, melamine, seafood toxins, Cu)

Matrices

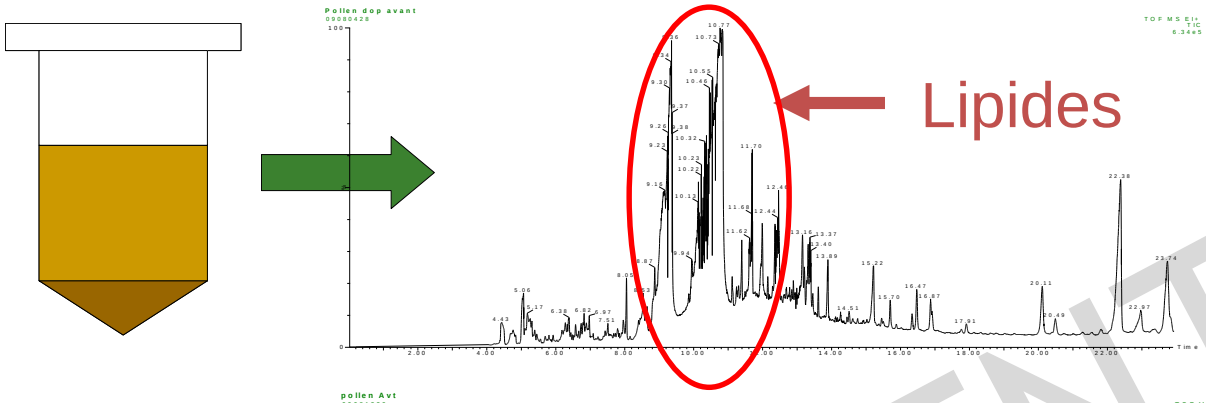
Fruits/Vegetables, Grains, Processed Foods (*e.g.* baby food), Honey, Nuts, Animal Tissues (meat, liver, kidney), Fish/Seafood, Milk, (Vegetable) Oils, Soil/Sediment, Dried Fruits, Feeds, Eggs, Tea, Juices/Beverages, Plants, Wine/Beer, Mushrooms, Blood/Serum, Water, Cactus, Compost, Roots, Tobacco, Dietary Supplements, Chinese Medicines

Lehotay (2013)

QuEChERS: suppliers

« Modified » QuEChERS

- Addition of apolar solvent

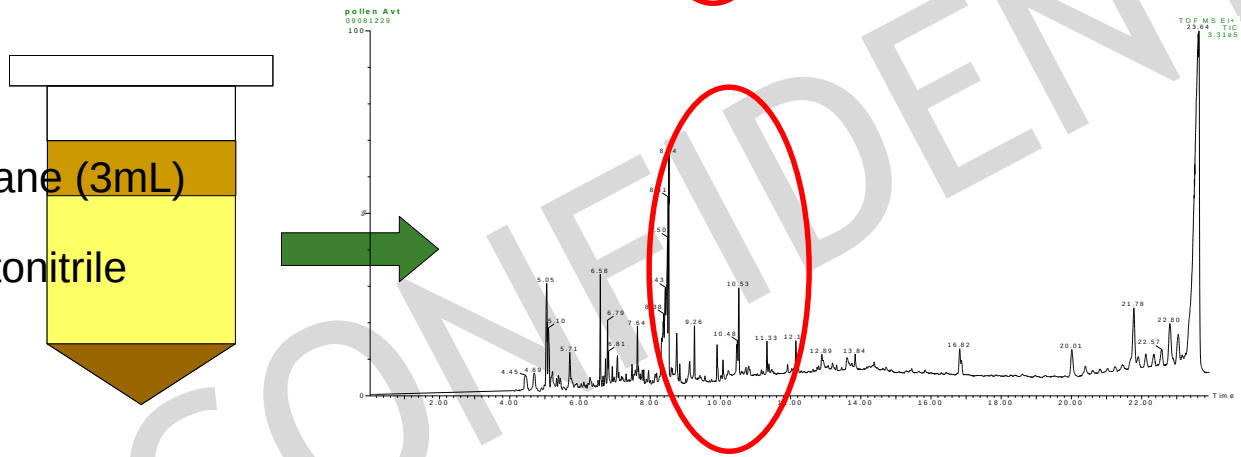


Lipides

Compounds: pesticides

Matrix : pollen

Analyse: GC-ToF



Hexane (3mL)

Acetonitrile

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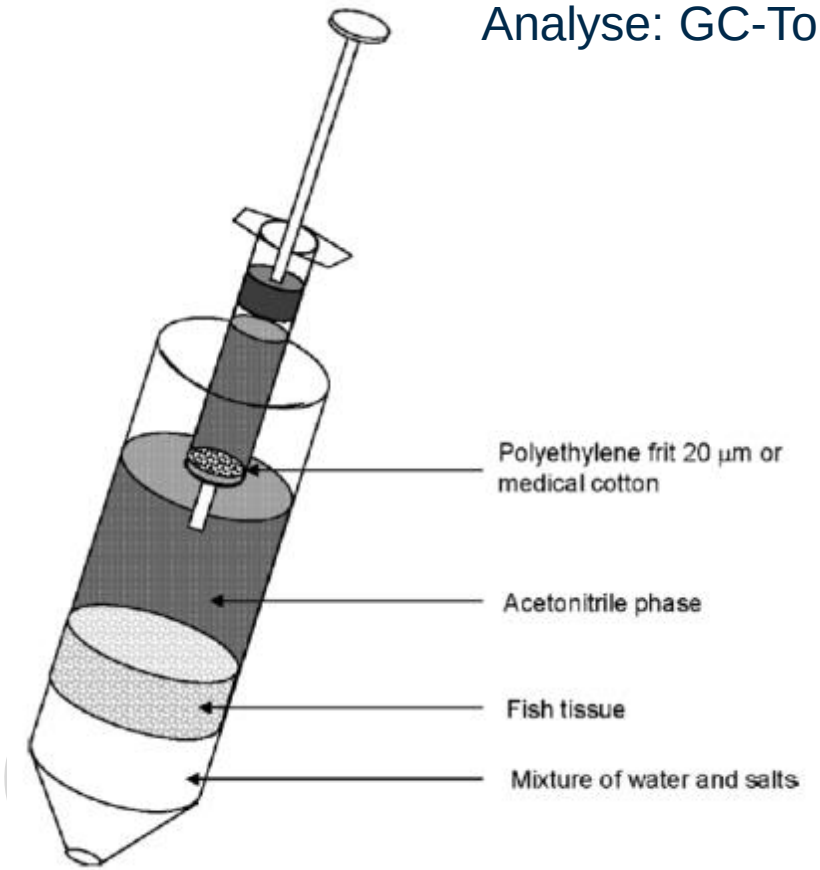
- Effect of freezing

Compounds: organochlorine pesticides and PCB

Matrix: Fish

Analyse: GC-ToF



Norli et al. (2011)

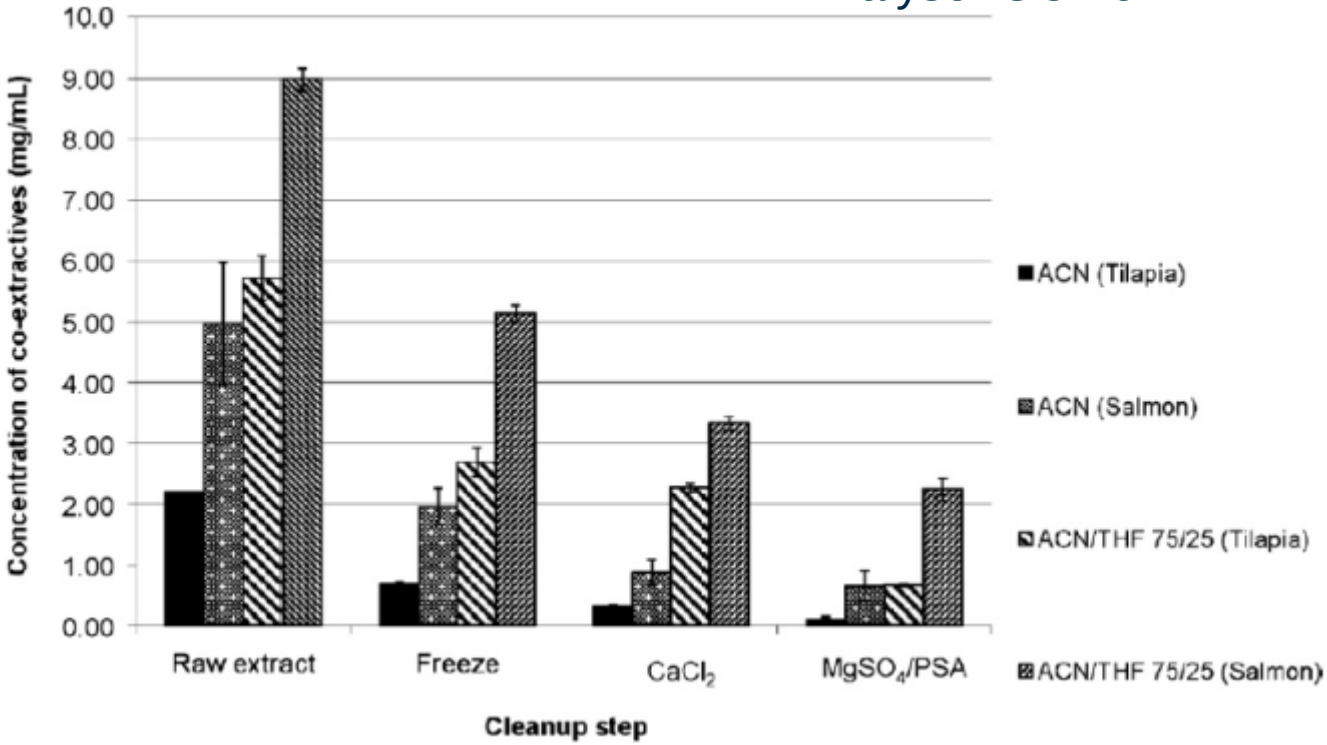
« Modified » QuEChERS

- Effet de la congélation

Compounds: organochlorine pesticides and PCB

Matrix: Fish

Analyse: GC-ToF



Norli et al. (2011)

Solid-Liquid Extraction

Optimisation

- **Solvent selection**
- **Particule size**
- **Energy input:**
  - Agitation
  - High frequency waves: ultrasound, microwave
  - Temperature
  - Pressure

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Solid Matrix: Extraction PLE (or ASE or PFE)

Accelerated hot solvent extraction under pressure

Solvent heated to high temperature and high pressure.

Increase of the solvation power
Increase of the diffusion power

Increased solubility of compounds
Increase of mass transfer

keeping the solvent in liquid form

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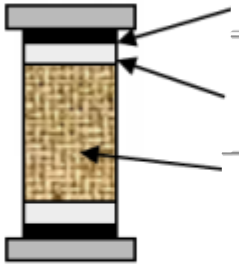
Solid Matrix: Extraction PLE (or ASE or PFE)



Accelerated hot solvent extraction under pressure

Solvent heated to high temperature and high pressure

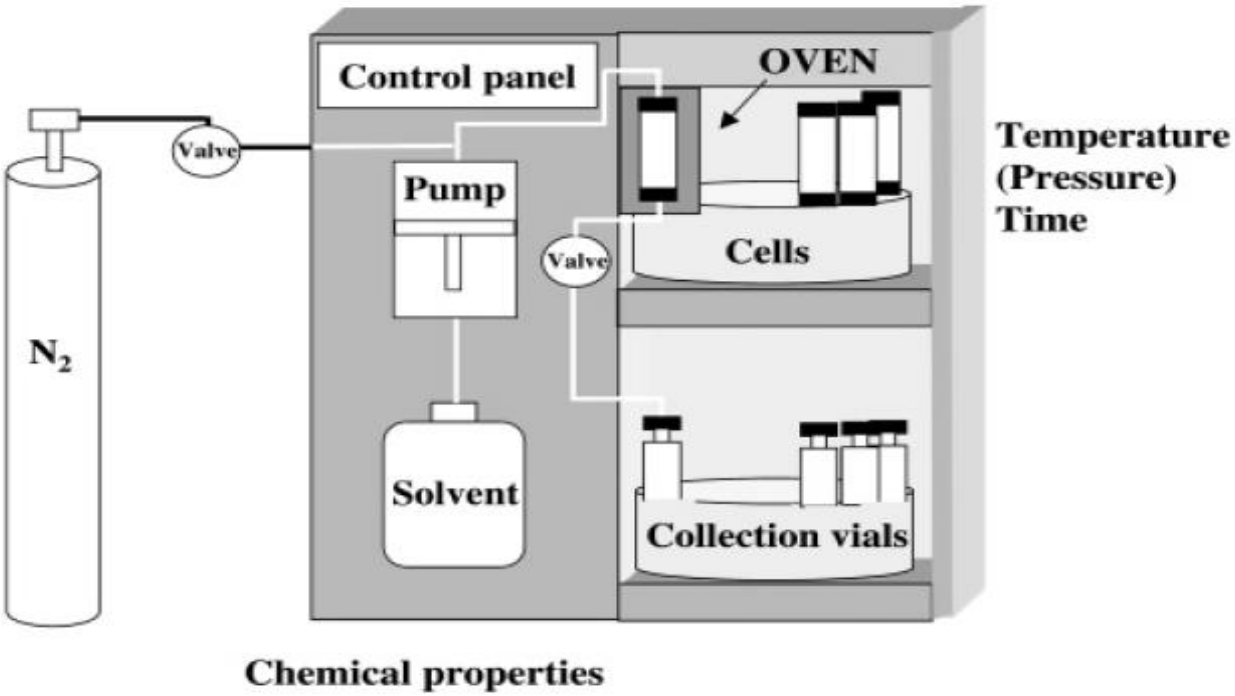


Fig. 3 Principle of a PFE system and influencing parameters.

Analyst, 2001, **126**, 1182–1193

Purification step required

Solid Matrix: Extraction PLE

Accelerated hot solvent extraction under pressure

Sample preparation:

- Dispersion of the sample in the cell with an inert material

  increase of the "accessible" surface of the sample

- Drying of the sample

Examples

SAMPLE TYPE	MATRIX MASS	DISPERSING AND DRYING
Soil / moist sediment	10 g	5 g diatomaceous earth + 20 g sodium sulfate
Fish (80% water)	3 g	5 g diatomaceous earth + 15 g sodium sulfate
Cream / lotion	2 g	3 g diatomaceous earth
fruits / vegetables	10 g	5 g diatomaceous earth + 10 g sodium sulfate
ground polymer	1 - 3 g	1-3 g sand

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Solid Matrix: Extraction PLE

Accelerated hot solvent extraction under pressure

key parameters :

Solvent : acetonitrile /acetone / ethyl acetate …

Pression: 40-100 bars (fixed at 100 bars)

Temperature: 50-100°C

Number of cycles

Time

↳ Optimization with an experimental design

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Solid Matrix: Extraction PLE : example

Phtalates

459 000 T / year used in Europe

Dibutylphtalate (DBP)

Cosmetic used

Diethylhexylphtalate (DEHP)

Bisphenol A

700 000 T / year used in Europe

Plastics industry

(protective films, bottles, toys...)

DEHP and DBP banned in plastic toys and baby bottles since 2003

• Alkylphenols

Estimated world consumption to more than 400 000 T / year

Nonylphenols

Octylphenols

And ethoxylates forms

Emulsifying and dispersing agents used in detergents, cosmetics, paints, pesticides formulation...

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- Insecticides

3 000 T / year used in France

Organophosphates

Dimethoate

Methamidophos

Carbamates

Propoxur (Baygon)

Prosulfocarbe

Organochlorines

DDTs (DDD and DDE)

Banned since 1964 in France but highly persistent

Methoxychlor and its metabolite HPTE

(2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane)

- Fungicides

41 000 T / year used in France

Triazole Fungicides

Bromuconazole

Fluquinconazole

Tetraconazole

Tebuconazole

Hexaconazole

Morpholine Fongicide

Fungicide pyridazinone

Pyridabene

Residue of dithiocarbamate pesticides

2-Imidazolidinethione

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Homogeneization - Gringing

Lyophilisation

sieving

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Solid Matrix: Extraction PLE : example

***Sample quantity**:*
Several hundred grams

Complexity and heterogeneity of the matrix

Break up/break down agglomerates and aggregates

Homogeneisation - Grinding

Grinding by knives in 3-second pulses for about 10 minutes

Gently dehydrate

Lyophilisation

Low dryness sample

Lyophilization for 24 hours at 0.002 mbar and -80°C

sieving

Sieving at 250 µm

Homogeneous, finely divided and dry sample

Homogeization - Grinding	***Grinding by knives in 3-second pulses for about 10 minutes***
Lyophilisation	***Lyophilization for 24h at 0.002 mBar / -80°C***
Sieving	***Sieving at 250 µm***
Extraction - ASE	

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Solid Matrix: Extraction PLE : example

Sample quantity:
1 to 10 grams

Solubilization and extraction of analytes

Selection of optimal ranges of the

4 ASE parameters

40 to 80 °C
40 to 120 bars
5 to 15 minutes
1 to 3 cycles

Synergistic model of the response

Main effects and interaction terms

Solvent selection

Design of experiments

Selection of extraction parameters

Selective and maximum extraction of the desired analytes

Homogeization - Grinding

Grinding by knives in 3-second pulses for about 10 minutes

Lyophilisation

Lyophilization for 24h at 0.002 mBar / -80°C

Sieving

Sieving at 250 mm

Extraction - ASE

Extraction by acetonitrile/isopropanol, 1/1, v/v

Temperature : 80°C

Pressure : 40 bar

Static time : 10 minutes

Number of cycles : 1

Purification - SPE

Conditioning: heptane/acetone, 3/7, v/v
Sample loading: ~1 mL at 1 mL.min-1
Additional collection: 3 mL heptane/acetone, 3/7, v/v

Concentration - Evaporation

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Solid Matrix: Extraction PLE : example

Sample quantity:
~3 mL heptane/acetone, 3/7, v/v

Analyte concentration

Dry residue of sludge extract difficult to solubilise

Concentration - Evaporation

Loss of analytes

Interferents concentration

Effect study:
White extract doped then reduced from 0.5 to 1.5 g
Mud extract evaporated from 0.5 to 1.5 g then spiked
Mud extract spiked then reduced from 0.5 to 1.5 g

Evaporation up to 1 g

Increase in the amount of signal produced x 2

Homogeization - Grinding	***Grinding by knives in 3-second pulses for about 10 minutes***
Lyophilisation	***Lyophilization for 24h at 0.002 mBar / -80°C***
Sieving	***Sieving at 250 mm***
Extraction - ASE	***Extraction by acetonitrile/isopropanol, 1/1, v/v*** ***Temperature : 80°C*** ***Pressure : 40 bar*** ***Static time : 10 minutes*** ***Number of cycles : 1***
Purification - SPE	***Conditioning: heptane/acetone, 3/7, v/v*** ***Sample loading: ~1 mL at 1 mL.min-1*** ***Additional collection: 3 mL heptane/acetone, 3/7, v/v***
Concentration - Evaporation	***Concentration to ~1 g***
Analysis	***22 compounds analyzed by LC-MS/MS***

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Accelerated hot solvent extraction under pressure

Advantages

- Drastic extraction
- Advantage for strongly matrix-bound analytes
- Rapid extraction
- Reduced solvent volume

Disadvantages

- Extraction of many interferents: need for a purification step
- Degradation of thermo-sensitive compounds

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Extraction/purification coupling

- Pre-treatment

- Solvent extraction

- Purification
  - Dispersive SPE
  - Liquid Liquid Extractions & Co
  - MSPD
  - SPLE

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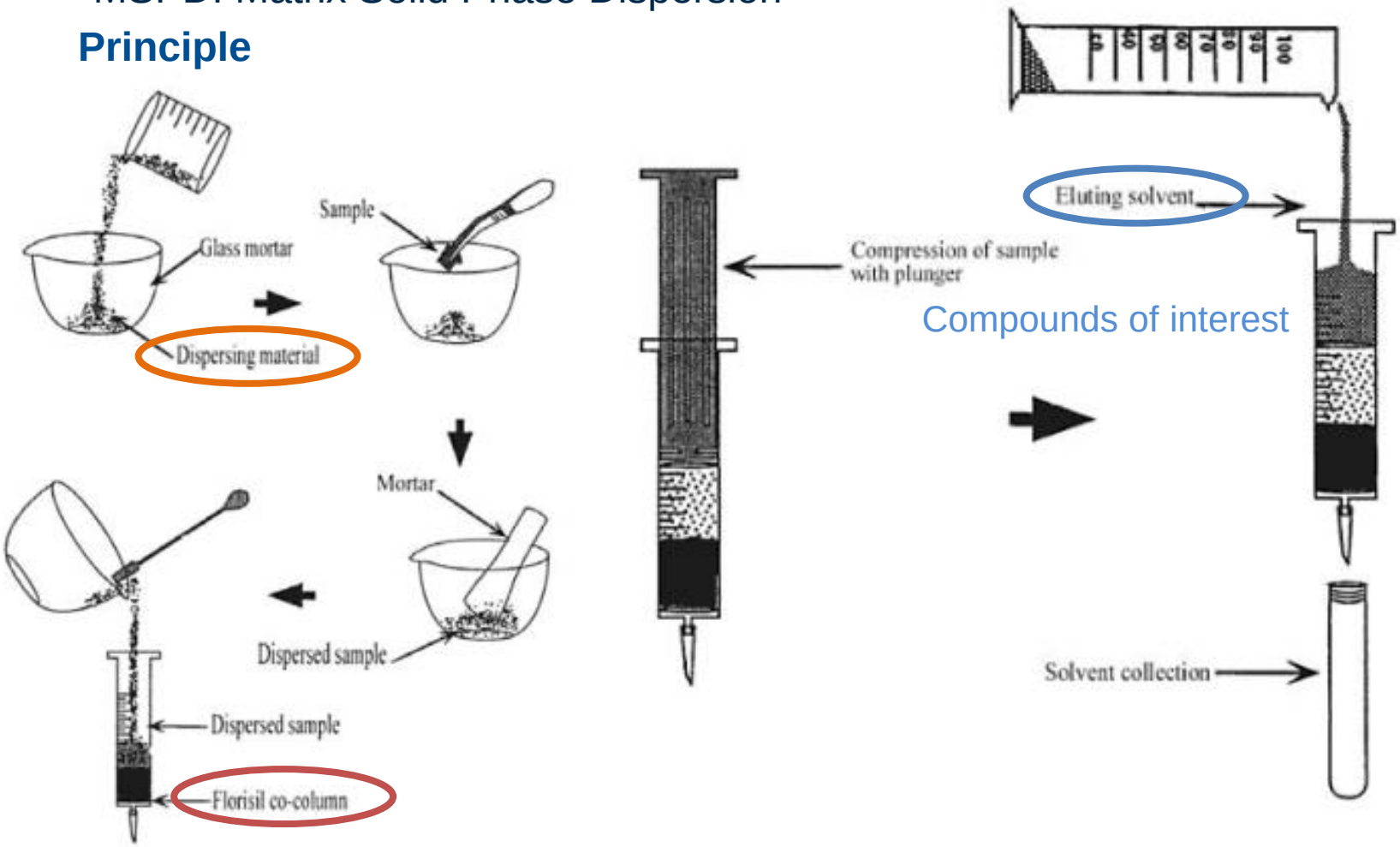
Solid matrices: New approaches: Extraction and Purification coupling

MSPD: Matrix Solid Phase Dispersion

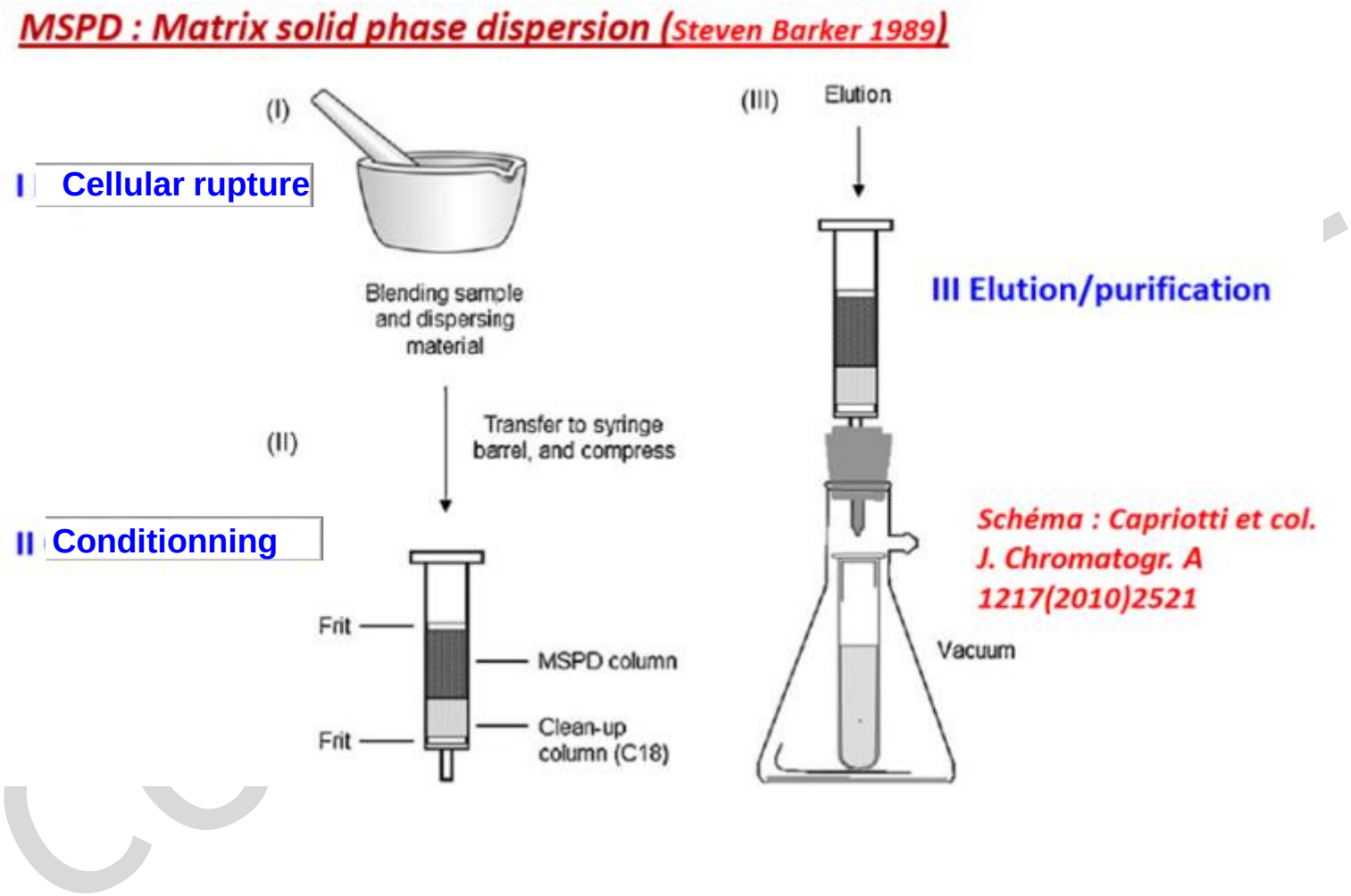
Principle



Interferences

Solid matrices: New approaches: Extraction Purification coupling

MSPD



Solid matrices: New approaches: Extraction Purification coupling

MSPD

Parameters to optimize :

- SPE phase: alumina, silica gel or florisil
- Loading solvent
- Elution solvent
- Evaporation : dry ? -> use of a keeper solvent
- Adsorbent and quantity

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Solid Matrix : New approaches : Extraction Purification coupling

Purification

- SPE dispersive
- DLLME

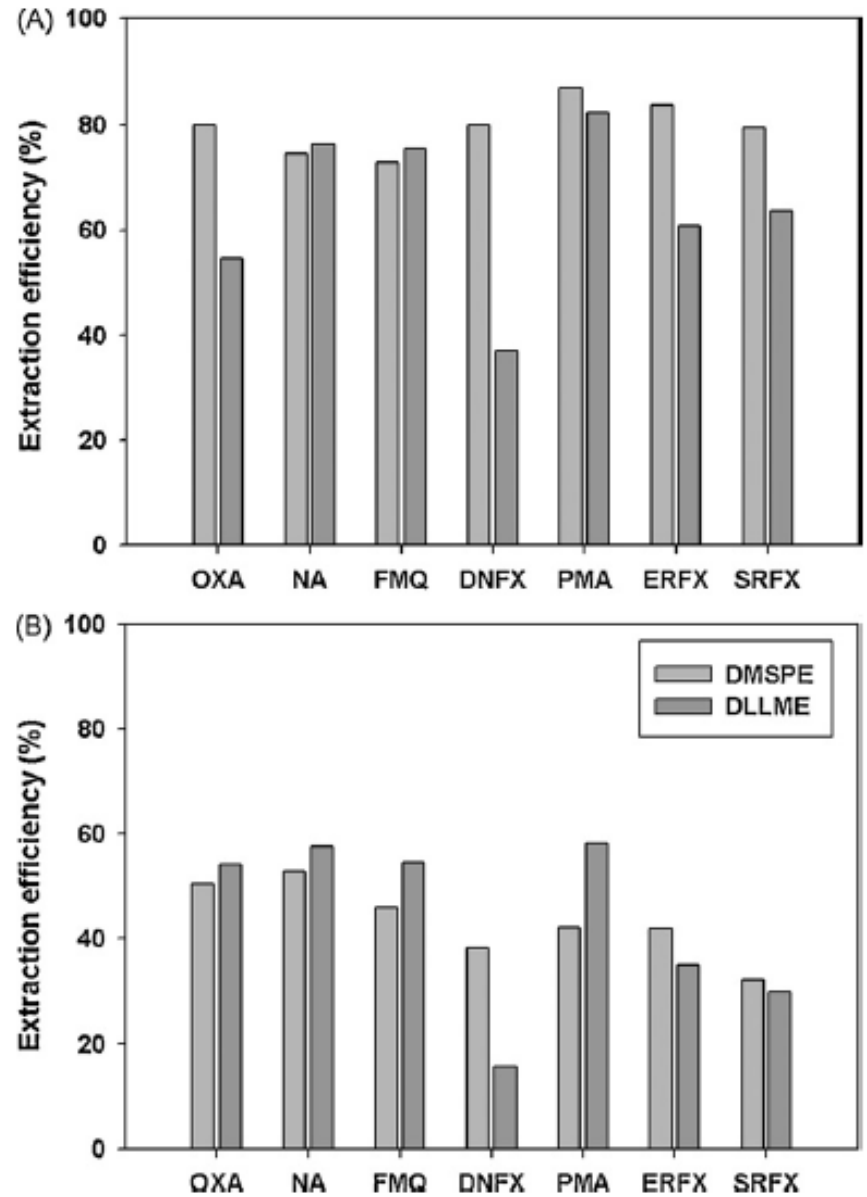

Fig. 4. Comparison of extraction efficiencies from water (A) and muscle matrix (B) processed using DMSPE and DLLME approaches.

Tsai et al. (2009)

CONFI

Solid Matrix : New approaches : Extraction Purification coupling

Purification

- SPE dispersive
- DLLME

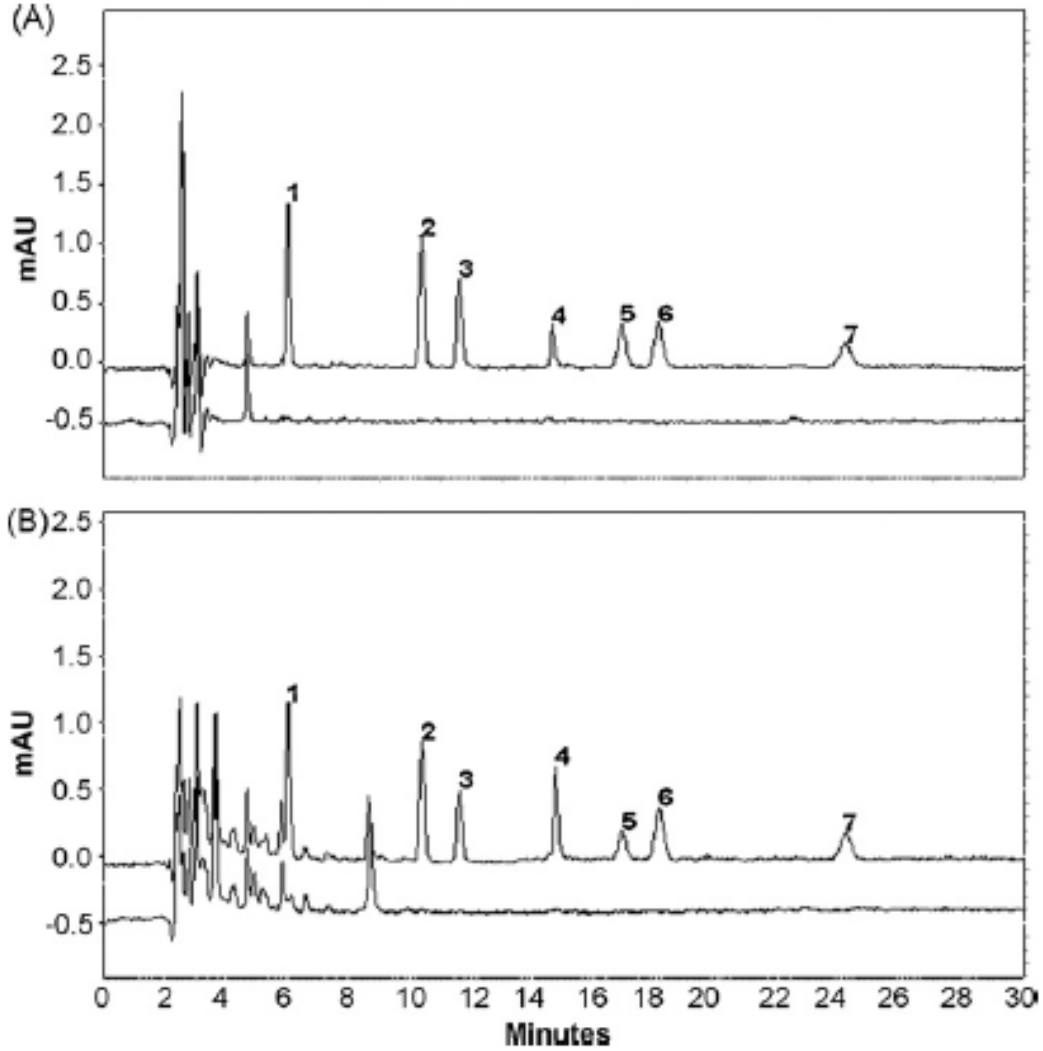

Fig. 5. HPLC chromatograms of blank swine muscle (bottom curve) and of a sample spiked with 100 μg kg^{-1} of quinolones (top curve) by DLLME (A) and DMSPE (B) approaches. The peaks were (1) OXA; (2) NA; (3) FMQ; (4) DNFX; (5) PMA; (6) ERFX and (7) SRFX.

Tsai et al. (2009)

CONF

Thank you for your attention
Questions ?

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