



Innovative sample preparation  
technology to reduce bottleneck  
in a measurement process

Mahitti Puanggam

# Steps in an analysis

## Define the Problem

- **What needs to be found?** Qualitative and/or quantitative?
- What will the information be used for?
- How accurate and precise does it have to be?
- The analyst (the problem solver) should consult with the client to plan a useful and efficient analysis, including how to obtain a **useful sample**.

## Select a Method

- Sample type
- Size of sample
- **Sample preparation needed**
- Concentration and range (**sensitivity needed**)
- **Selectivity needed** (interferences)
- **Accuracy/precision needed**
- **Tools/instruments available**
- Cost
- Speed
- Are methods available in the chemical literature?
- Are standard methods available?
- Are there regulations that need to be followed?

## Prepare the Sample for Analysis

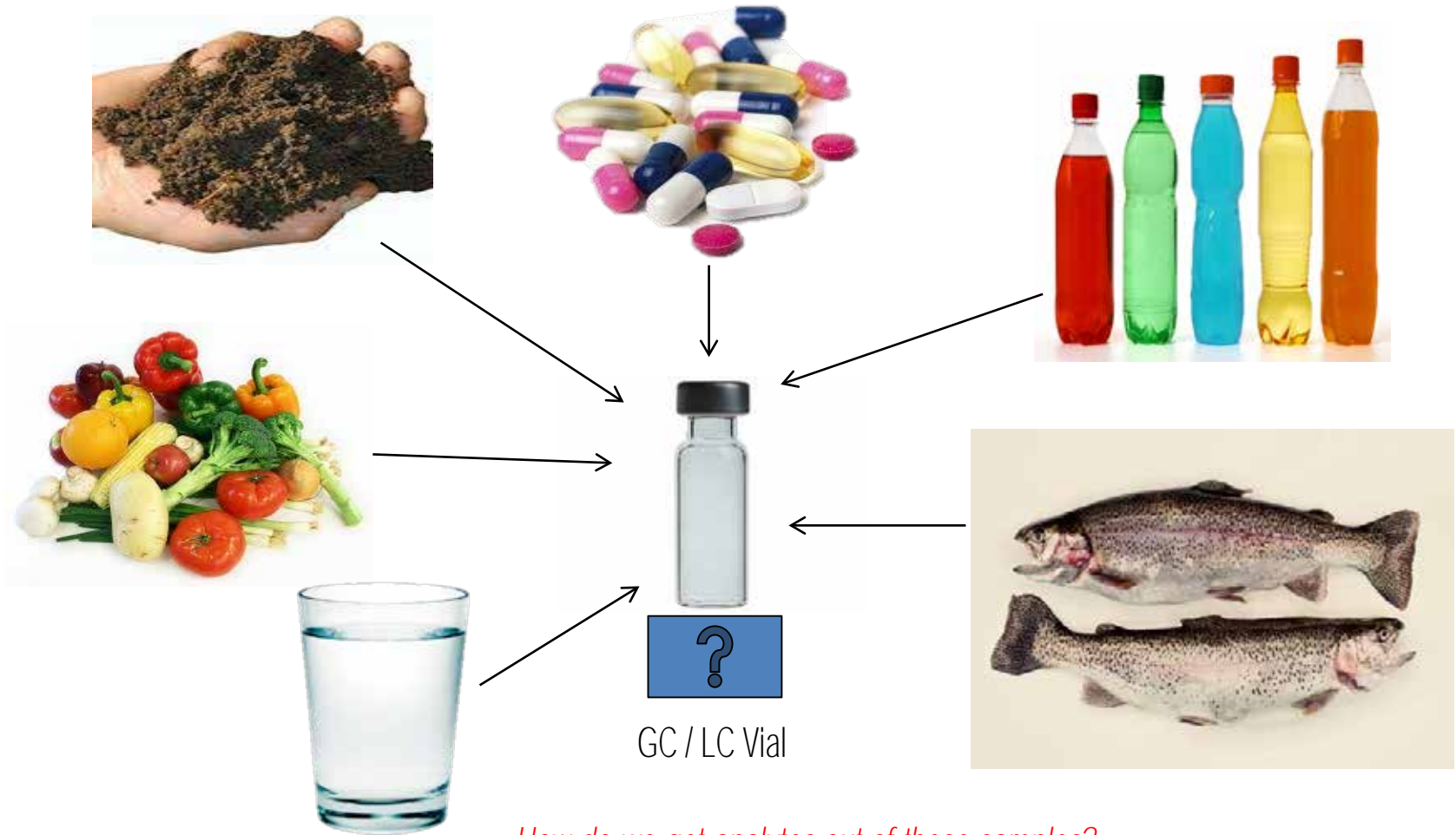
- Solid, liquid, or gas?
- Ash or digest?
- Precipitation
- Solid phase extraction
- Chemical separation or masking of interferences needed?
- Need to concentrate the analyte?
- Need to adjust solution conditions (pH, add reagents)?
- Need to change (derivatize) the analyte for detection?
- Dissolve?
- Distillation
- Solvent extraction

Perform the Measurement  
Calculate the Results and Report

## Obtain a **Representative Sample**

- Sample type/homogeneity/size
- Sampling statistics/errors

# The Challenge for Analysis



*How do we get analytes out of these samples?*

## Solvent extraction or Liquid-Liquid Extraction (LLE)



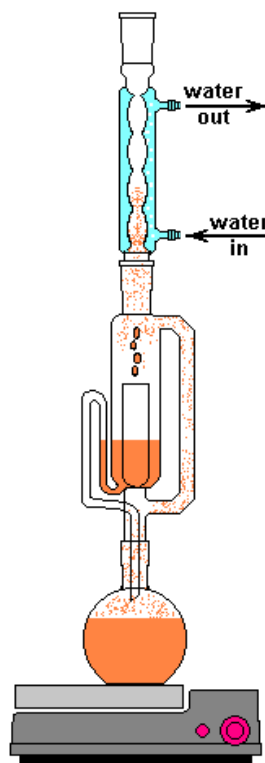
### Distribution Coefficient

$$K_D = \frac{[S]_1}{[S]_2}$$

where  $K_D$  is the distribution coefficient and the subscripts represent solvent 1 (e.g., an organic solvent) and solvent 2 (e.g., water). If the distribution coefficient is large, the solute will tend to be quantitatively partitioned in solvent 1.

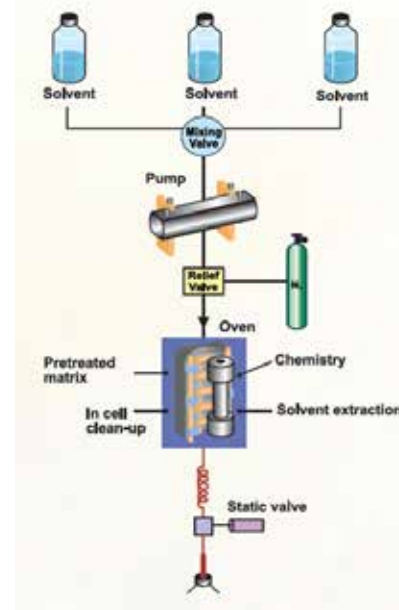
*Analytical Chemistry, 7th Edition, Gary D. Christian,  
Pumendu K. Dasgupta, Kevin A. Schug ©2014*

## Soxhlet extraction



Wikipedia

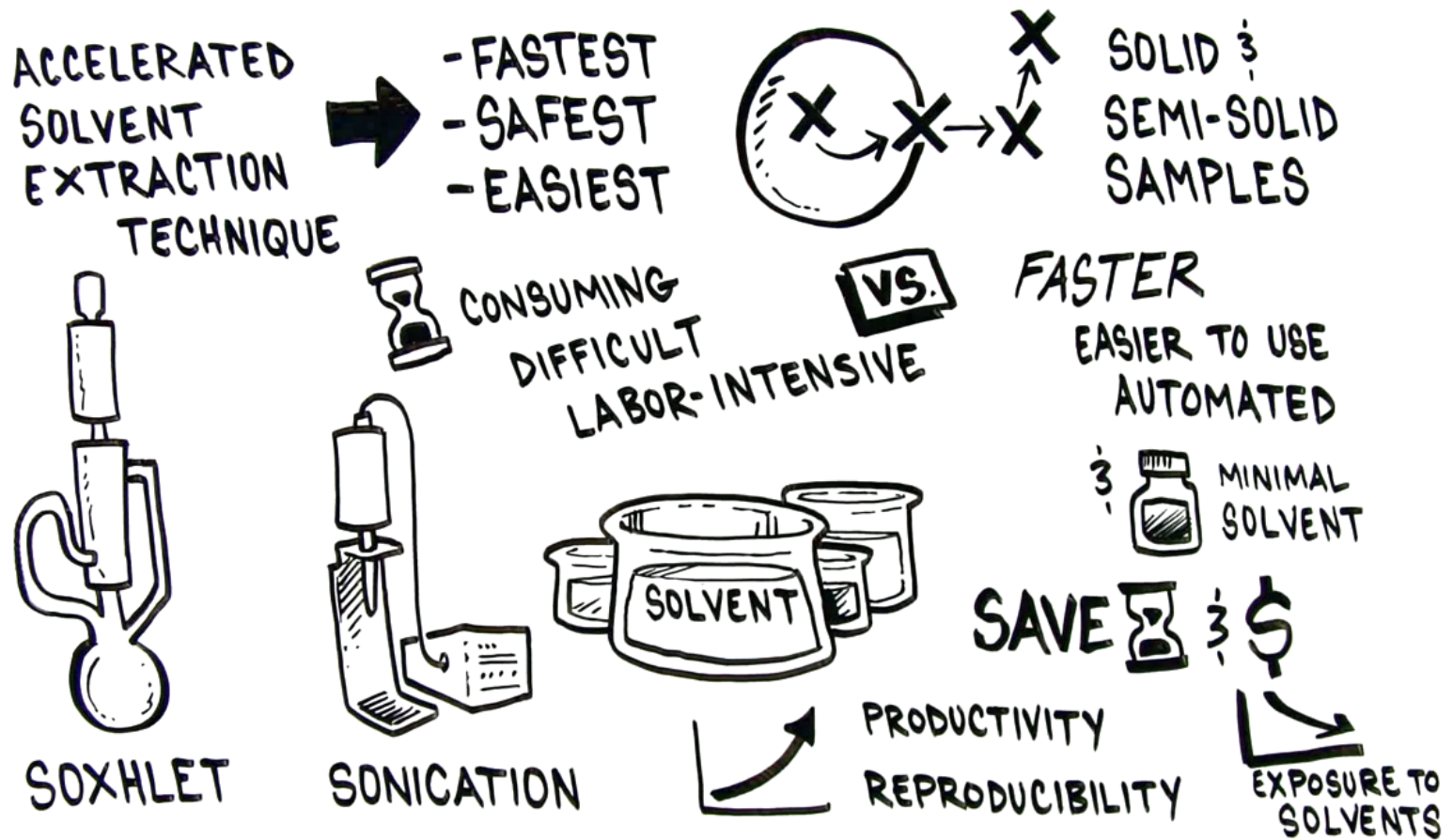
## Accelerated and Microwave-Assisted Extraction



Accelerated solvent extraction is a technique for the efficient extraction of analytes from a solid sample matrix into a solvent. The sample and solvent are placed in a closed vessel and heated to 50 to 200°C. The high pressure allows heating above the boiling point, and the high temperature accelerates the dissolution of analytes in the solvent. Both time of extraction and the volume of solvent needed are greatly reduced over atmospheric extraction.

# Accelerated Solvent Extraction

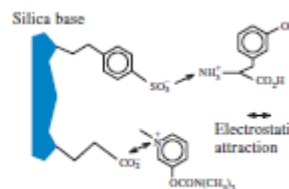
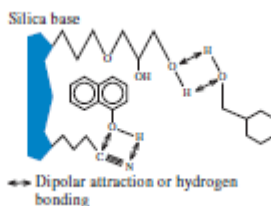
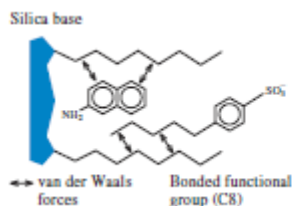
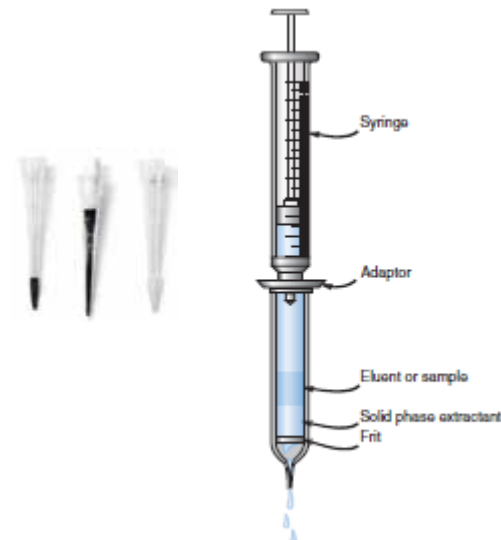
Accelerated Solvent Extraction



## Solid-Phase Extraction

The extracting solvents are limited to those that are water immiscible (for aqueous samples). Emulsions tend to form when the solvents are shaken, and relatively large volumes of solvents are used that generate a substantial waste disposal problem. The operations are often manually performed and may require a back extraction.

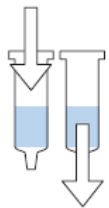
Solid-Phase Extraction (SPE), which has become a widely used technique for sample cleanup and concentration prior to chromatographic analysis in particular.



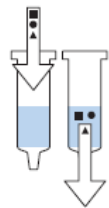
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## Solid-Phase Extraction

Typical sequence in a solid-phase extraction.

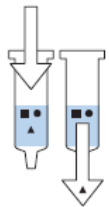


**CONDITIONING**  
Conditioning the sorbent prior to sample application ensures reproducible retention of the compound of interest (the isolate).



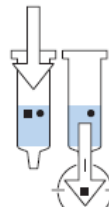
**RETENTION**

- Adsorbed isolate
- Undesired matrix constituents
- ▲ Other undesired matrix components



**RINSE**

- ▲ Rinse the columns to remove other undesired matrix components



**ELUTION**

- Purified and concentrated isolate ready for analysis
- Undesired components remain

*Analytical Chemistry, 7th Edition, Gary D. Christian,  
Pumendu K. Dasgupta, Kevin A. Schug ©2014*

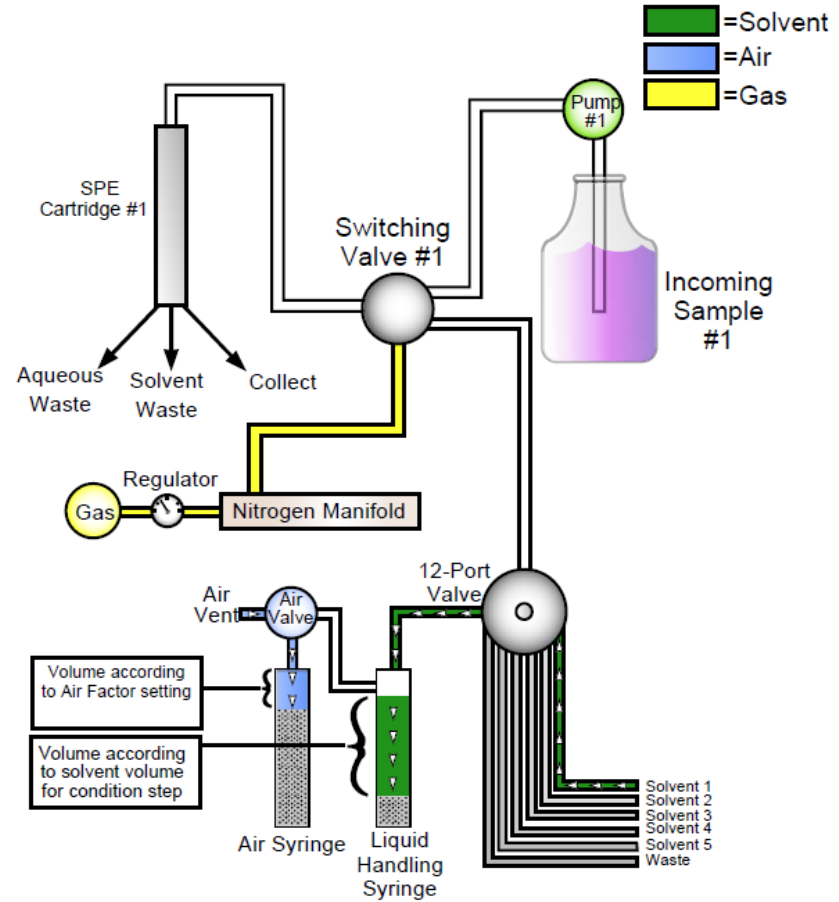
Solid phase extraction is a form of liquid chromatography used in processing samples to selectively isolate constituents of interest from other compounds that may interfere with the analysis. Before a solid sample can be processed by SPE, it must first be extracted by soxhlet extraction, pressurized fluid extraction (ASE), or other method, to produce a solution that contains the analytes of interest.



More details about SPE

<https://www.nist.gov/video/solid-phase-extraction>

## Automated Solid-Phase Extraction





## Solvent Evaporation

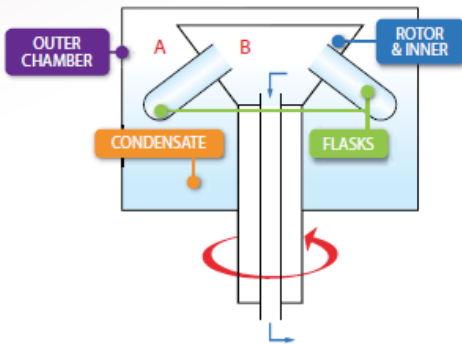
After extraction and cleanup process, remaining solvent typically larger than required volume. In order to obtain better sensitivity of analysis, solvent have to be reduced.



# Centrifugal Evaporators

## The Rocket Evaporator

Sample evaporation with walkaway capability.



## Solid-Phase MicroExtraction (SPME)

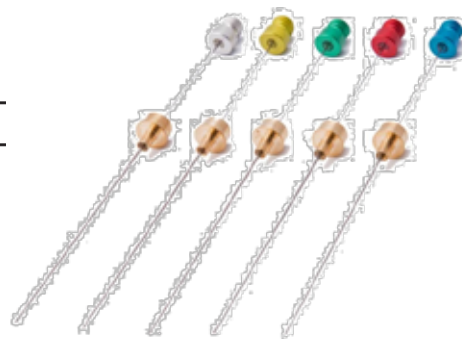
SPME is a solvent-free extraction technique, typically used for analyte collection for determination by gas chromatography. The key feature of this device is an extraction fiber, protected inside the needle of a syringe. A typical SPME fiber is made of fused silica coated with a thin layer (7  $\mu\text{m}$  to 100  $\mu\text{m}$  thick) of immobilized polymer or a solid adsorbent, or a combination. In a solution or headspace (vapor in equilibrium with the solution in a closed system) analytes are exposed to the fiber and distribute between the sample matrix and the fiber coating during extraction.

### Commercial SPME fiber coatings and their applications<sup>a</sup>

Fiber coating	Analytes
Polydimethylsiloxane (PDMS)	Nonpolar analytes
Polydimethylsiloxane/ Divinylbenzene (PDMS/ DVB)	Many polar compounds (esp. amines)
Polyacrylate	Highly polar (ideal for phenols)
Carboxen/ Polydimethylsiloxane (CAR/PDMS)	Gaseous/volatile analytes
Carbowax/ Divinylbenzene (CW/DVB)	Polar analytes (esp. alcohols)
DVB/CAR/PDMS	Broad range of polarities (good for C3-C20 range)
Carbowax/ Templated resin (CW/TPR)	For HPLC applications

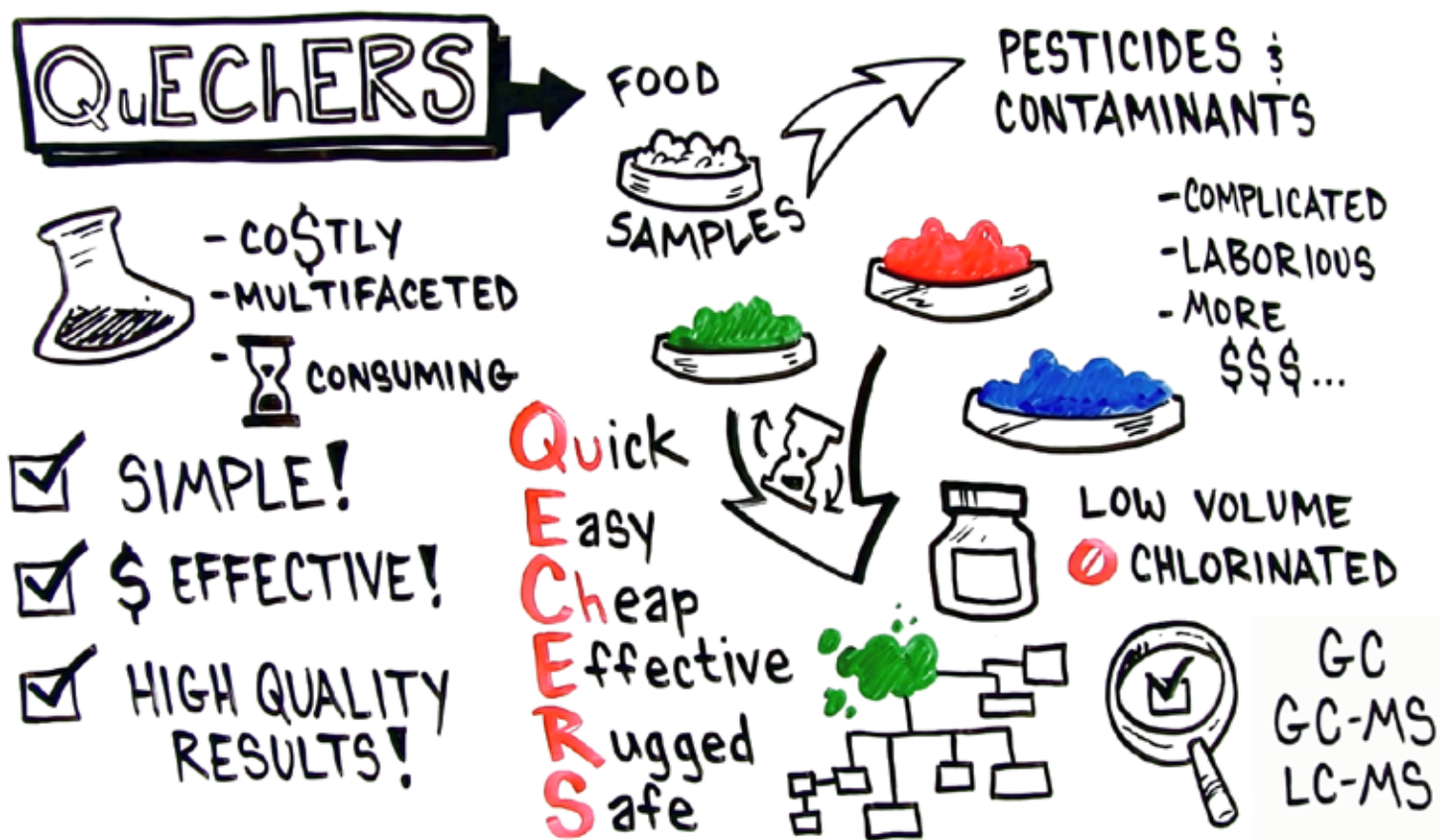
<sup>a</sup>Information adapted from Supelco application note

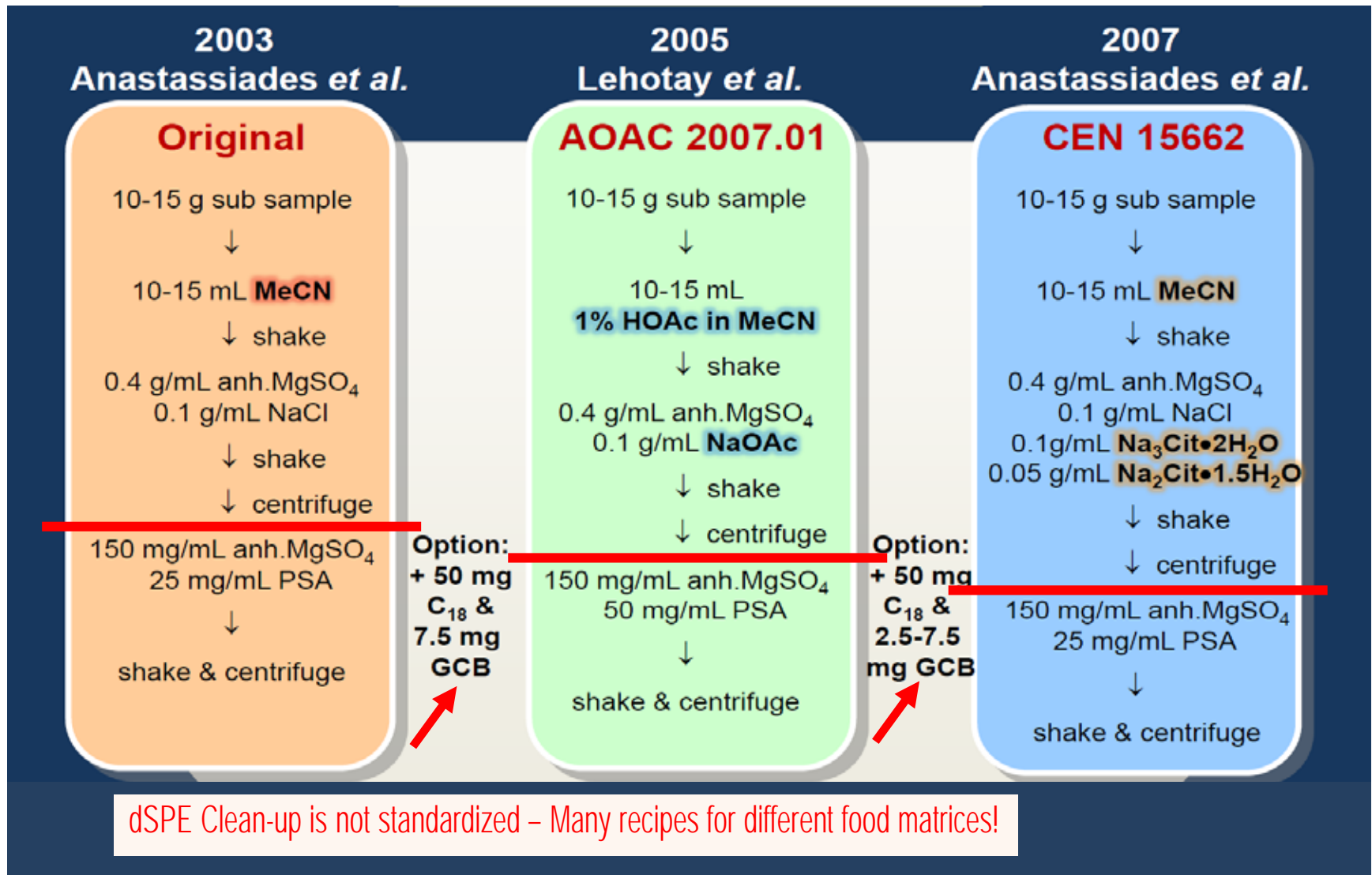
“like dissolves like”



# QuEChERS

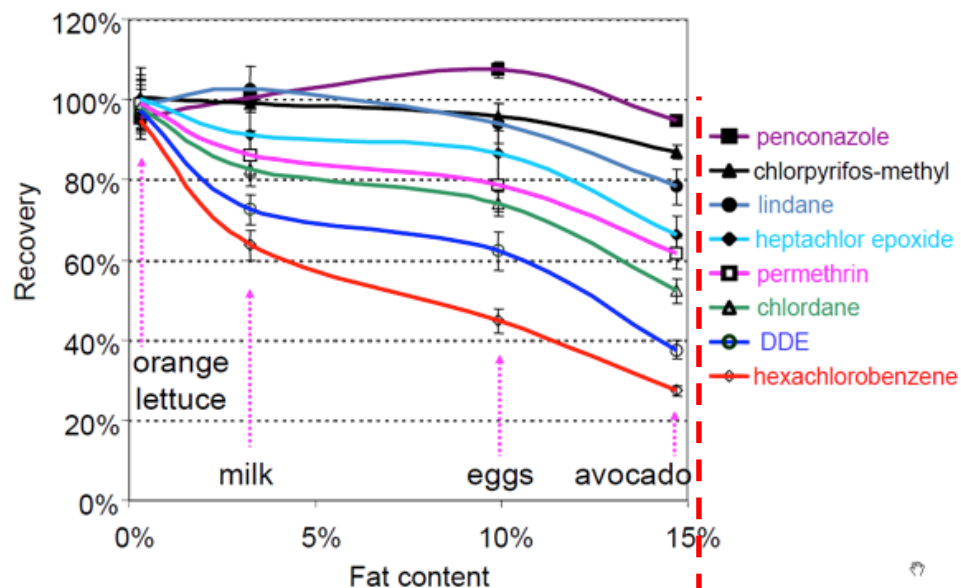
QuEChERS (pronounced "catchers"), an acronym for Quick, Easy, Cheap, Effective, Rugged and Safe, covers a variety of sample preparation and clean-up techniques for the analysis of multiple pesticide residues in agricultural matrices.





Lehotay, S.J. "Revisiting the Advantages of the QuEChERS Approach to Sample Preparation." Sep Science 2013.

- Too many modified versions
- Cereals require a separate protocol
- Still problems with captan, folpet, captafol
- Spices, tea, and oils give problems
- Matrix effects in complicated matrices
- Low recovery for fatty samples
- Even simpler sample prep possible Solutions by S. Lehotay published:  
Use  $\mu$ SPE with PAL3 System autosampler



Recovery drop with increased fat content!

## Compare to the classical cartridge SPE



### Classical SPE

- *Limited* selectivity
  - High sample and solvent volumes
  - Requires evaporation with  $N_2$
  - End volume  $\gg 100 \mu\text{L}$  in vial
- *Vacuum* operated
- *Drying* before elution
- *Evaporation step required*
  - Sample dilution
- *Manual operation*
  - Time consuming
  - Low sample throughput
  - Batch processing
- *No QA/QC*
  - As of manual operation



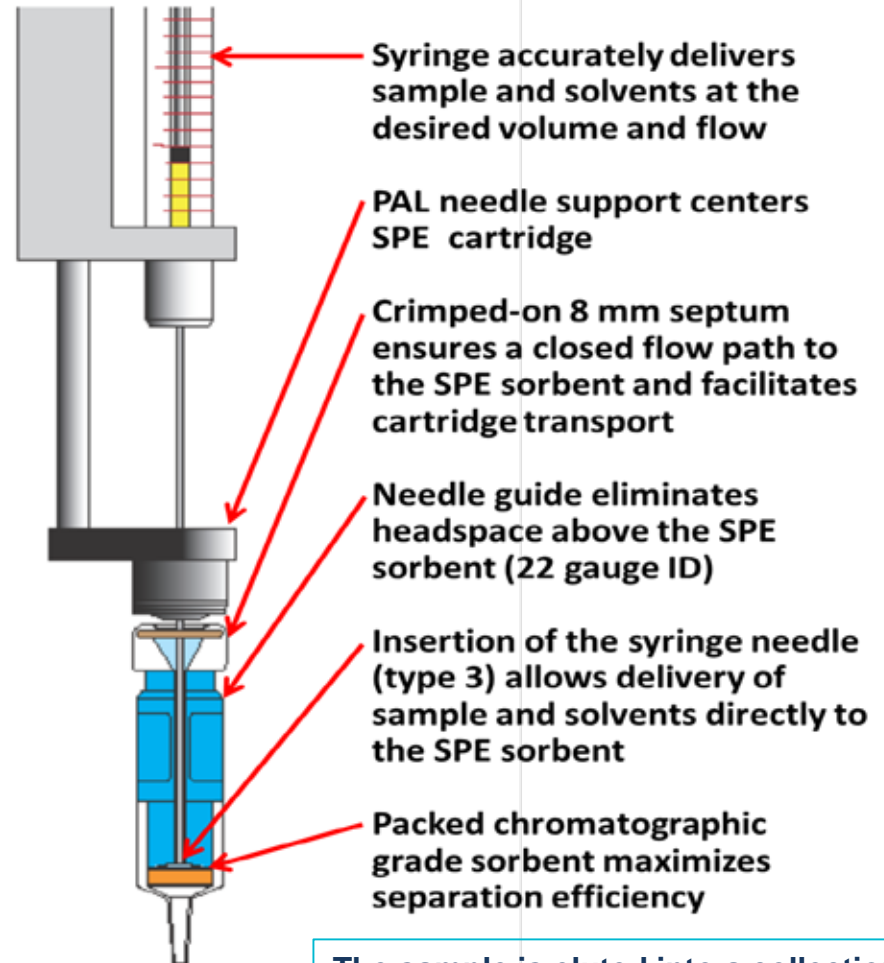
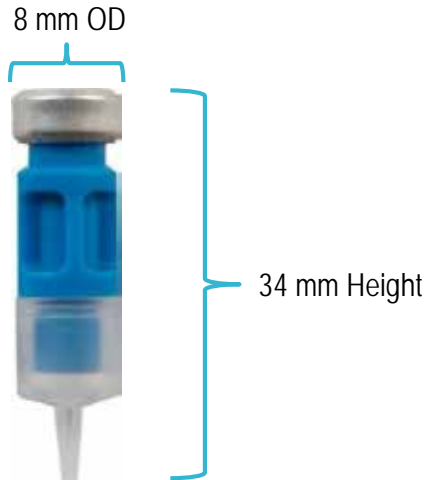
### $\mu$ SPE

- *High* selectivity
  - Controlled elution (flow/separation),
  - Sharp elution peak profile, no concentration
  - Final volume  $< 100 \mu\text{L}$  (or online)
- *Positive pressure* with liquid syringe
- *No drying* step
- No evaporation
  - Sample concentration maintained
- Walk away automation
  - Fast with  $< 10$  min
  - High productivity
  - Prep on chromatographic timescale
  - Online to LC-MS and GC-MS
- Traceable
  - Processing well documented



Controlled elution from low particle size sorbent bed

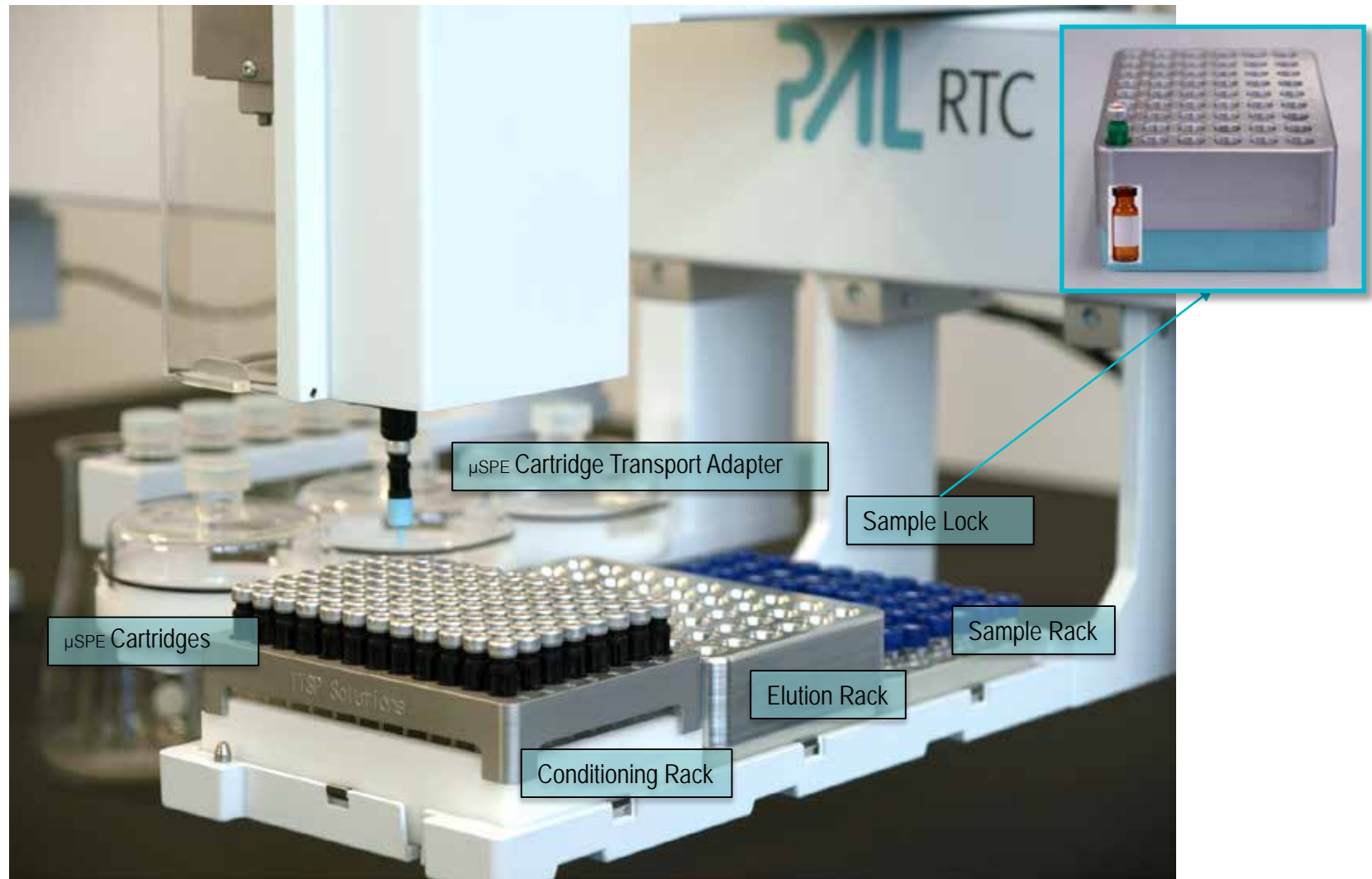
Solvent and sample flow rates are precisely controlled with the syringe plunger.



The sample is eluted into a collection plate or vial and then injected into the LC injection valve or GC inlet.



# PAL-RTC $\mu$ SPE QuEChERS clean-up workflow setup



## A fully automated clean-up workflow

10 g of homog. sample

➔ Add 10 mL Acetonitrile  
Add ISTD (Triphenylphosphate)

Salt out

➔ Add chemical kit, EN15662

Shake 15 min

Centrifuge

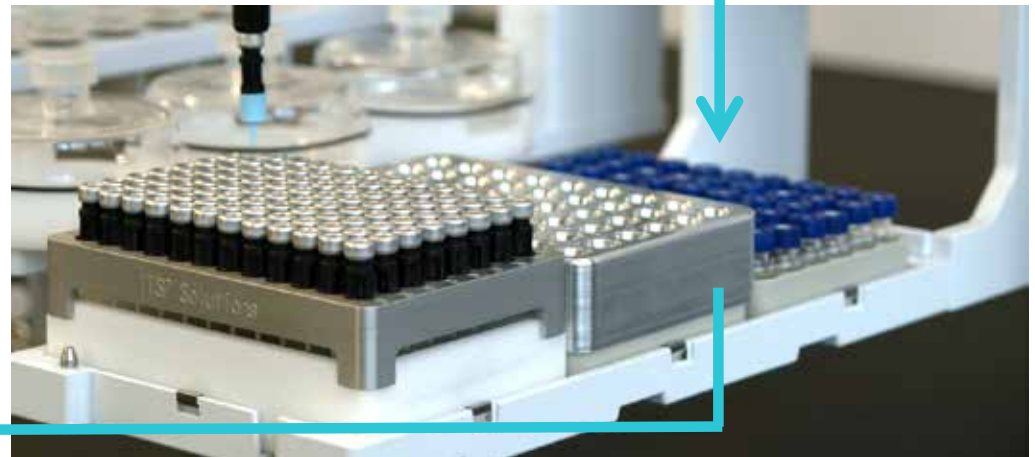
➔ Take 1 mL for  $\mu$ SPE clean-up

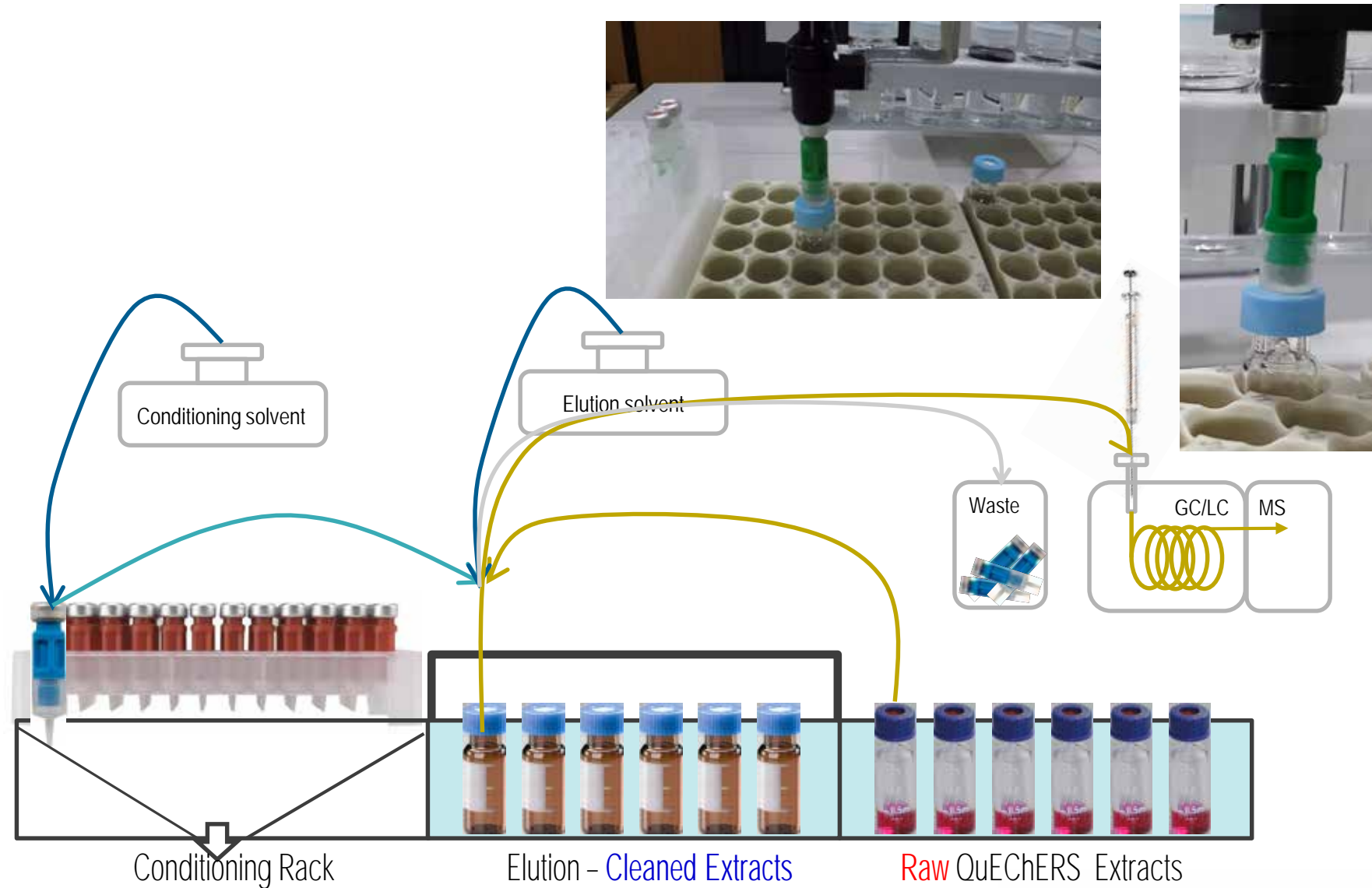
~~Dispersive SPE~~

~~Shake 1 min~~

~~Centrifuge~~

Automated Injection  
GC-MS and LC-MS





## PAL3 automated clean-up procedure\*

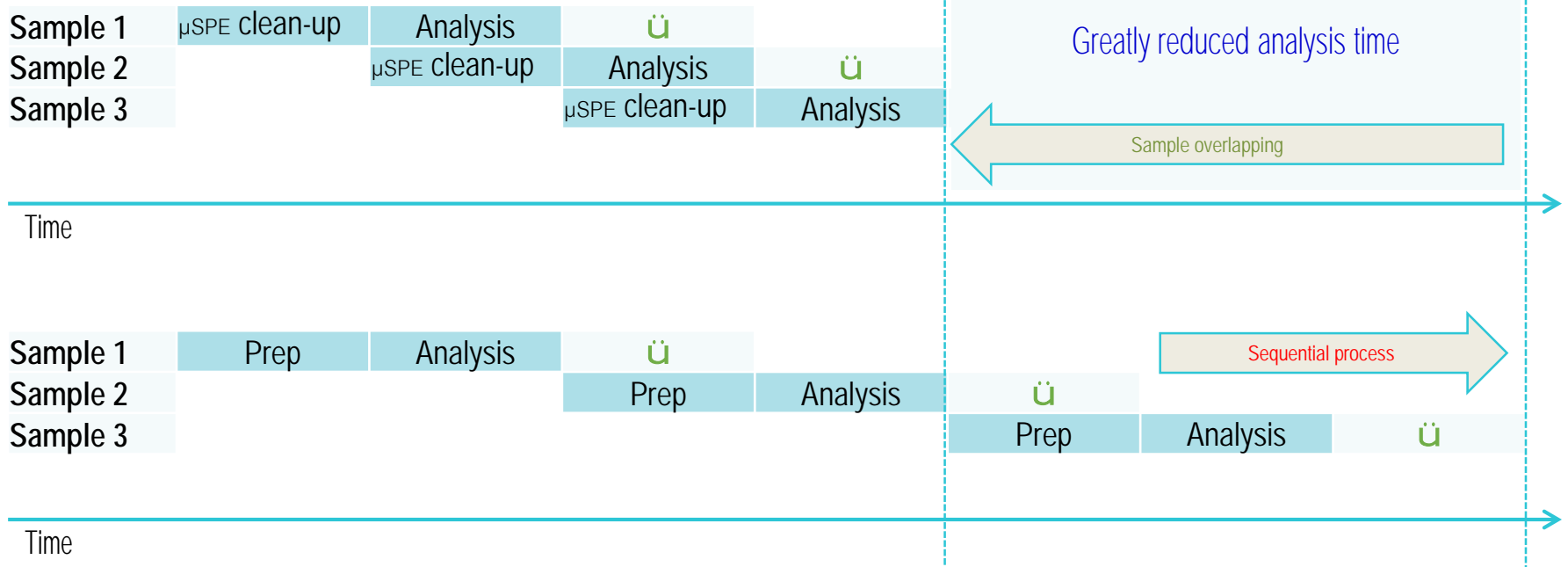
- All steps are program controlled, customizable
- 7 min to injection only - Prep-ahead clean-up while previous sample gets analyzed

Procedure step*	LCMS	GCMS
Clean syringe with elution solvent		
Condition $\mu$ SPE cartridge in the conditioning rack	150 $\mu$ L	200 $\mu$ L
Transfer cartridge to the elution rack		
Load QuEChERS extract from the sample vial onto the cartridge**	150 $\mu$ L	100 $\mu$ L
Clean syringe with elution solvent		
Elute the cartridge with elution solvent	150 $\mu$ L	150 $\mu$ L
Collected eluents in 2 mL vial, total volume:	300 $\mu$ L	250 $\mu$ L
Discard cartridge to waste baker		
LCMS: Dilute combined extract and mix with syringe	1200 $\mu$ L	
GCMS: Add analyte protectant solution		30 $\mu$ L
GCMS: Add Ethyl Acetate and mix with syringe		250 $\mu$ L
Inject to GCMS or LCMS	10 $\mu$ L	3 $\mu$ L

\* As of Bruce D. Morris and Richard B. Schriener, J. Agric. Food Chem. 2015, 63, 5107–5119. \*\* Lehotay Han apply 300  $\mu$ L raw extract, no separate elution required

Sample prep and analysis in parallel

Prep-ahead mode!



The PAL3 performs sample prep and analysis in parallel. As a result, no time is lost in the continuous analysis of samples requiring HS sampling or other time-consuming pretreatments. The MS units works in maximum duty cycle.

## References

- Morris, Schriener 2014 - Eliminating the need for Matrix-matched Calibration Standards\_QuEChERS Cleanup Poster
- Morris, Schriener 2015 – Automated Column SPE Cleanup of QuEChERS Extracts Using a Zirconia-Based Sorbent for Pesticide Residue Analyses by LC-MS/MS, J Agriculture Food Chem
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- Lehotay et al. 2016 - Automated Mini-Column SPE Cleanup for High-Throughput Analysis of Chemical Contaminants in Foods by GC-MS/MS, Chromatographia, DOI 10.1007/s10337-016-3116-y.

Automated micro-SPE Clean-up of QuEChERS Extracts for Multi-Residue Pesticide Analysis

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**Abstract** This study demonstrated the application of an automated high-throughput mini-cartridge solid-phase extraction (mini-SPE) cleanup for the rapid low-pressure gas chromatography—tandem mass spectrometry (LPGC-MS/MS) analysis of pesticides and environmental contaminants in QuEChERS extracts of foods. Cleanup efficiencies and breakthrough volumes using different mini-SPE sorbents were compared using avocado, salmon, pork loin, and kale as representative matrices. Optimum extract load volume was 500  $\mu\text{L}$  for the 45 mg mini-cartridge containing 20% (w/w) and 40% (w/w)  $\text{MgSO}_4 \cdot 2\text{H}_2\text{O}$  (primary secondary amine)/ $\text{C}_{18}$ /CarbonX sorbents used in the final method. In method validation to demonstrate high-throughput capabilities and performance trends, 230 spiked extracts of 10 different foods (apple, kiwi, carrot, kale, orange, black olive, wheat grain, dried bean, pork, and salmon) underwent automated mini-SPE cleanup and analysis over the course of 5 days. In all, 325 analyses for 54 pesticides and 43 environmental contaminants (1 analyzed together) were conducted using the 10 min LPGC-MS/MS method without changing the liner or refueling the instrument. Merely, 1 mg equivalent sample injected achieved  $0.1 \mu\text{g g}^{-1}$  limits of quantification. With the use of internal standards, method validation results showed that 91 of the 94 analytes including pairs achieved satisfactory results (70–120 % recovery and RSD  $\leq 25\%$ ) in the 10 tested food matrices ( $n = 160$ ). Matrix effects were typically less than  $4\pm 20\%$ , mainly due to the use of analyte protectants, and minimal human review of software data processing was needed due to summation function integration of analyte peaks. This study demonstrated that the automated mini-SPE + LPGC-MS/MS method yielded accurate results in rugged, high-throughput operations with minimal labor and data review.

**Keywords** High-throughput automation · Solid phase extraction cleanup · Pesticide residue analysis · QuEChERS sample preparation · Fast GC-MS/MS · Analyte protectants · Environmental contaminants · Foods

**Introduction**

Trade of food products continues to increase globally [1], which is leading to greater food safety concerns [2, 3], and recent legislation [4] places greater emphasis on a higher rate of monitoring by private as well as regulatory laboratories to test for pesticide residues and other contaminants in the commodities. However, the cost of monitoring adds to the price of the food to the consumer, and delays in the analysis of perishable items reduces shelf life and sales of the product. Yet, more pesticides are being registered monthly for different crops worldwide [5], while human health and

# Automated workflow for the determination of fatty acid methyl esters (FAME) in fat and fat containing food samples

The analysis of oils, fat and fat containing food via fatty acid methyl esters (FAME) is a common task in governmental, quality control (QC) or contract research laboratories (CRO). Most often the samples are processed manually, which is labor intensive and exposes the lab personnel to potentially hazardous chemicals

การเตรียมตัวอย่างแบบอัตโนมัติ  
ด้วยเครื่อง PAL-RTC  
สำหรับการวิเคราะห์ FAMES ใน  
ตัวอย่างไขมัน และไขมันในอาหาร  
โดยใช้ Sodium-Methoxide  
ในกระบวนการ  
Transesterification  
ก่อนทำการวิเคราะห์ด้วยเครื่อง GC-FID

# Automated workflow for the determination of fatty acid methyl esters (FAME) in fat and fat containing food samples



Heat 90 - 100 °C



200 **mL** Extracted sample or 20 **mL** oil sample +  
Methanol : Hexane (4:1) 2 mL + 200 **mL** Acetyl Chloride



Cool down at  
room temperature



Add 6%  $K_2CO_3$  5 mL



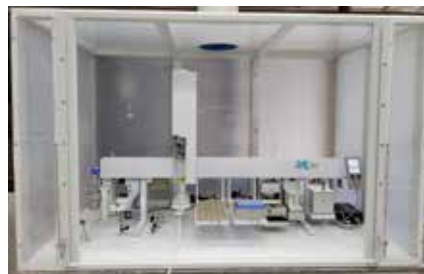
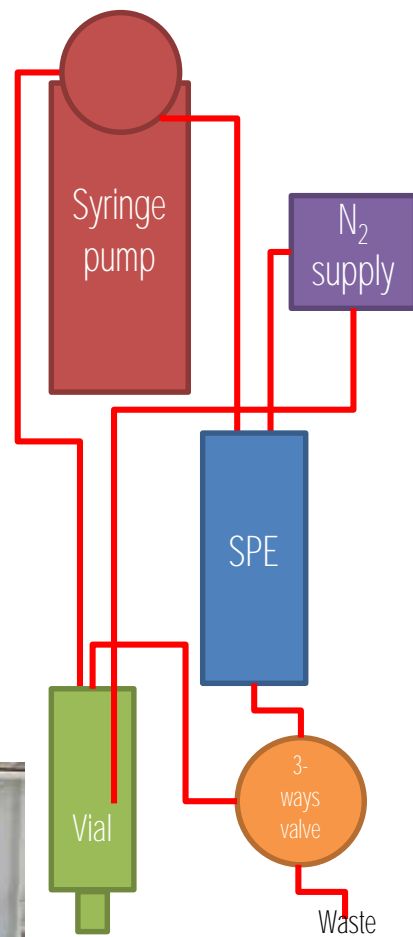
Transfer the supernatant  
(hexane) to vial



Inject into GC



# Automated workflow: large volume sample clean up and pre-concentration for Nitrosamine determination in rubber samples

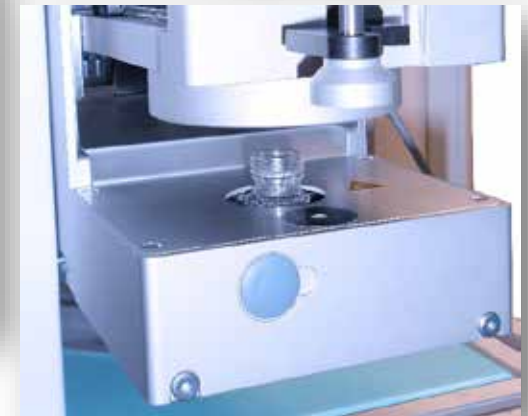


- Load sample through SPE (25 mL)
- ↓ ~ 1 mL/min.
- Flush SPE with N<sub>2</sub>
- ↓
- Rinse sample container with 30 mL DCM
- ↓
- Elute SPE with 30 mL DCM and collect
- ↓ ~ 1 mL/min.
- Rinse sample container with 30 mL DCM
- ↓
- Elute SPE with 30 mL DCM and collect
- ↓ ~ 1 mL/min.
- Flush SPE with N<sub>2</sub>
- ↓
- Add 1 mL Ethanol in collection vial
- ↓
- Concentrate sample with N<sub>2</sub> blow down

PAL SYSTEM



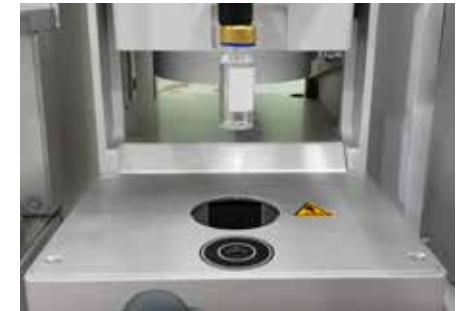
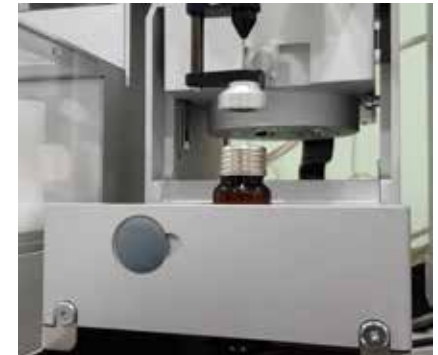
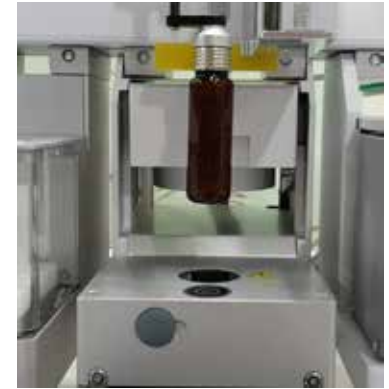
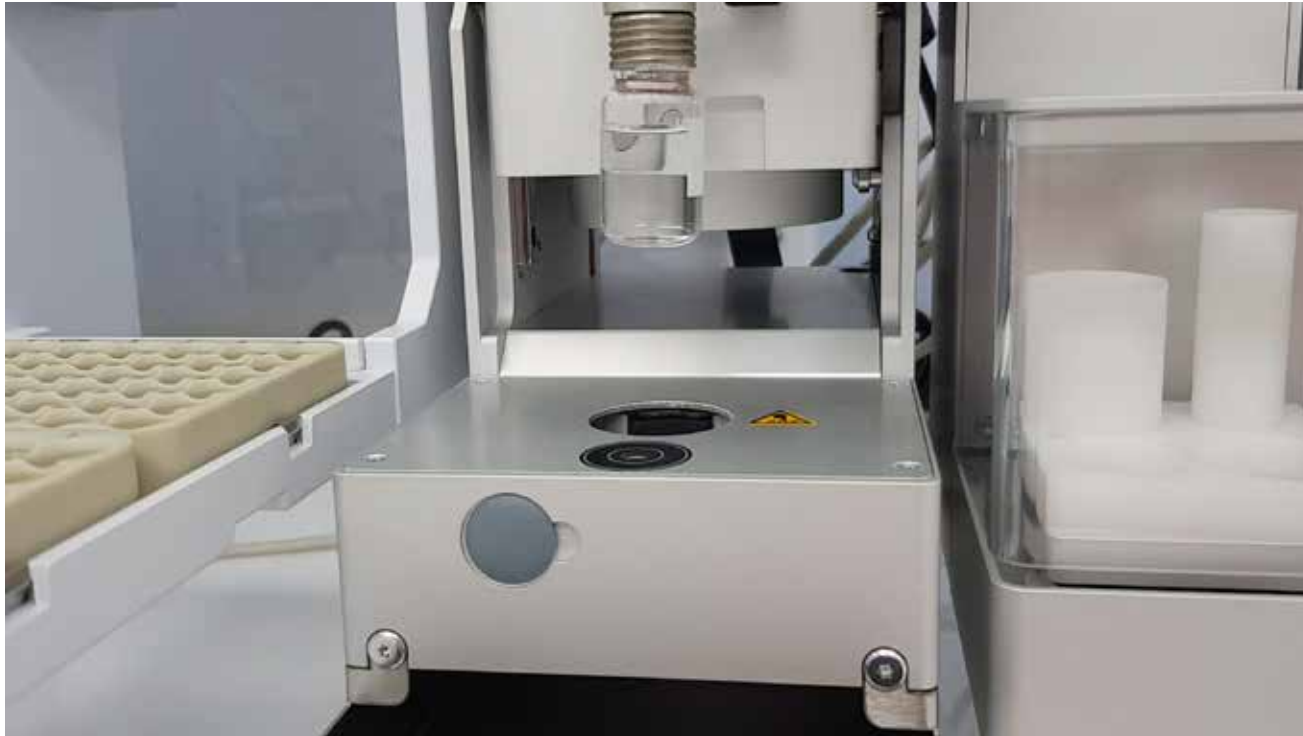
## New tools



μSPE



Decapper and Pipette tool in action



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