Innovative sample preparation technology to reduce bottleneck in a measurement process

Mahitti Puanngam

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?

Obtain a Representative Sample

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

Prepare the Sample for Analysis

- Solid, liquid, or gas?
- Dissolve?
- Ash or digest?
- Distillation
- Precipitation
- Solvent extraction
- 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?

Perform the Measurement

Calculate the Results and Report

The Challenge for Analysis

GC / LC Vial

How do we get analytes out of these samples?

Solvent Extraction

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.

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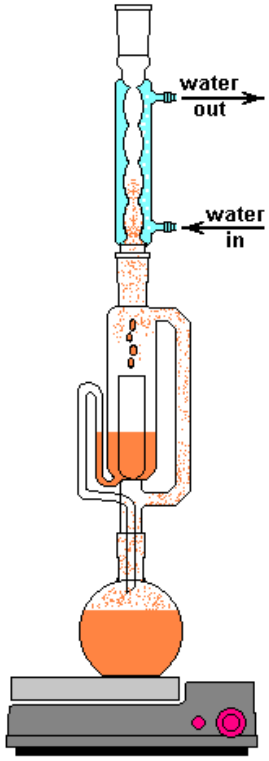
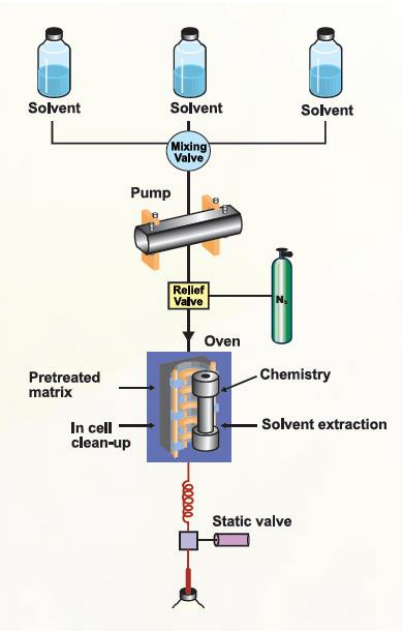
Soxhlet extraction



Wikipedia

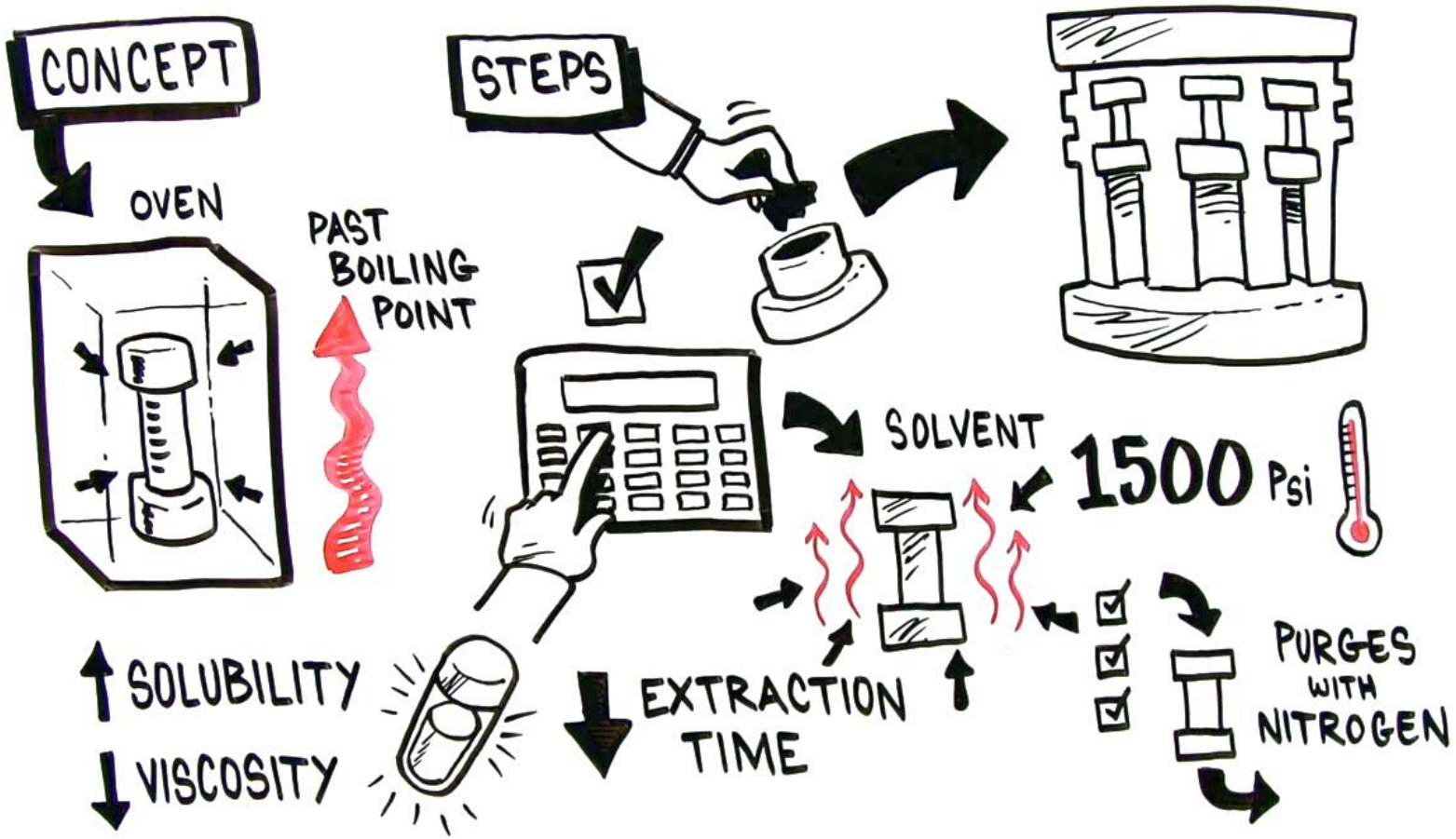
Accelerated and Microwave-Assisted Extraction



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

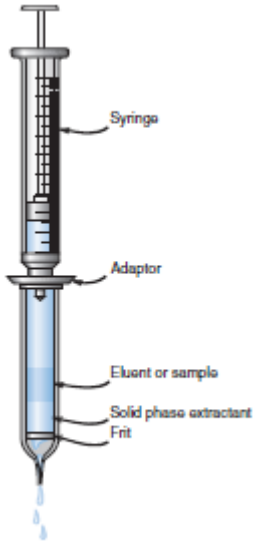


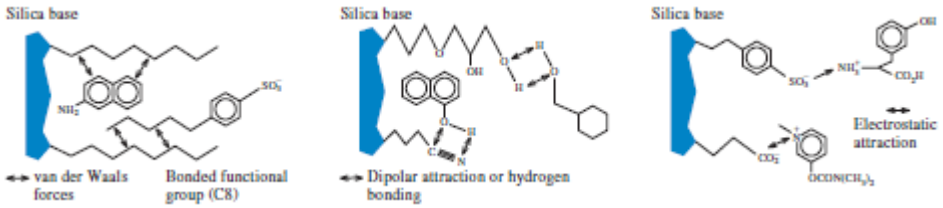
Solid-Phase Extraction

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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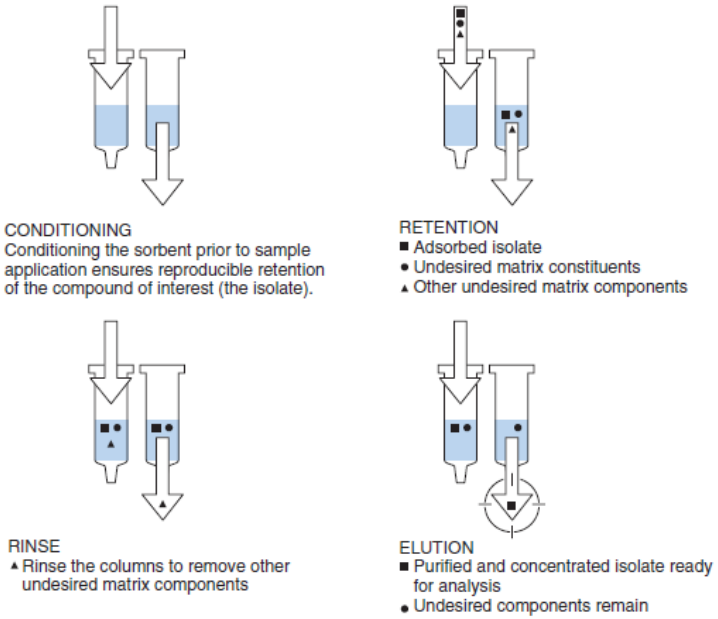
Automated Solid-Phase Extraction

Solid-Phase Extraction

Typical sequence in a solid-phase extraction.



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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.

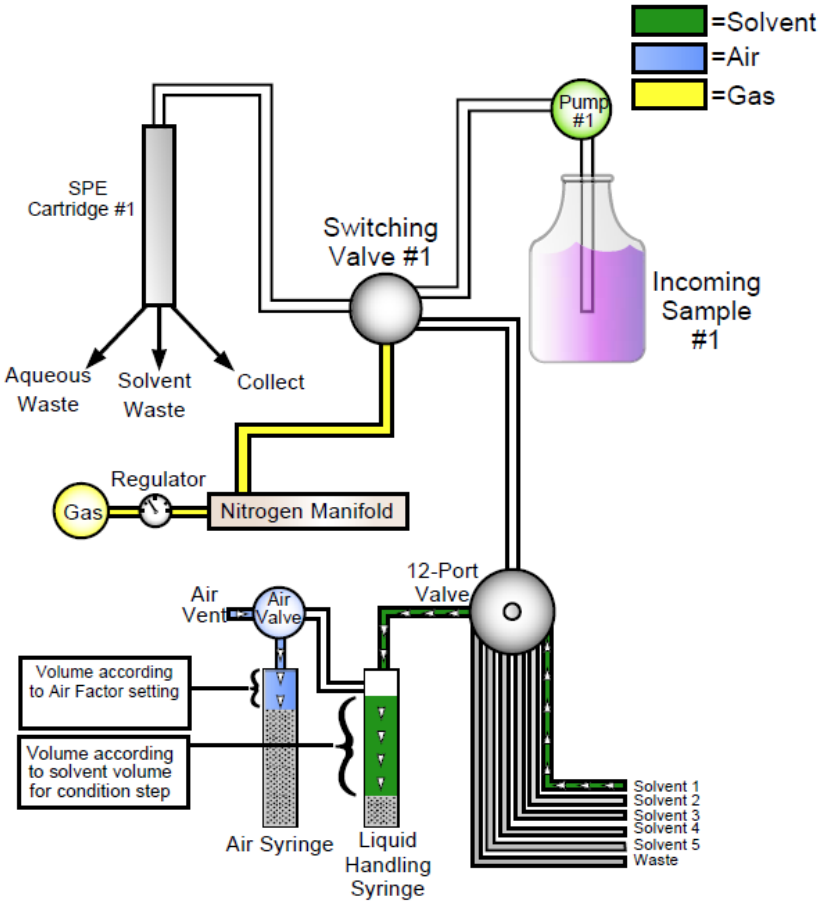
Before a solid sample can be processed by SPE, it must first be extracted by soxhlet extraction, pressurized fluid extraction (ASE), or other method, to produce a solution that contains the analytes of interest.

More details about SPE

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

Automated Solid-Phase Extraction

Automated Solid-Phase Extraction



Solvent Evaporation

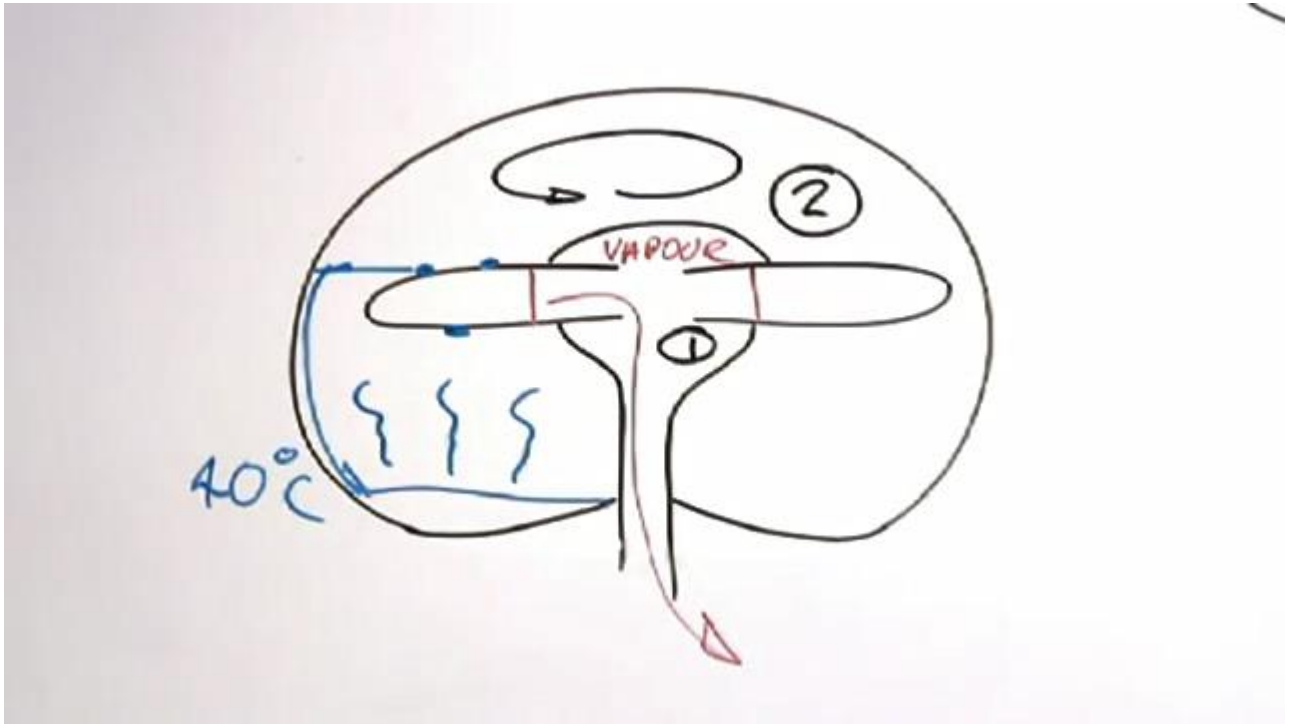
Solvent Evaporation

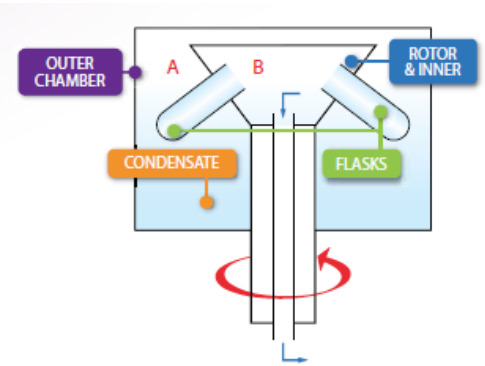
After extraction and cleanup process, remaining solvent typically larger than required volume. In order to obtain better sensitivity of analysis, solvent have to be reduced.

Centrifugal Evaporators

The Rocket Evaporator

Sample evaporation with walkaway capability.





Solid-Phase MicroExtraction

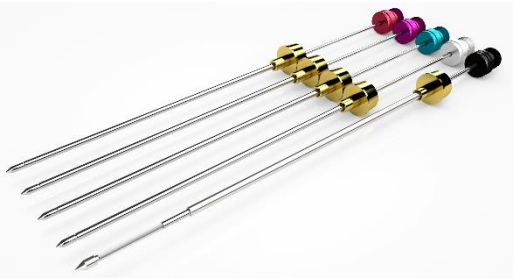
Solid-Phase MicroExtraction (SPME)

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 μm to 100 μm 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[a]

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

[a]Information adapted from Supelco application note



"like dissolves like"

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Solid-Phase MicroExtraction

HiSorb, sorptive extraction

An innovative, labour-saving sampling system for the analysis of volatile and semi-volatile organic compounds (VOCs and SVOCs) in liquids and solids by TD–GC–MS.

Simple workflow for maximum productivity

1

Probe insertion:
Two probe lengths allow immersive or headspace sampling in 20 or 10 mL vials.

2

Analyte extraction:
The HiSorb Agitator efficiently mixes and heats the sample.

3

Probe washing:
Probes are washed and dried to remove residual matrix.

4

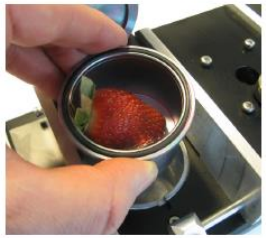
Analysis:
The HiSorb probe is inserted into a standard TD tube for analysis by TD–GC–MS.

Micro-Chamber / Thermal Extractor

Fast and flexible sampling of chemicals and odours released from materials and foods

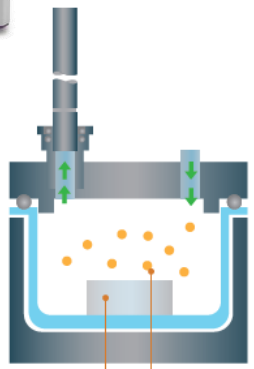
Simultaneously collect volatile and semi-volatile organic compounds (VOCs and SVOCs) from up to six samples

1 Load the material



2 Set the conditions

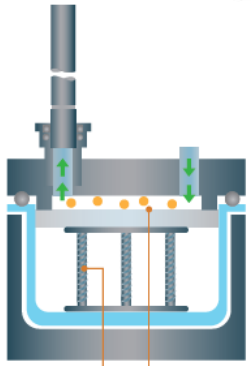
3 Collect the volatiles



Samples are placed straight into the chambers.

Vapours swept from the entire sample are collected.

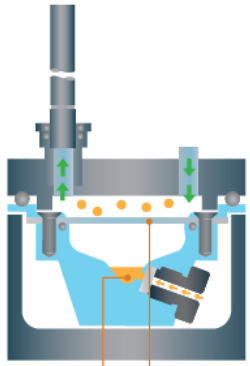
Bulk emissions testing



Sprung spacers raise planar samples to the top of the chamber.

A seal forms when the lid is closed, so only vapours released from the sample's surface are collected.

Surface emissions testing



Liquid samples are injected through a septum into the well under a sealed sample of test material.

Vapours diffuse through the test material into the chamber.

Permeation testing

Thermal Desorption

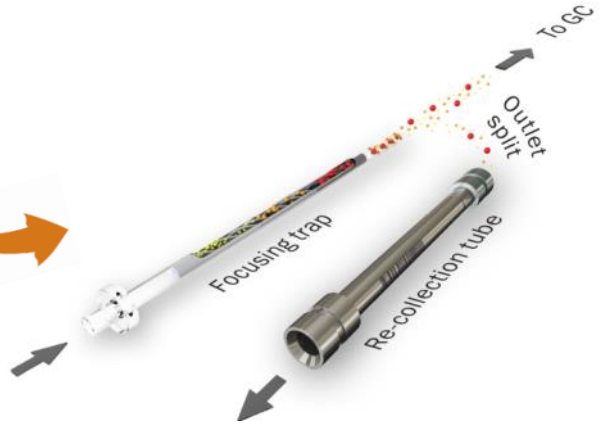
Thermal desorption (TD) is the process of heating a material to release adsorbed compounds from it.

As an analytical method, TD is used as a pre-concentration technique for gas chromatography (GC), making GC compatible with low-concentration analytes that would otherwise be impossible to detect with this method.

Tube desorption and inlet split

Sample tube heated in a flow of carrier gas and analytes swept onto an electrically cooled focusing trap, typically held between Ambient and –30°C.

Trap desorption and outlet split



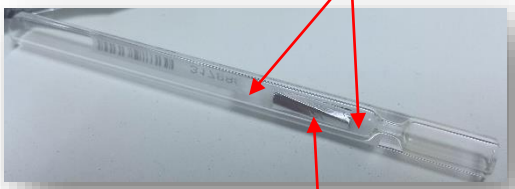
Focusing trap rapidly heated (up to 100°C/s) in a reverse flow of carrier gas (‘backflush’ operation), to transfer the analytes to the GC column.

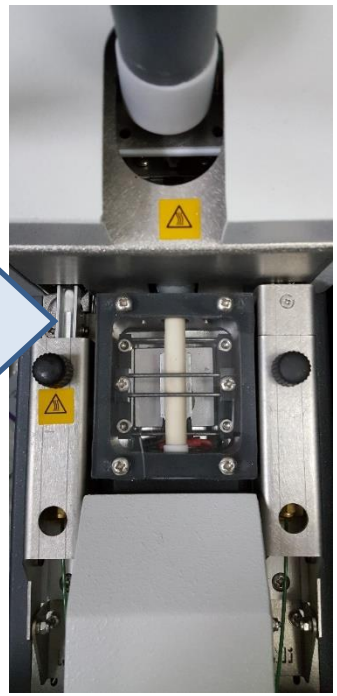
Thermal Desorption (Direct desorption)

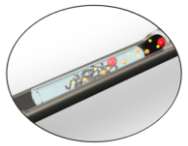
Direct desorption (Dynamic Headspace)

Provides a ‘gas extraction’ or ‘dynamic headspace’ alternative to conventional solvent extraction

Weight sample





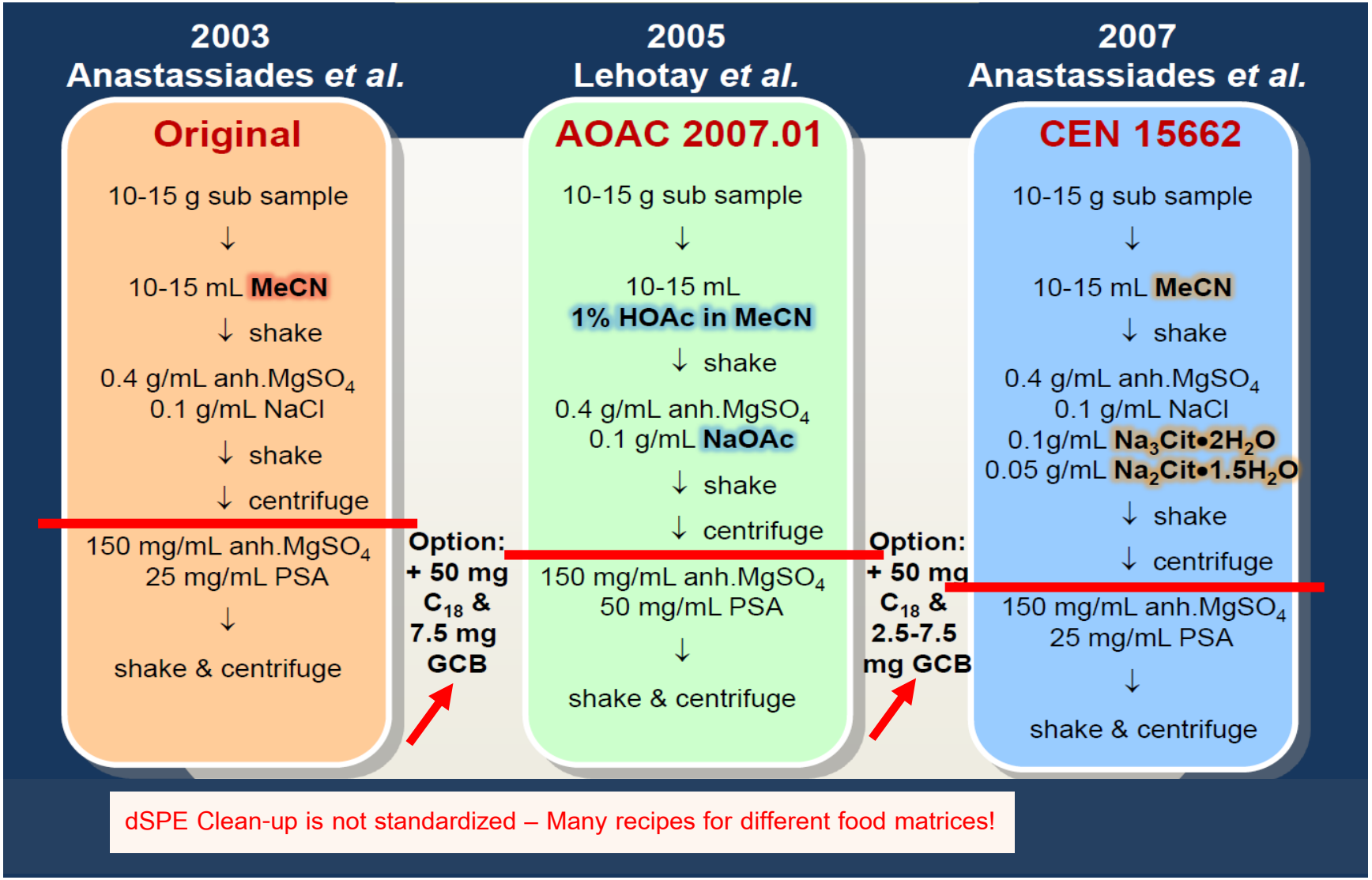


Your Scientific Specialist

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.

QuEChERS Methods – AOAC vs. CEN



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

Current Limitations of QuEChERS

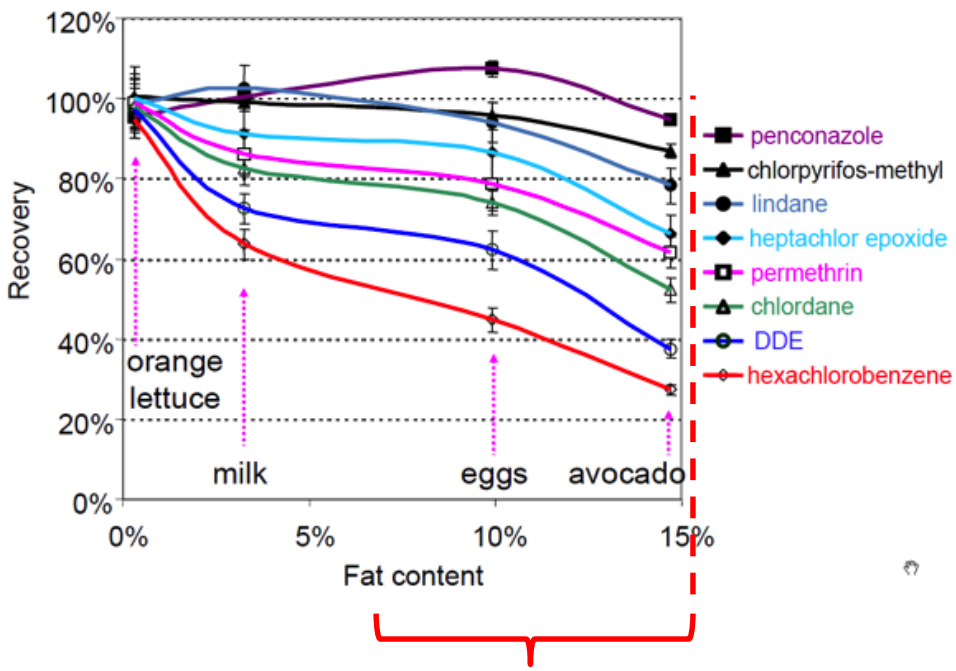
As reported by Steven Lehotay

- 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 µSPE with PAL3 System autosampler



Recovery drop with increased fat content!

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

Automated µSPE Clean-up of QuEChERS Extracts

for online GC-MS and LC-MS Multi Residue Pesticide Analysis

Compare to the classical cartridge SPE

Classical SPE

- *Limited* selectivity
  - High sample and solvent volumes
  - Requires evaporation with N_2
  - End volume >>100 µL 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

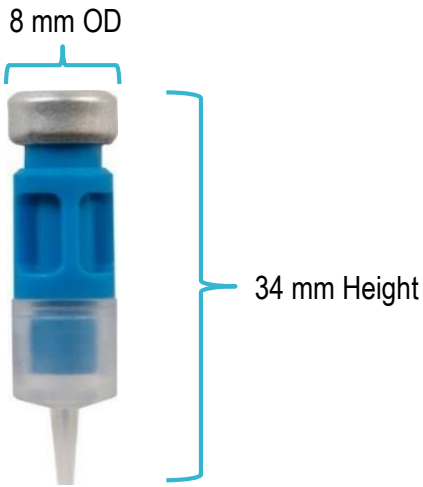
µSPE

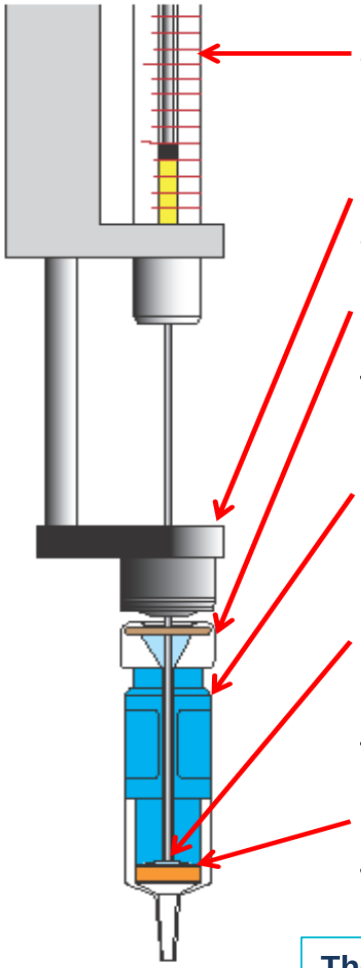
- *High* selectivity
  - Controlled elution (flow/separation),
  - Sharp elution peak profile, no concentration
  - Final volume < 100 µL (or online)
- *Positive pressure* with liquid syringe
- *No drying* step
- No evaporation
  - Sample concentration maintained
- Walk away automation
  - Fast with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

Automated µSPE

Controlled elution from low particle size sorbent bed

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





Syringe accurately delivers sample and solvents at the desired volume and flow

PAL needle support centers SPE cartridge

Crimped-on 8 mm septum ensures a closed flow path to the SPE sorbent and facilitates cartridge transport

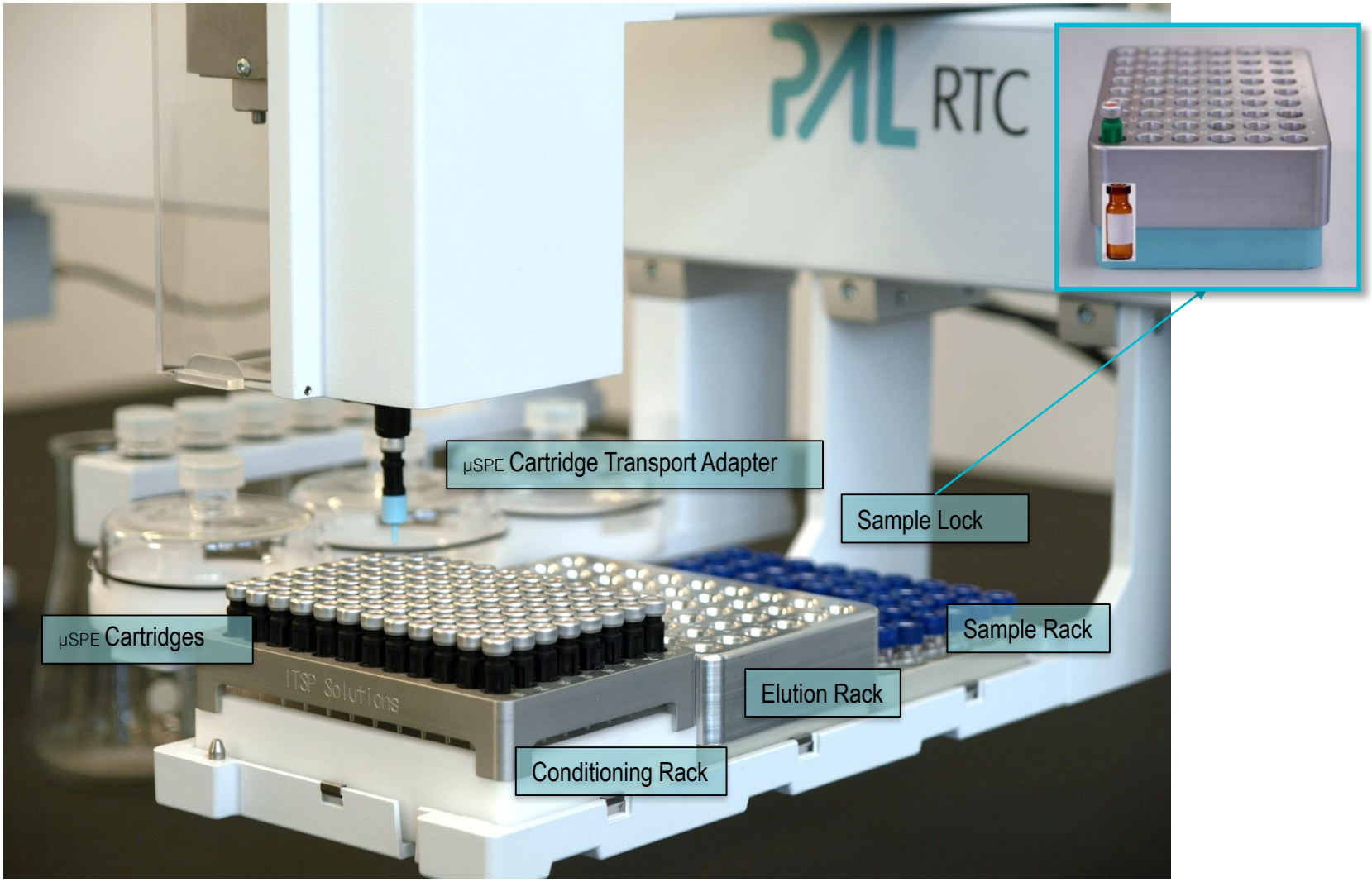
Needle guide eliminates headspace above the SPE sorbent (22 gauge ID)

Insertion of the syringe needle (type 3) allows delivery of sample and solvents directly to the SPE sorbent

Packed chromatographic grade sorbent maximizes separation efficiency

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

PAL-RTC µSPE QuEChERS clean-up workflow setup



PAL-RTC µSPE QuEChERS clean-up workflow

A fully automated clean-up workflow

10 g of homog. sample → Add 10 mL Acetonitrile
Add ISTD (Triphenylphosphate)

Salt out → Add chemical kit, EN15662

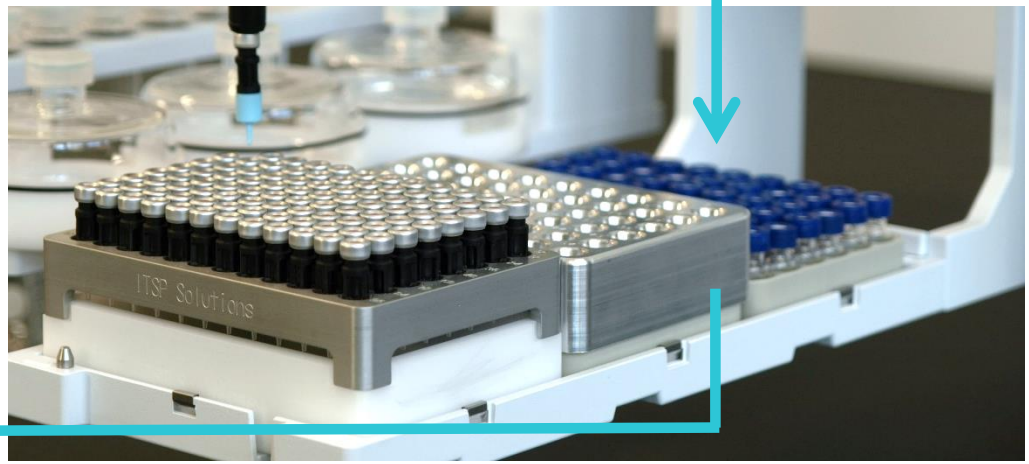
Shake 15 min

Centrifuge → Take 1 mL for µSPE clean-up

~~Dispersive SPE~~

~~Shake 1 min~~

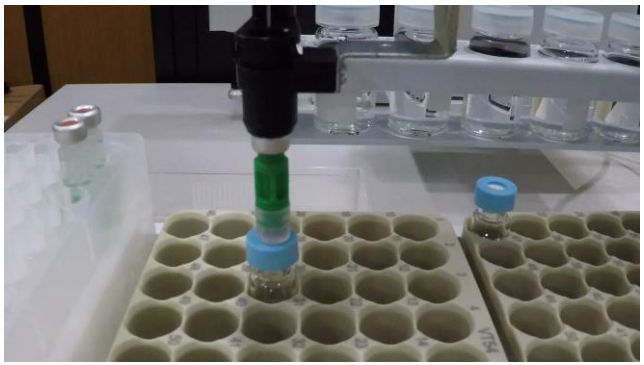
~~Centrifuge~~

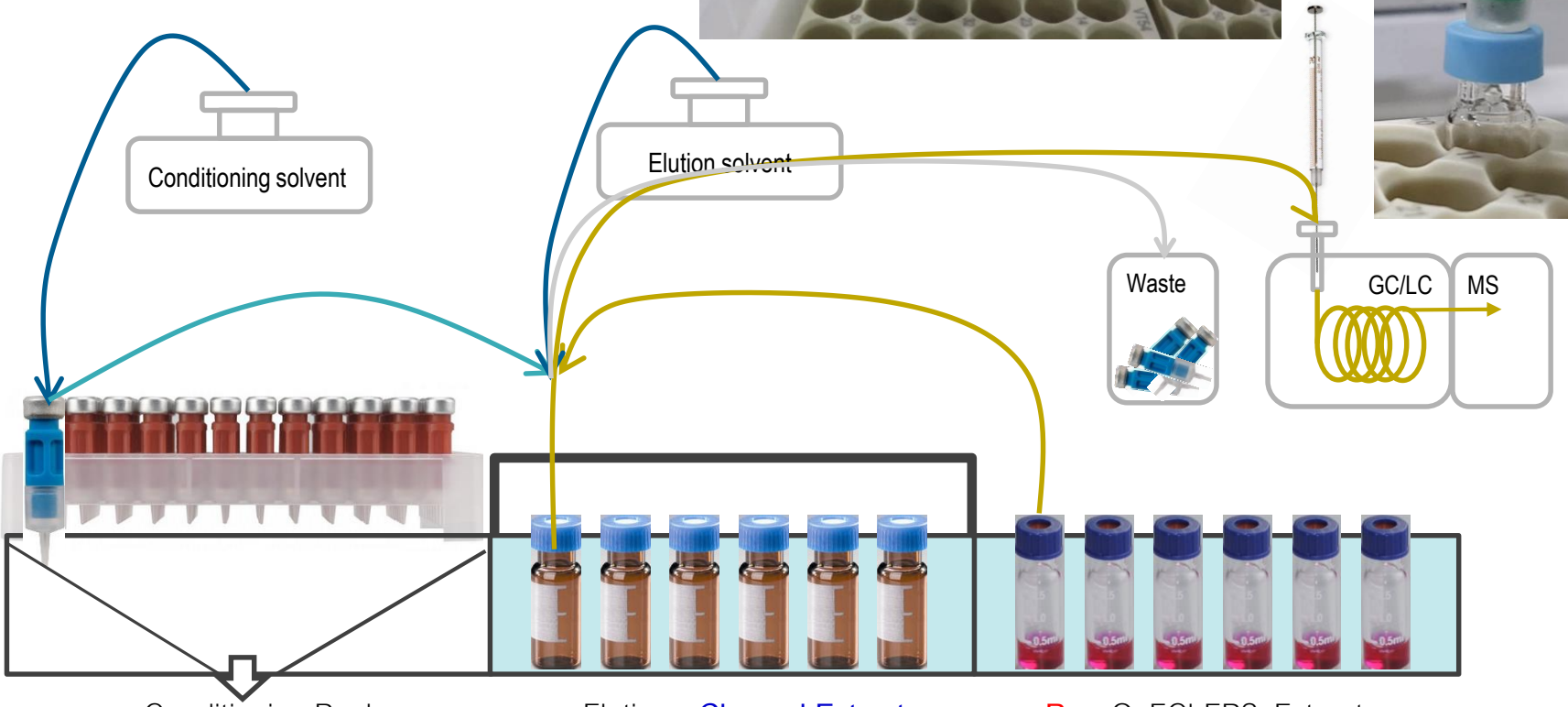


Automated Injection
GC-MS and LC-MS

Automated Clean-up Workflow

Step-by-step operation





Automated Clean-up Workflow

PAL3 automated clean-up procedure*

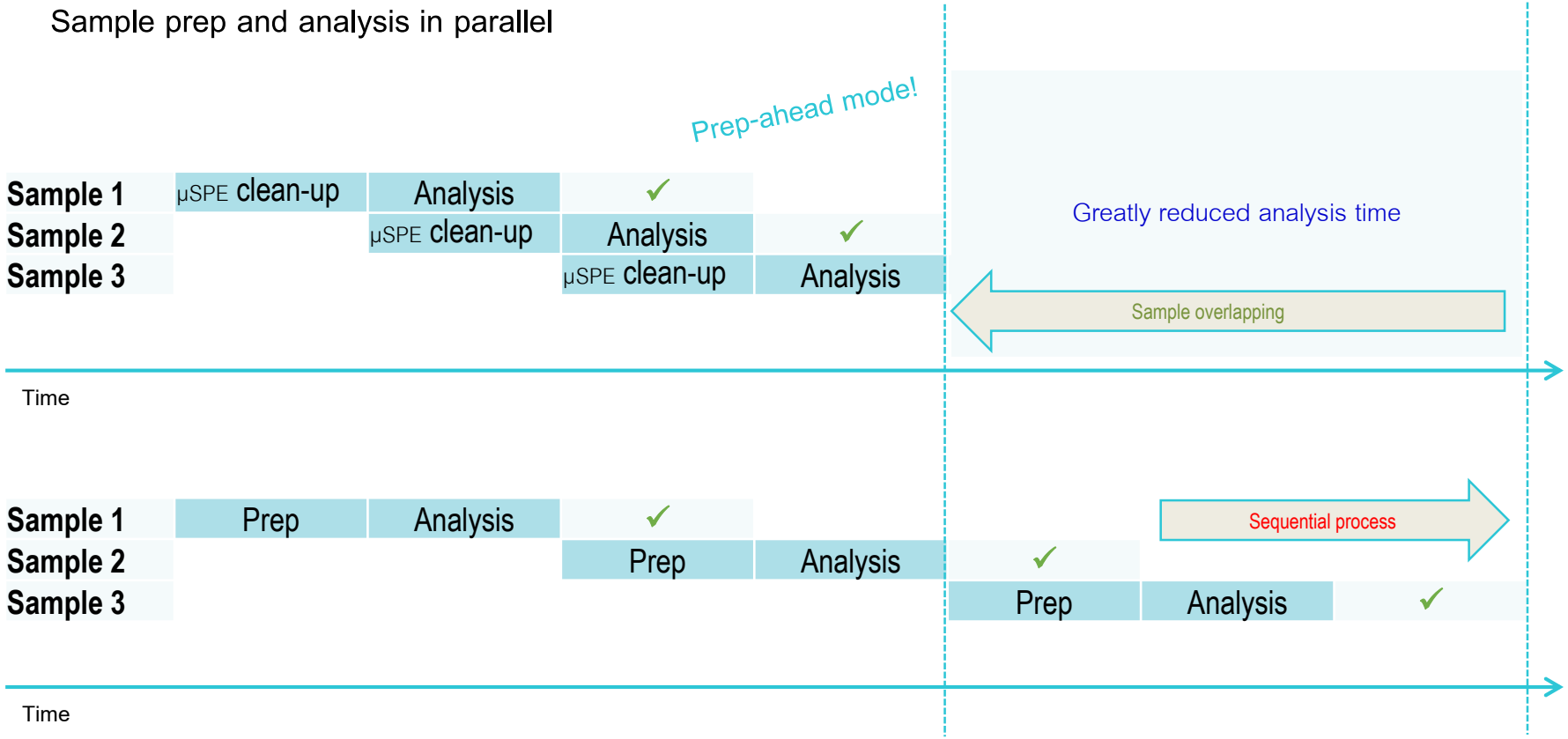
- All steps are program controlled, customizable
- 7 min to injection only - Prep-ahead clean-up while previous sample gets analyzed

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

* As of Bruce D. Morris and Richard B. Schriner, J. Agric. Food Chem. 2015, 63, 5107−5119. ** Lehotay Han apply 300 µL raw extract, no separate elution required

Automated Clean-up Workflow

Prep-ahead for Increased Throughput



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.

Automated QuEChERS Extract Clean-up

References

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

Chromatographia
DOI 10.1007/s10337-016-3116-y

ORIGINAL

Automated Mini-Column Solid-Phase Extraction Cleanup for High-Throughput Analysis of Chemical Contaminants in Foods by Low-Pressure Gas Chromatography—Tandem Mass Spectrometry

Steven J. Lehotay · Lijun Han · Yelena Sapozhnikova

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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 300 µL for the 45 mg mini-cartridges containing 20/12/12/1 (w/w/w/w) anh. $MgSO_4$/PSA (primary secondary amine)/C_{18}/CarbonX sorbents used in the final method. In method validation to demonstrate high-throughput capabilities and performance results, 230 spiked extracts of 10 different foods (apple, kiwi, carrot, kale, orange, black olive, wheat grain, dried basil, 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 (3 analyzed together) were conducted using the 10 min LPGC-MS/MS method without changing the liner or retuning the instrument. Merely, 1 mg equivalent sample injected achieved <5 ng 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 ≤ 25 %) in the 10 tested food matrices (n = 160). Matrix effects were typically less than ±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

Mention of brand or firm name does not constitute an endorsement by the US Department of Agriculture above others of a similar nature not mentioned. USDA is an equal opportunity provider and employer.

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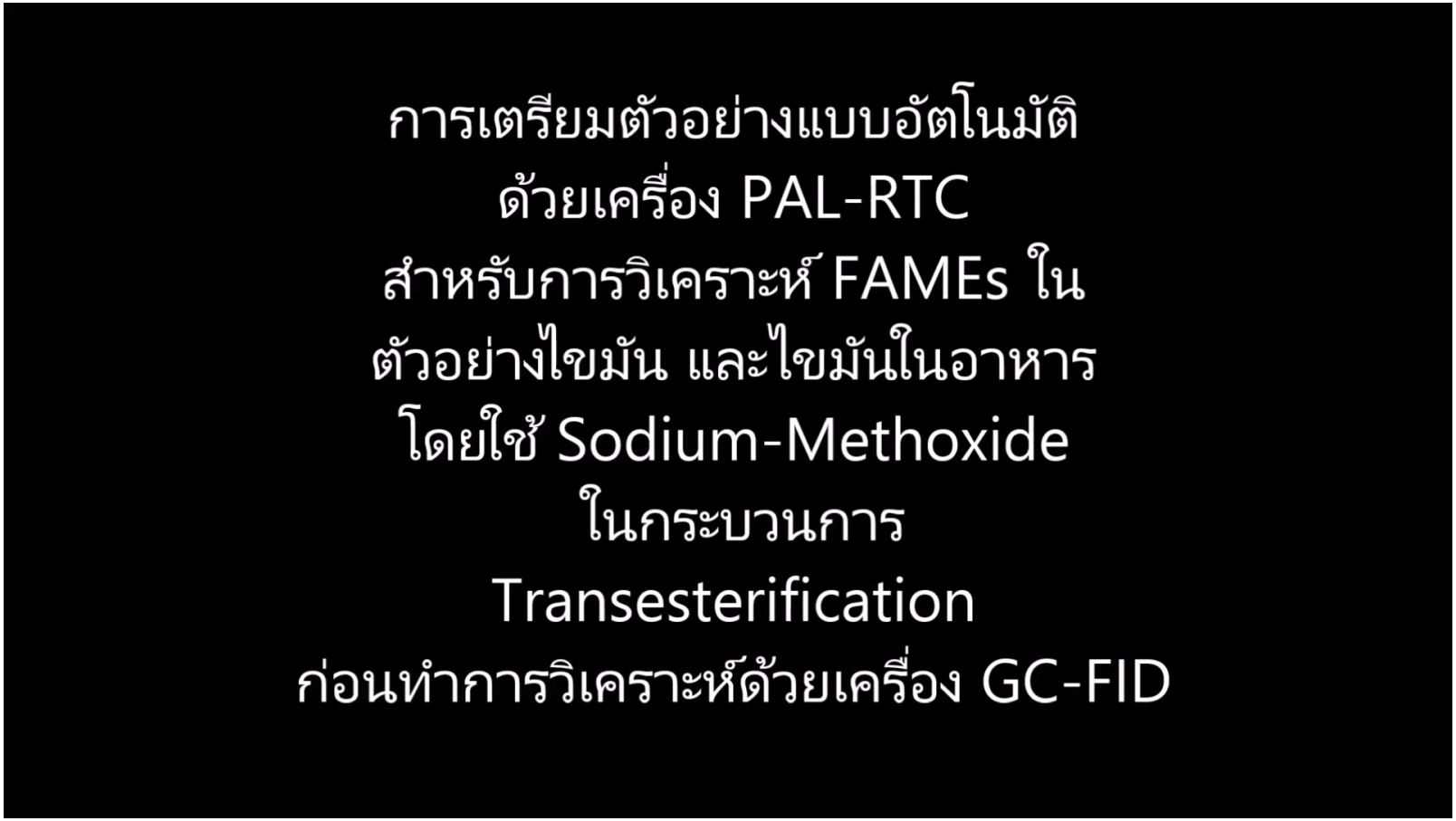
Springer

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



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

200 µL Extracted sample or 20 µL oil sample +

Methanol : Hexane (4:1) 2 mL + 200 µL Acetyl Chloride

Heat 90 – 100 °C

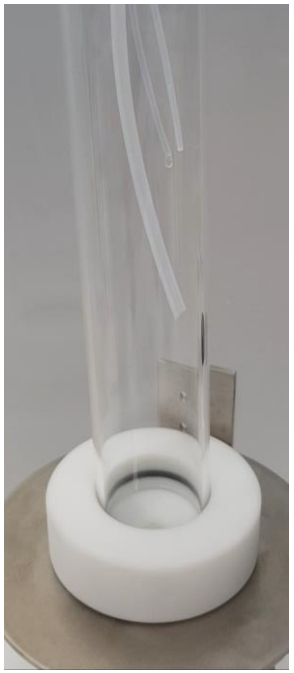
Cool down at room temperature

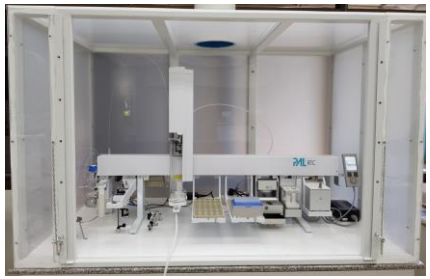
Add 6% K_2CO_3 5 mL

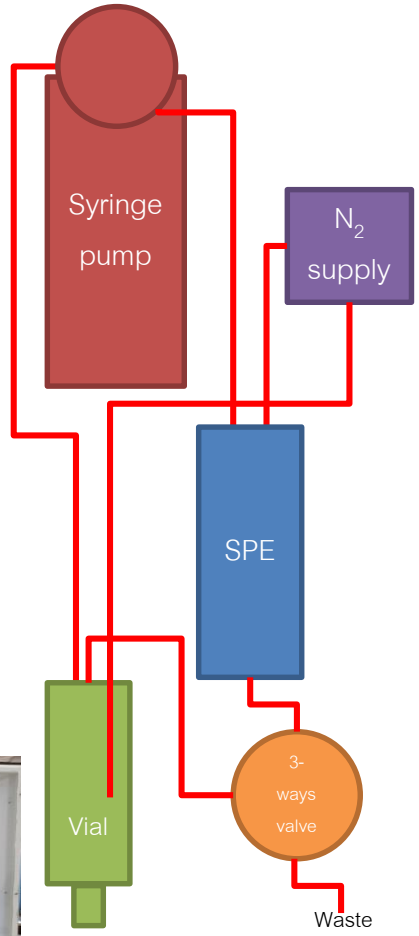
Transfer the supernatant (hexane) to vial

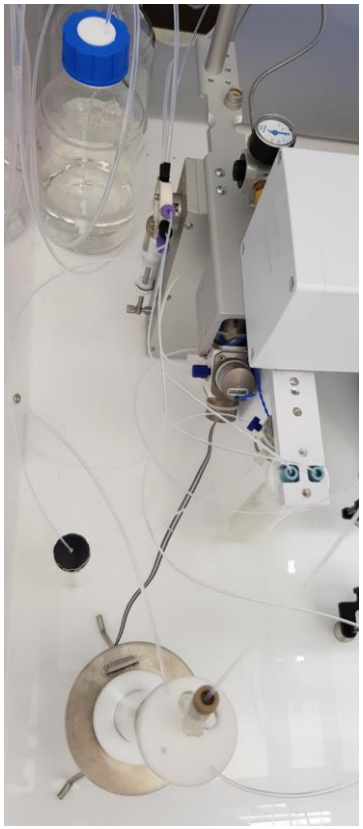
Inject into GC

Automated workflow: large volume sample clean up and preconcentration for Nitrosamine determination in rubber samples









Load sample through SPE (25 mL)

↓ ~ 1 mL/min.

Flush SPE with N_2

↓

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_2

↓

Add 1 mL Ethanol in collection vial

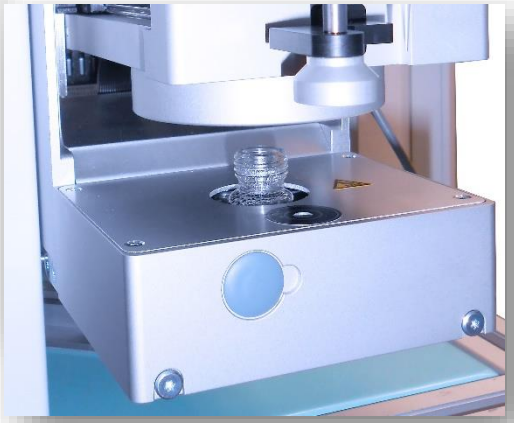
↓

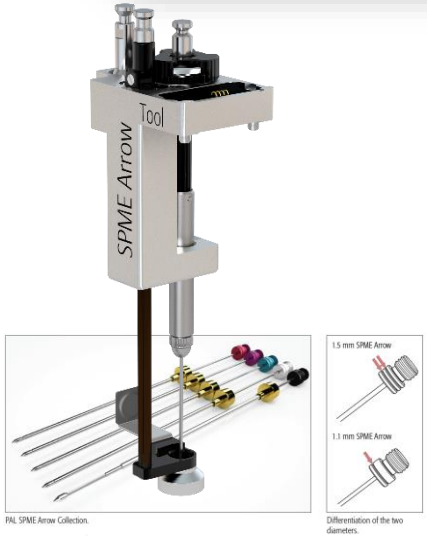
Concentrate sample with N_2 blow down

Introduction to PAL-RTC for automated sample preparation

Introduction to PAL-RTC for automated sample preparation

New tools

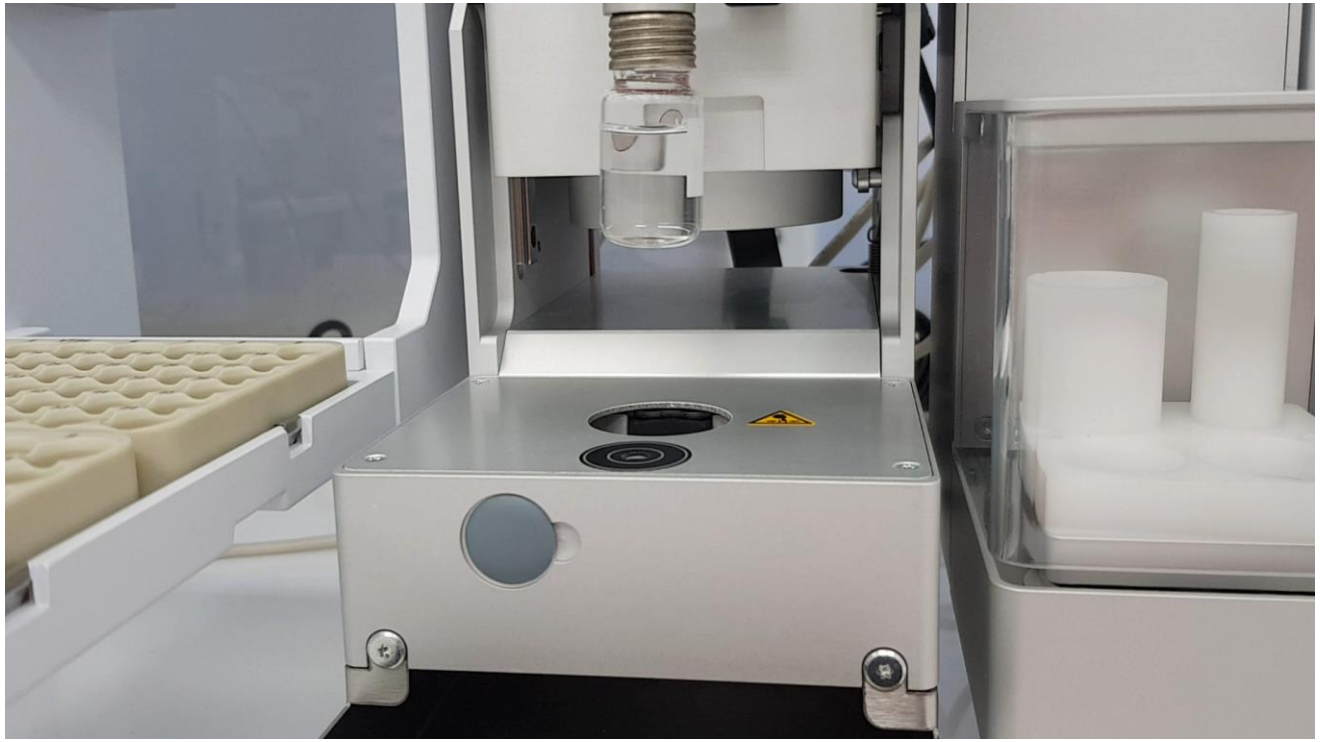


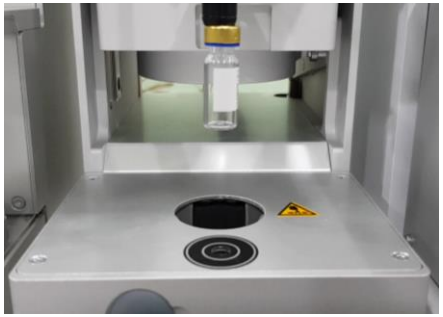


µSPE

Introduction to PAL-RTC for automated sample preparation

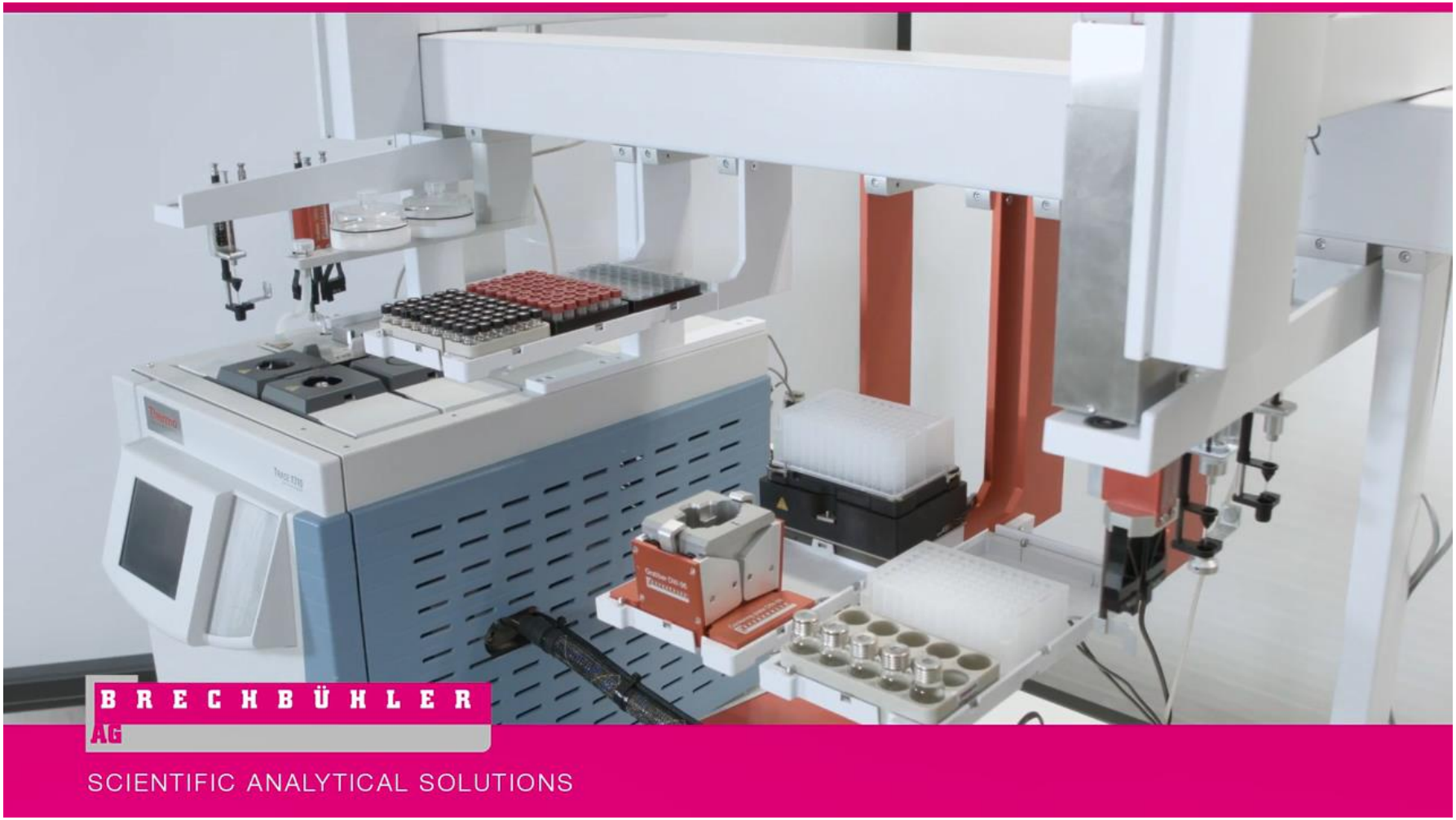
Decapper and Pipette tool in action





Liquid
Liquid
Extraction

Introduction to PAL-RTC for automated sample preparation



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