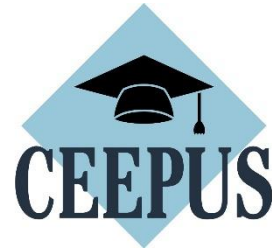


**Summer School and International Symposium
„Smart Sample Preparation: The Heart of Green Analytical, Food and Environmental Chemistry”
within the CEEPUS network RS-1816-03-2526
July 15 – 18, 2026, Blagoevgrad, Bulgaria**



SMART SAMPLE-PREPARATION STRATEGIES 1

Innovative sample preparation techniques



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INTRODUCTION



- **Smart sample preparation strategies** are the cornerstone of the modern analysis, aimed at transforming labor-intensive laboratory protocols into efficient, green, and automated workflows.
- Various samples for analysis: food samples, beverages, biological fluids and tissues, environmental samples, natural waters, wastewater, medicinal plants, etc.
- **Complex, heterogeneous and challenging matrixes**, with different physical properties and a wide variety of components that can interfere with the analytical procedure.
- Contain compounds of diverse chemical nature in wide concentration ranges, such as: proteins, lipids, vitamins, fatty acids, carbohydrates, polyphenols, amino acids, aroma compounds, biogenic amines, organic acids, various metabolites, elements, etc.
- **Choosing the right sample preparation technique is crucial to achieving the required accuracy and precision.**

INTRODUCTION

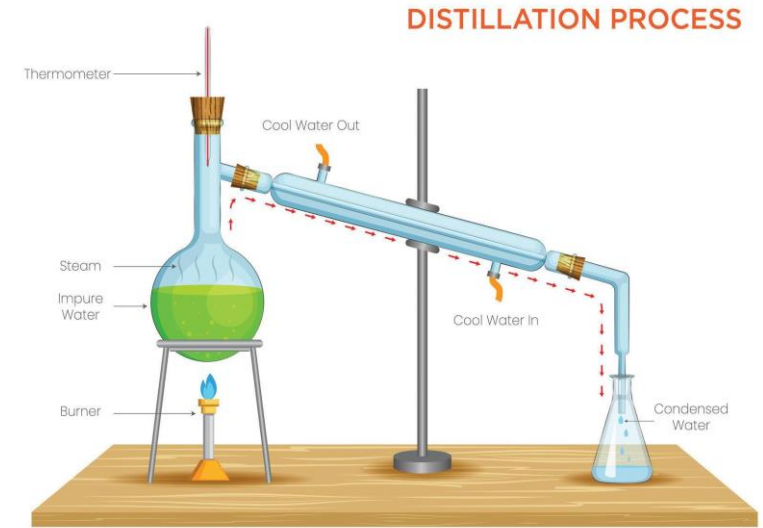


- In modern analytical protocols, sample preparation no longer serves merely a necessary preliminary step; it provides a distinct advantage significantly enhancing the over all performance of an analytical method.
- Particularly promising is the use of task-specific substances and materials designed to address specific analytical challenges, called: state-of-the-art, sustainable, tunable or tailored, advanced, functional, and **smart** materials. **Among these, the term “smart” most accurately and concisely captures the essence of these systems.**

Extraction techniques

Sample treatment, involves both:

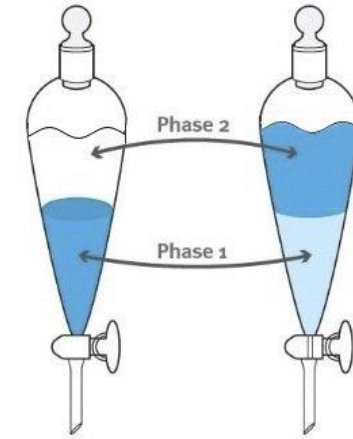
- ✓ **Extraction** of the analytes, and
 - ✓ **Purification** of the sample extract obtained.
-
- ✓ It is the most **labour-intensive and time-consuming analytical step**, accounting for 60–80% of the total analysis time.
 - ✓ These steps can affect both, main (accuracy and precision) and green analytical features of analytical methods.
-
- ✓ Traditional sample preparation methods for liquid samples are **liquid-liquid extraction** or **solid-phase extraction (SPE)**.



Liquid-liquid extraction

- ✓ Liquid-liquid extraction is one of the **oldest** extraction techniques.
- ✓ Based on the **partition coefficient of analytes** between two **immiscible solvents**, one of them usually an aqueous solution.
- ✓ This technique is very **simple** and requires **no complex** analytical instrumentation.
- ✓ Extraction can be performed **manually**, by mixing the liquid sample with an organic solvent in a separatory funnel or **automatically**, in Erlenmeyer flask(s) placed on a magnetic stirrer, allowing multiply samples to be mixed at the same time.

Liquid-Liquid Extraction (LLE)



Liquid-liquid extraction

Advantages:

- Easily accessible
- Can be performed in almost any laboratory
- Simple and inexpensive method for extracting components of interest.

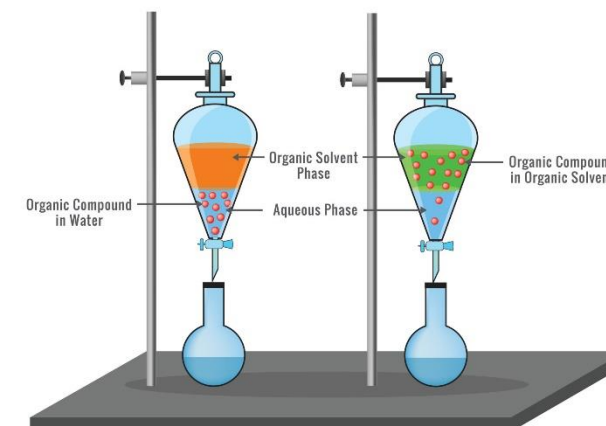
Disadvantages:

- Can take a long time,
 - Unsuitable for many polar components,
 - It has low selectivity and low extraction yields,
 - Requires a large amount of organic solvents, resulting in the generation of large quantities of solvent waste that can cause environmental problems.
 - There is a risk of forming emulsions, which leads to incomplete phase separation,
 - Lower analytical yields, and
 - The use of easily breakable glassware.
-
- Because the use of hazardous solvents should be avoided from the green and sustainable point of view, selective and environment friendly extraction procedures.



EXTRACTION

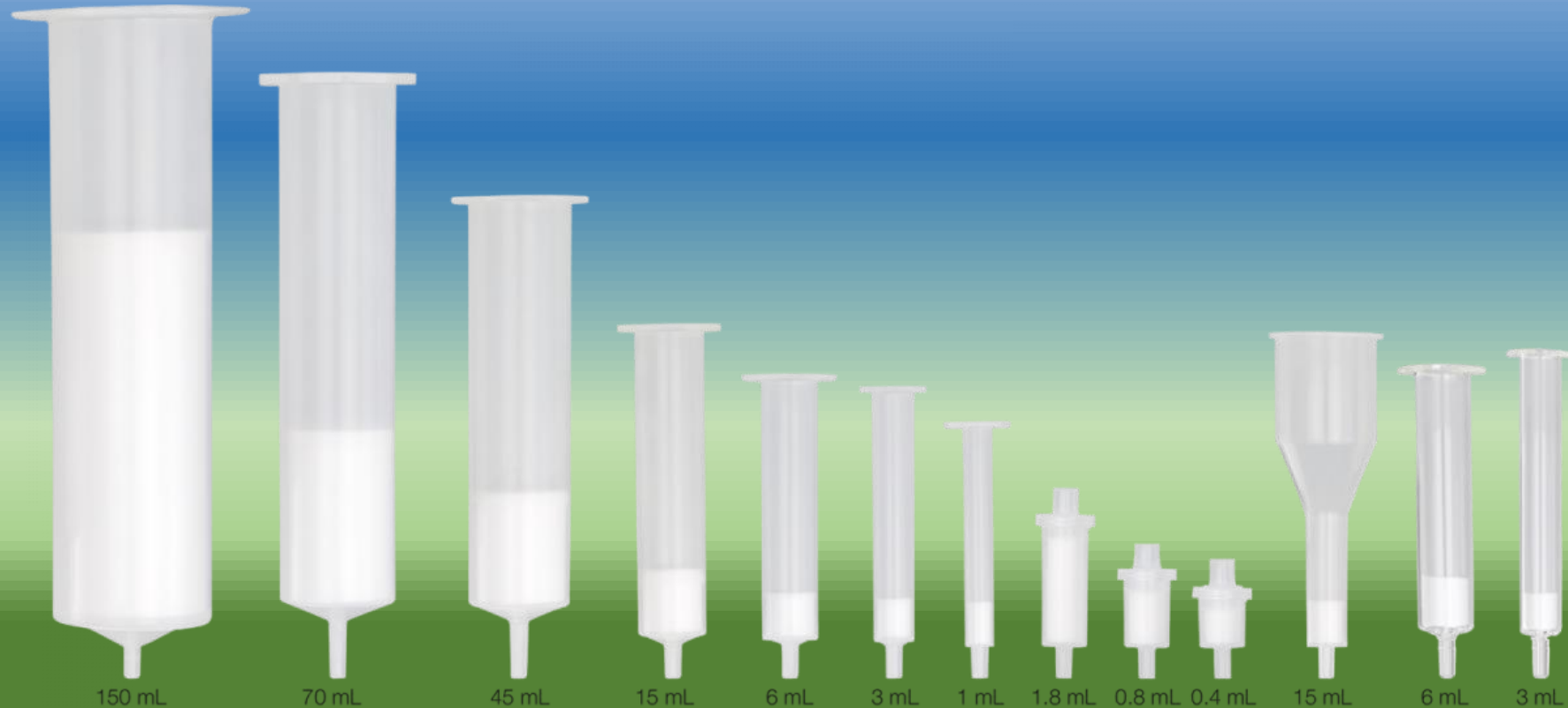
Before Extraction → Shake → After Extraction



Solid-phase extraction (SPE)

The principle of SPE is based:

- On a **strong**, but **reversible** interaction between the analyte and the surface of the solid phase.
- Sorption of analytes on a solid solvent contained into a polypropylene cartridge.
- Elution with a suitable volume of an appropriate organic solvent, simultaneously achieving extraction and enrichment of the analytes.



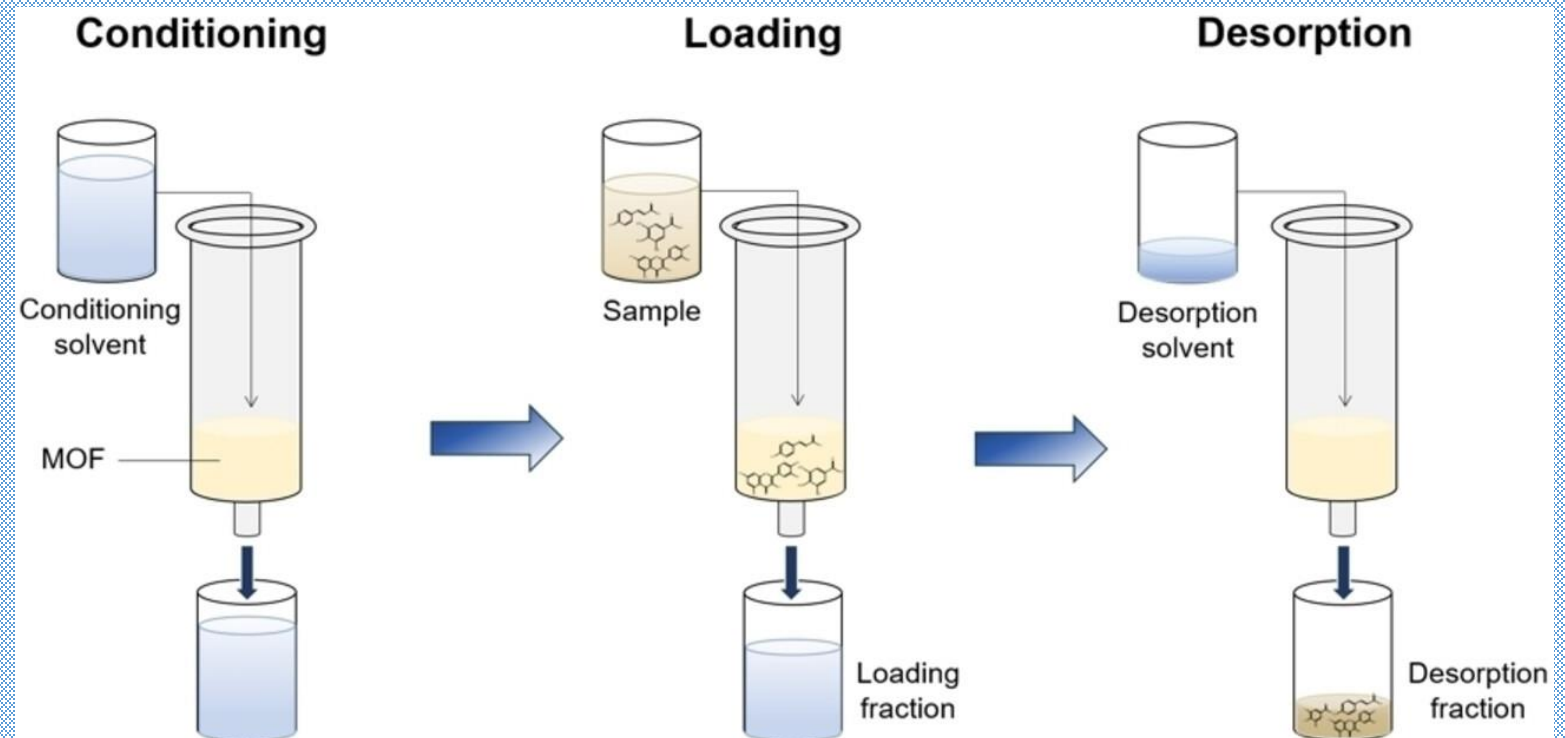
Solid-phase extraction (SPE)

Depending on the type of interaction between the analyte and the solid phase, there are several types of solid-phase extraction:

- **Reverse-phase extraction** where a polar liquid phase is used, and the solid phase is non-polar modified.
- **Normal-phase extraction** where the analytes are retained on a polar modified solid phase, and the liquid phase is non-polar.
- **Ion-exchange extraction**,
- **Adsorption extraction**.

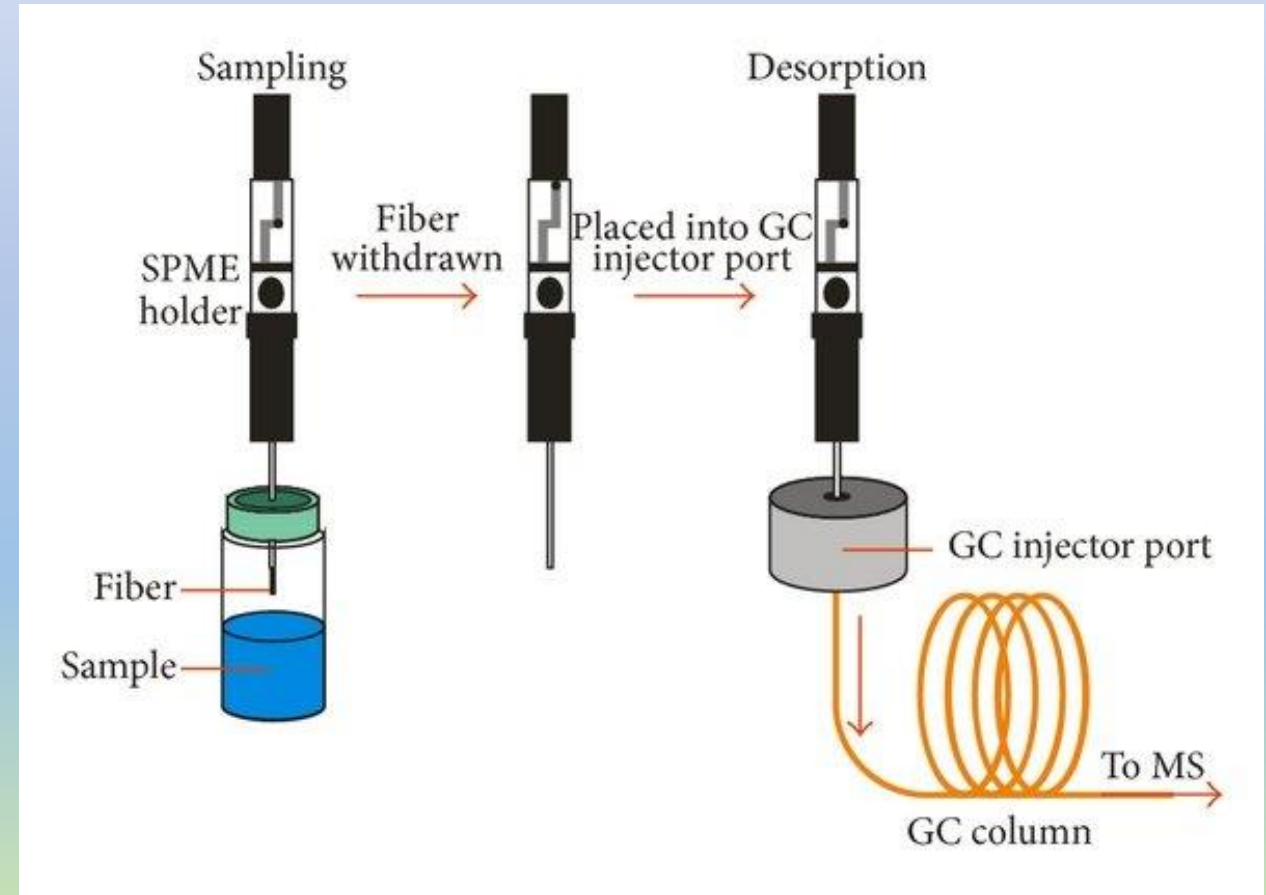
➤ The main **advantages** of SPE are:

- automation degree,
- relatively simple and inexpensive equipment,
- improved accuracy, precision, and laboratory throughput.



Solid-phase microextraction (SPME)

- SPME is a simple, fast, universal, sensitive, solventless, and economical technique for the preconcentration and sampling of analytes derived from various types of samples.
- SPME is a **non-exhaustive** sample preparation technique, which **combines extraction, enrichment, and sample injection into a single step**.
- Main advantages:
 - **Simplicity, rapidity, accuracy, and low organic solvent consumption** (trapped analytes can be directly introduced to analytical instruments).
 - **Reliability, sensitivity, and selectivity of this technique.**



Solid-phase microextraction (SPME)

- It has been widely used to analyze target compounds from gaseous and liquid matrices using chromatography coupled to hyphenated detection techniques.
- SPME allows the detection of semi-volatile and nonvolatile compounds and benefits from the constant development of new sorption coatings.
- This procedure can be performed in different modes:
 - (i) **Headspace SPME (HS-SPME) mode** in which the analytes are adsorbed/absorbed from the gas phase in equilibrium with the samples (as the temperature is a parameter with a significant effect on the kinetics of the process, this is the most adequate for volatiles extraction).
 - (ii) **Direct mode (DI)** in which the SPME fiber is immersed directly into the bulk sample. In this case, the agitation is an important experimental parameter to facilitate the transport of the analytes from the solution to the fiber.
 - (iii) In the third mode, **membrane extraction**, the extraction of less volatile compounds is facilitated using a protected membrane.



Headspace SPME coupled to GC-MS

Green Analytical Chemistry

Analytical methodologies can have side effects:

- energy consumption,
- wastes that could create risks for operators and damage the environment, etc.

These side effects of analytical methods have become the key features considered during development of **green analytical process**.

Principles of Green Chemistry, directly relevant to:

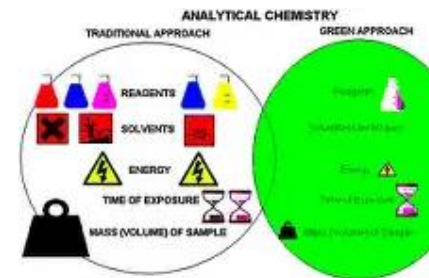
- sample preparation, including the use of safer solvents,
- reduced energy consumption, and
- designing processes that minimize hazardous byproducts or wastes.



- Major efforts of scientists have been devoted to **miniaturization** of the extraction device; development of new sorbent materials with advanced properties; reduction/replacement/elimination of toxic and hazardous organic solvents; reduction of sample volume and extraction time, and maximizing extraction efficiency and selectivity.

Green Analytical Chemistry

- In modern analytical protocols, sample preparation no longer serves merely a necessary preliminary step; it provides a distinct advantage significantly enhancing the overall performance of an analytical method.
- Particularly promising is the use of task-specific substances and materials designed to address specific analytical challenges, called: state-of-the-art, sustainable, tunable or tailored, advanced, functional, and **smart** materials. **Among these, the term “smart” most accurately and concisely captures the essence of these systems.**



Smart materials have to possess:

- high sensitivity,
- selective preconcentration and/or separation,
- suppression of matrix effects,
- reduced analysis time,
- simplified sample preparation procedures,
- potential for on-site application,
- automation, and
- compliance with the principles of green chemistry.

Materials that have demonstrated successful performance in the sample preparation of biological fluids and environmental materials:

- ionic liquids, eutectic solvents,
- nanoparticles, metal-organic frameworks,
- covalent organic frameworks, and
- molecularly imprinted polymers.

Green solvents

Green solvents have been defined as those that are: (1) available, (2) non-toxic, (3) biodegradable, (4) recyclable, (5) non-flammable, and (6) cheap.

Water

- ✓ Water is considered the **safest** and **least expensive green solvent** due to its availability, non-toxicity, renewability, safety, ease of handling, and ease of treatment compared to traditional organic solvents.
- ✓ It can be added directly to food and drug formulations.
- ✓ Water is widely utilized in sample preparation techniques due to its versatility across various stages, including cleaning materials during preparation for extraction, serving as the extraction medium either alone or in combination with other solvents, and in analytical processes for diluting extracts as well as the mobile phase in liquid chromatographic analyses.
- ✓ Water has been associated with different techniques aimed at increasing its efficiency for the extraction of natural compounds, mainly when their solubility in water is poor.
- ✓ Some examples consist in the addition of chemical agents such as salts, surfactants, co-solvents, complexing ligands, enzymes.

Green solvents

Solvent	Advantages	Disadvantages
Water	Widely available, non-toxic, non-volatile, renewable, safe, easy to handle, easy to treat, can be added directly to food and drug preparations .	Removal can be expensive and time-consuming, high efficiency only for extracting polar compounds, low selectivity.
Liquefied solvents (Dimethyl ether, Carbon dioxide)	Economically favorable conditions, safe (some cases), minimal traces in the extracts, easy separation, no need for prior drying stages (depending on the solvent), efficient, high selectivity.	Flammable in some cases, high cost of implementation depending on application.
Neoteric solvents (DES-Deep Eutectic Solvents, NADES-natural deep eutectic solvents, liquid mixtures)	Tunable, non-volatile, renewable, safe, can be added directly to food and drug preparations.	Removal can be expensive and time-consuming, high cost of some components, high viscosity.
Bio-based solvents (D-limonene, Cyclopentyl methyl ether, Ethanol)	Non-toxic, obtained from renewable sources (biodegradability, low carbon footprint), might be recyclable, high selectivity, high tunability when mixed.	May require purification steps, land competition with food crops, expensive due to limited market availability.

Direct Analysis of Samples - The Greenest Approach

- The clearest way to reduce wastes, energy, consumption of solvents, and reagents is through the **direct analysis of the samples**.
- This approach is not always possible since most of the samples need to be in solution or analytes of interest need to be selectively extracted and therefore, no direct determination can be done.
- Methodologies mostly related to direct analysis involve the use of MS-based procedures (like DART (Direct Analysis in Real Time)-MS, PTR (Proton Transfer Reaction)-MS, IM (Ion Mobility)-MS, and NMR (Nuclear Magnetic Resonance)).

- ✓ **NMR and MS-based techniques can be used for a direct analysis of the samples.**
- ✓ They are the most employed in **metabolomics** and **food** applications.

NMR

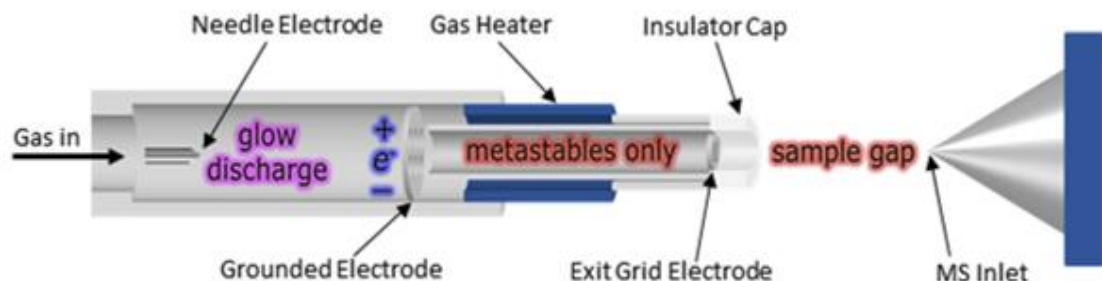


Direct Analysis of Samples - The Greenest Approach

MS-based methods are the most used for metabolite profiling/fingerprinting.

A new sample ionization source, called DART, has been developed allowing a direct and rapid identification of analytes in different types of samples without any sample treatment.

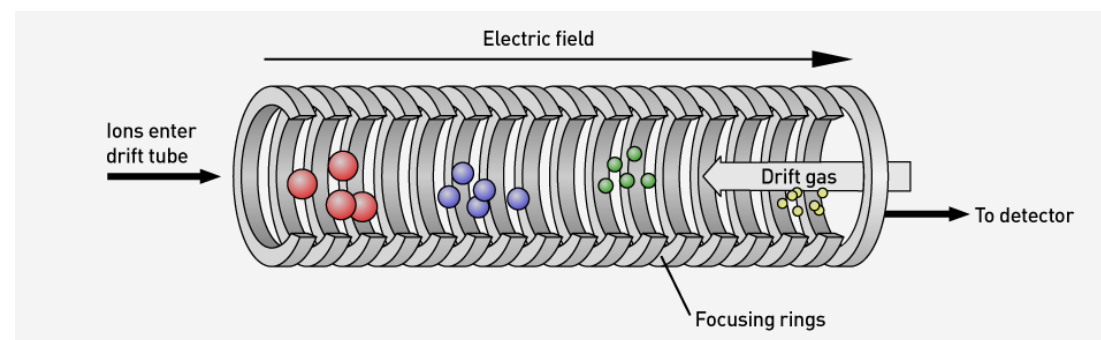
DART-MS (Direct Analysis in Real Time Mass spectrometry) is a powerful ionization technology that enables rapid, versatile analysis across solids, liquids and gases, all in their native forms. It allows for the direct analysis of solid, liquid or gas samples without extensive sample preparation.



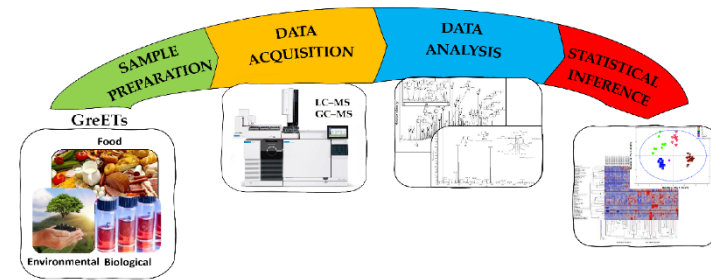
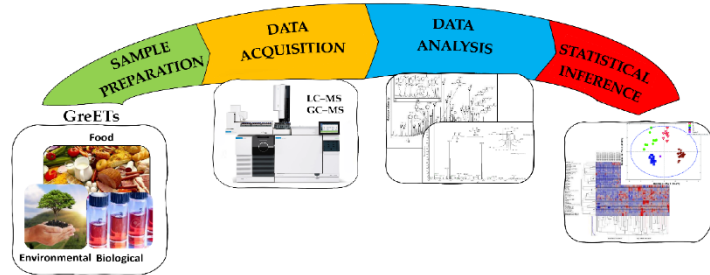
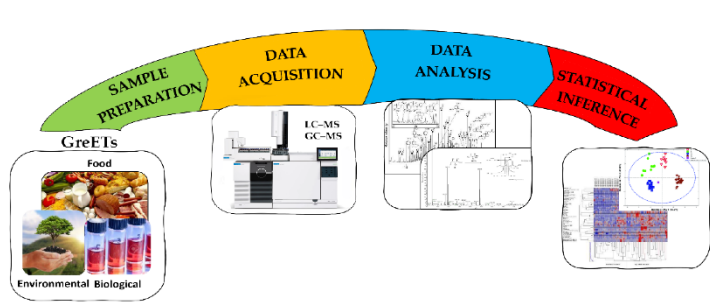
Sample ionization source DART

Ion-mobility spectrometry (IMS) allows direct introduction of solid and liquid samples into the MS analyzer by thermal desorption in which the vapor generated is ionized by atmospheric pressure chemical ionization (APCI) to produce ions.

This approach has been used, for metabolic profiling of human blood.



IMS



Green extraction techniques (GreETs)

- Green extraction techniques (GreETs) emerged recently as **sustainable alternatives to classical sample preparation methods**.
- They **enhance selectivity and sensitivity**, while **reducing harmful effects** on operators and the environment.
- These improved processes address inefficiencies and aim to minimize or eliminate harmful substances.
- This will promote more efficient use of energy and resources in close association with the principles supporting the concept of green chemistry.

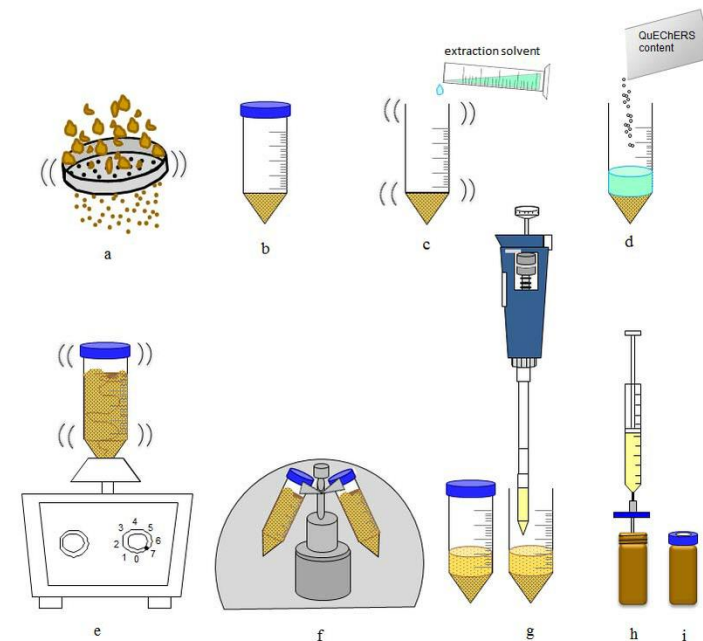
Approaches to improve and accelerate the extraction efficiency and to lower solvent consumption, include:

- 1) Sorbent-based techniques**, such as solid-phase microextraction (SPME) and fabric-phase sorbent extraction (FPSE), and
- 2) Solvent based techniques** (QuEChERS—microquick, easy, cheap, efective, rugged, and safe), ultrasound assisted extraction (UAE), and microwave-assisted extraction (MAE), supercritical fluid extraction (SFE) and pressurized solvent extraction (PSE).

QuEChERS: quick, easy, cheap, effective, rugged, and safe

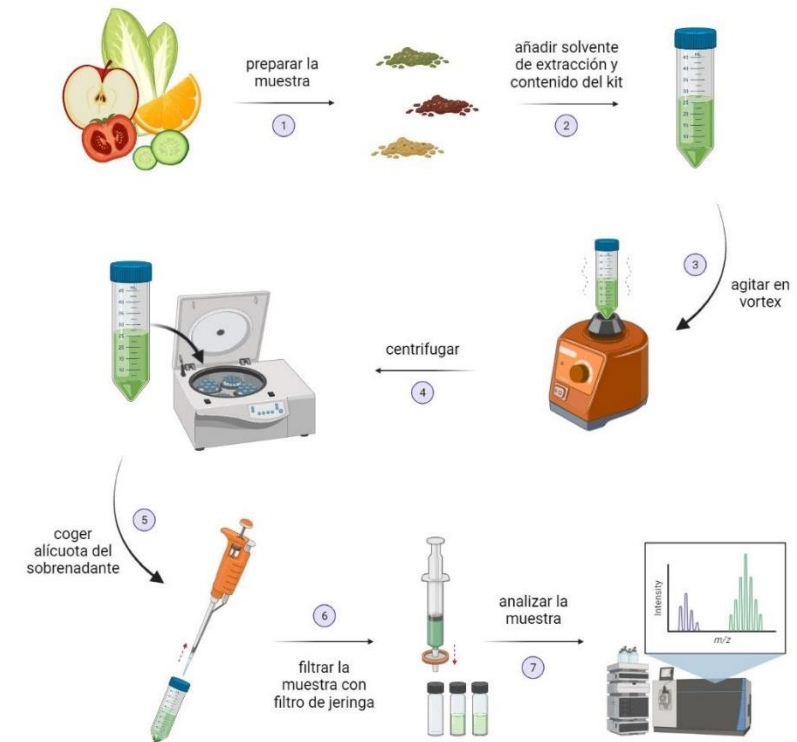
The QuEChERS method demonstrates:

- **high effectiveness** and **ruggedness** in the extraction and analysis of a wide range of analytes (e.g., pesticides, mycotoxins, pharmaceutical residues, illicit drugs, etc.) and solid and liquid matrices, including environmental, food, feed, pharmaceutical, biological, and forensic matrices.
- Based on the green chemistry principle of multiresidue analysis, multiclass methods have been frequently applied.
- The effectiveness of the method is strongly influenced by the **choice** of **extraction solvents** and **sorbents** since matrix interferences and co-extracted substances can affect the accuracy and reliability of the analysis and induce instrument damage.
- Thus, optimization of the method may be essential to overcome these challenges.
- In this regard, factors such as the extraction solvent, sample amount and sample/solvent ratio, pH, types and amounts of salts for solvent extraction, sorbent types and amounts, mode and time of agitation could be considered.



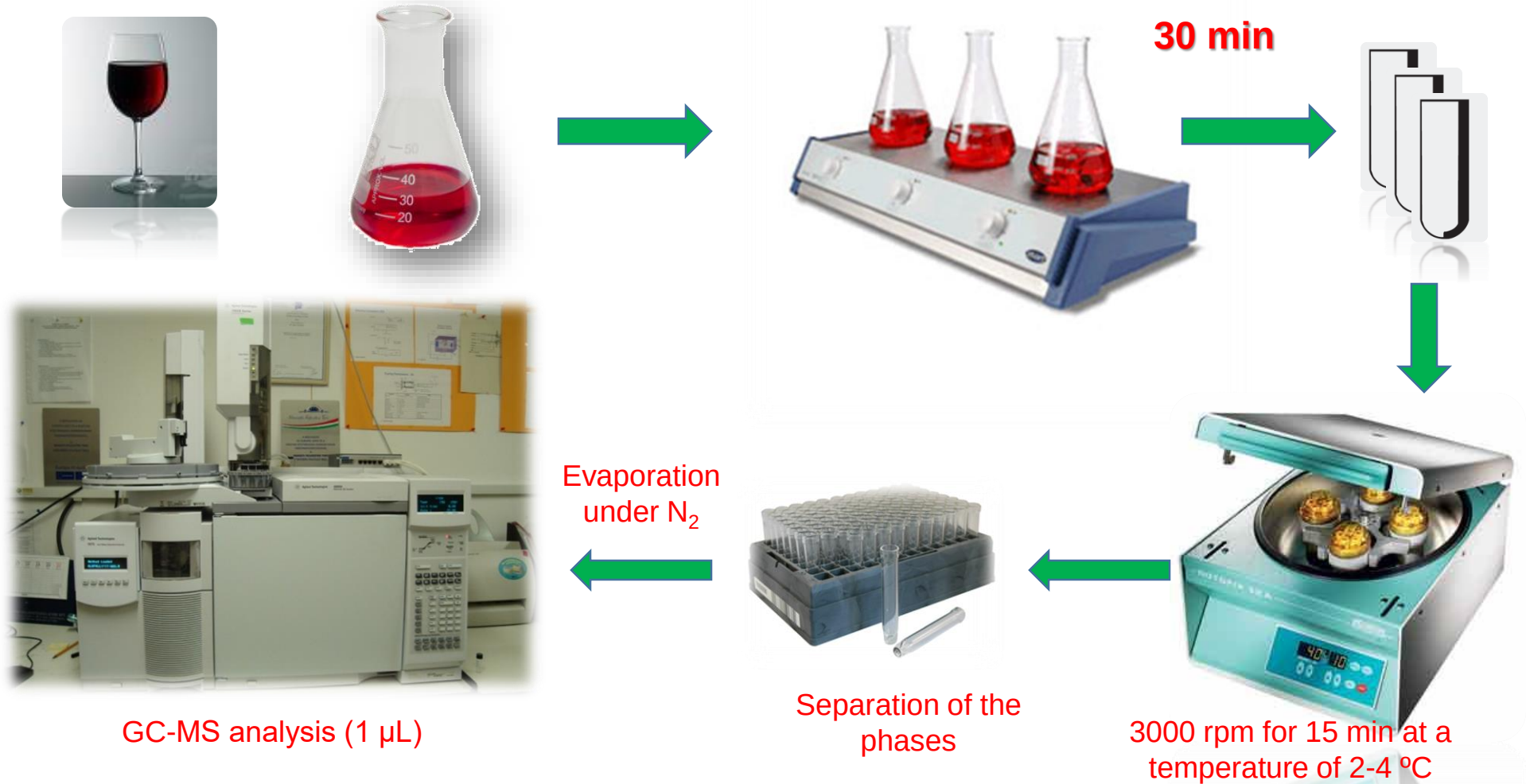
QuEChERS: quick, easy, cheap, effective, rugged, and safe

- QuEChERS method for the quantitative measurement of **pesticide residues in vegetables and fruits**.
- It is a **two-stage process** of solid–liquid partitioning with a salting-out effect and a DSPE. The extraction of the target analytes occurs in the first stage, when a mixture of salts is dispersed in the matrix and mixes thoroughly with an organic phase, often acetonitrile, till an equilibrium is reached. This is followed by a cleanup step (DSPE) using a different combination of porous sorbents and salts according to the matrix interferences that should be removed.
- Since its **invention**, **QuEChERS** has been applied for the analysis of a wide spectrum of analytes in different sample matrices, such as **fluoxetine** and **carbamazepine** in benthic invertebrates, **pharmaceuticals** and **personal care products** in **sewage** and **surface waters**, **sulfonamide residues** in **milk samples**, or **BPA** in **human urine**.
- More recently, several improvements to the original procedure were reported, notably, its miniaturization applied in different fields of research.



Liquid-liquid extraction of wine aroma compounds

50 mL wine + 25 mL dichloromethane + 200 µL internal standard of 1-octanol



GC-MS analysis of aroma compounds in wine

GC-MS analysis



Agilent 5975 Mass Spectrometer coupled to an Agilent 6890N Gas Chromatograph (Agilent)

Separation - polar capillary column, Carbowax type from Agilent, (30 m × 0.25 mm ID and 0.25 µm film thickness)

Working parameters:

Injector temperature 240 °C;

MS source 230 °C;

MS Quad 150 °C,

Transfer line 280 °C.

40 °C for 3 min

180 °C at 3 °C /min.

260 °C with 20 °C /min

260 °C for 10 min

Carrier gas - He with a flow rate of 1.5 mL/min.

Splitless mode for injection

Mass range of 50–400 m/z , recorded at 1 scan/s.

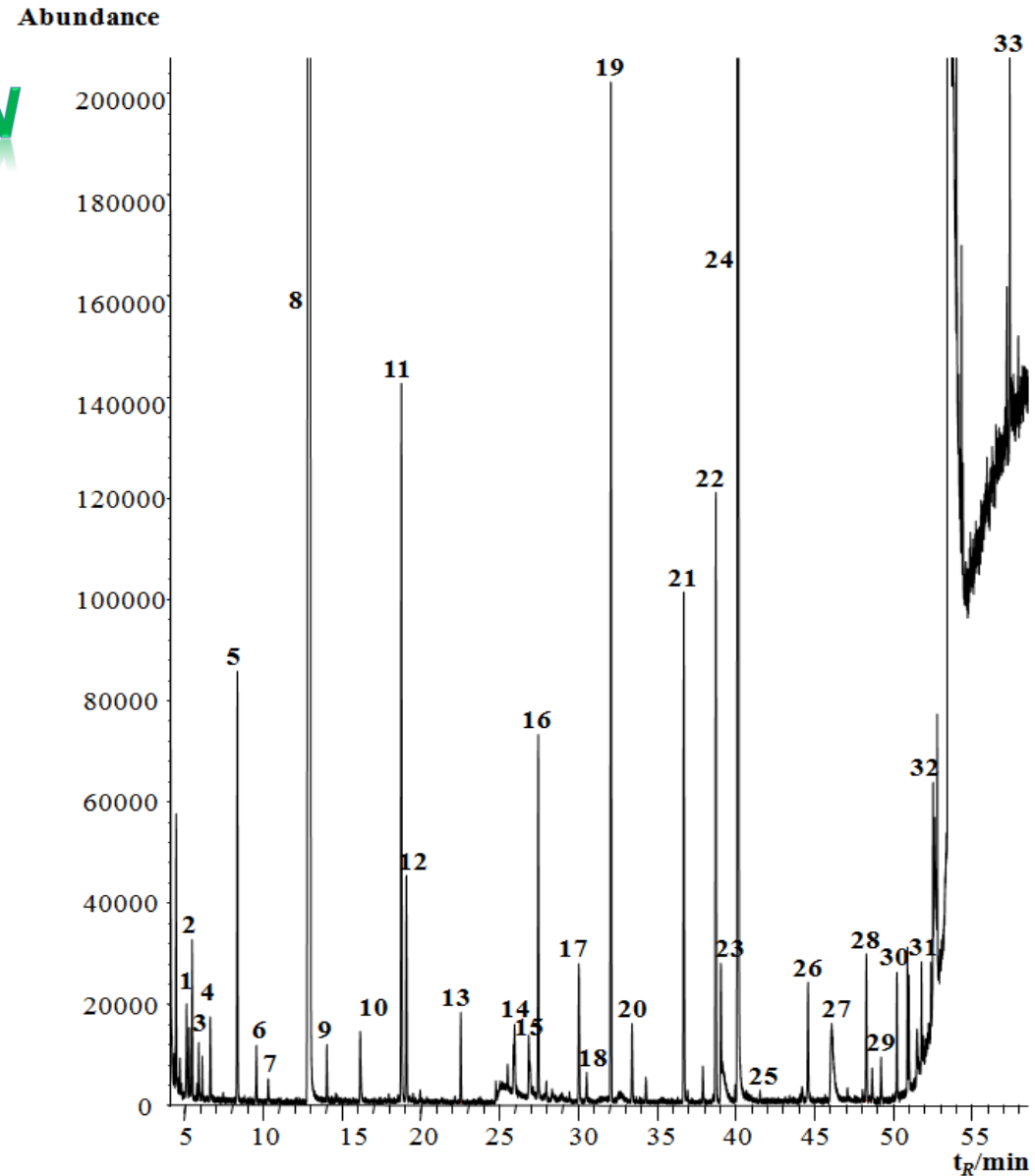
Ivanova et al. *Food Analytical Methods*, 5, 1427-1434, 2012

Ivanova et al. *Food and Bioprocess Technology*, 6(6) 1609-1617, 2013

METHOD APPLICATION

- Analysis of the volatile composition of Vranec wine
- 33 volatile compounds were identified and quantified

Ivanova et al. Food and Bioprocess Technology, 6(6) 1609-1617, 2013



GC/MS chromatogram of the volatile compounds in Vranec wine

HS-SPME-GC-MS analysis of aroma compounds

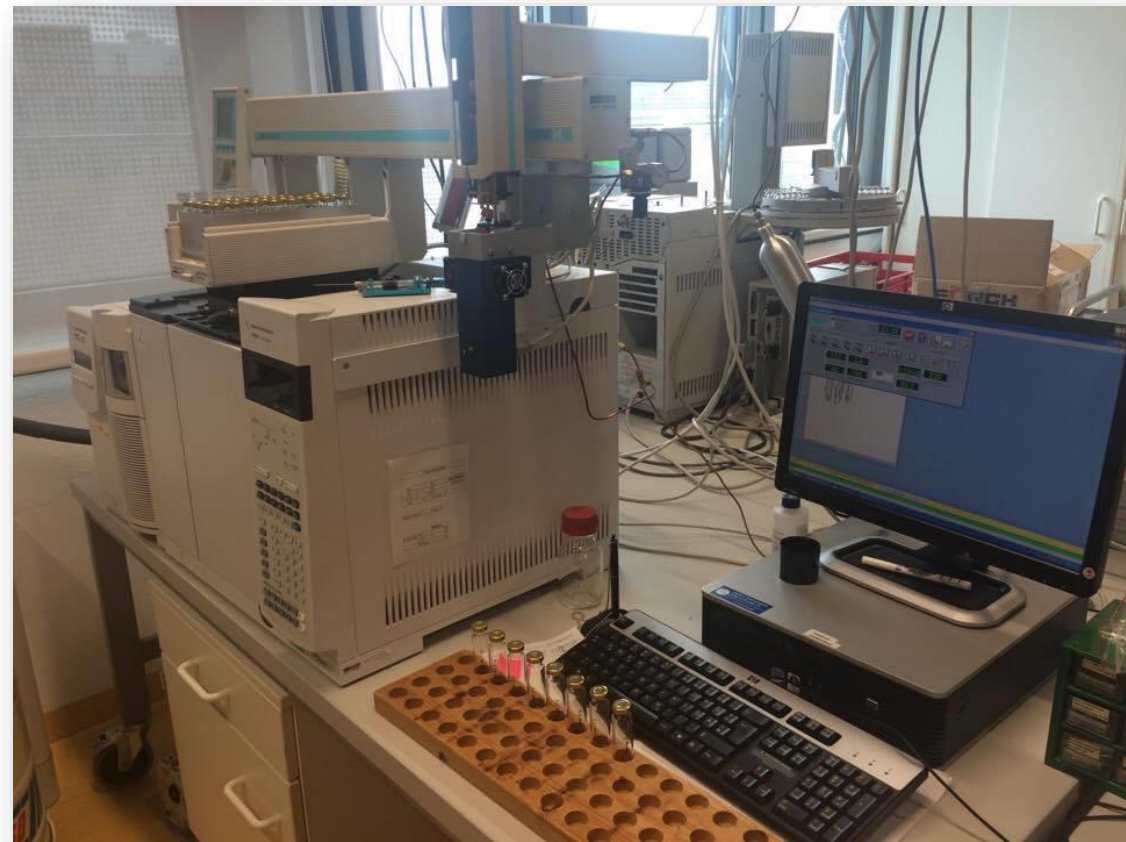
An automated HS-SPME combined GC-MS is highly **efficient separation technique** for extraction and separation of wine aroma compounds

- ✓ SPME fiber was used: **DVB/Carboxen/PDMS 50/30, 2 cm stable flex**
- ✓ the samples were equilibrated in the oven of the autosampler at 40°C for 5 minutes
- ✓ SPME fiber was exposed into the headspace of the sample for 20 minutes at 40°C
- ✓ transferred to the GC-injector for thermo-desorption at 270°C



GC-MS conditions

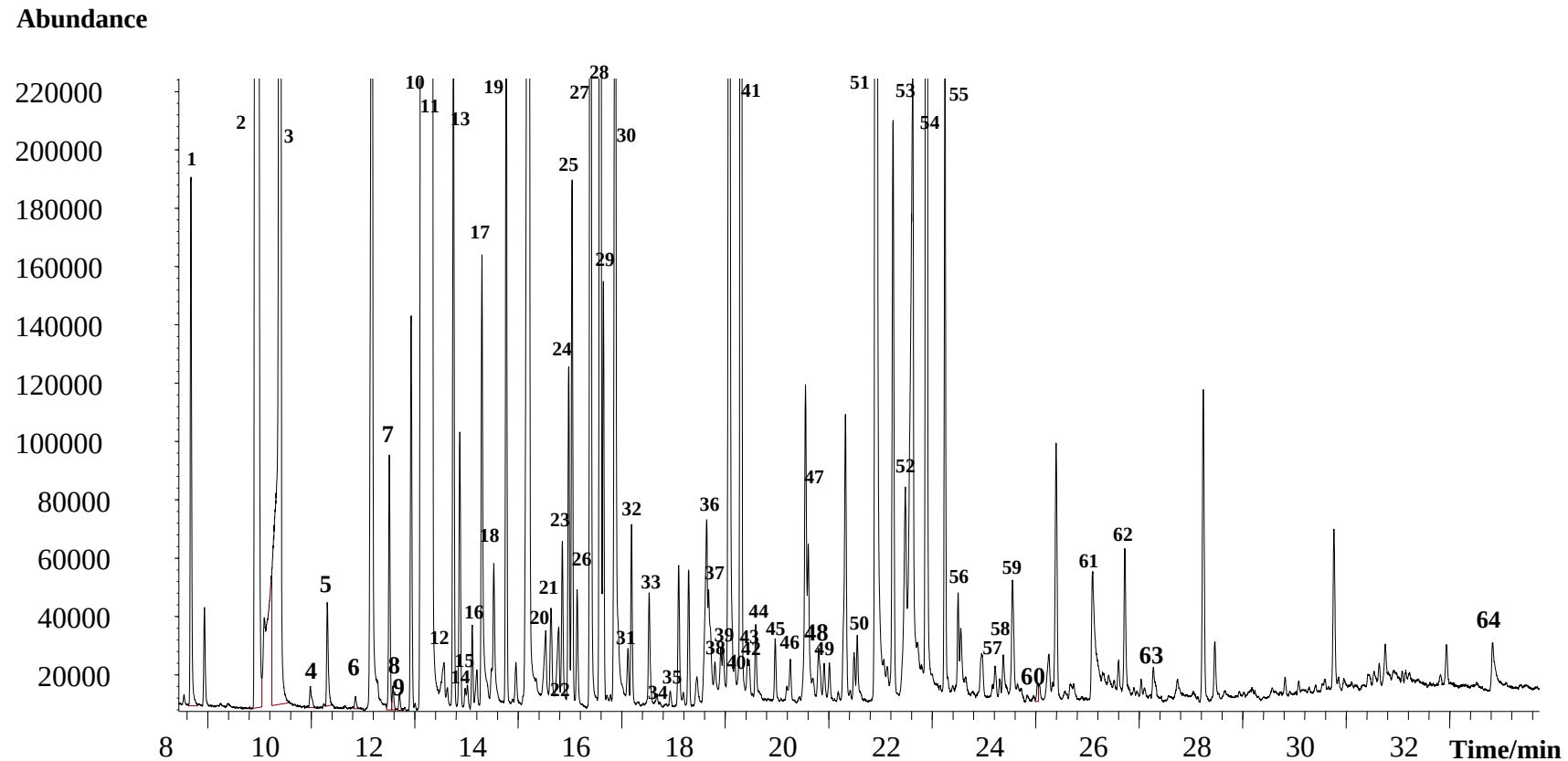
- Agilent system (GC 7890, MS 5975c VL MSD)
- Analytical column of medium polarity (HP5MS, 30m*0,25mm*1μm, Agilent Technologies)



Temperature program:

- 10 °C for 1 min with a temperature ramp of 8 °C min⁻¹ up to 270 °C (holding time 1 min).
- Detector temperature 230°C
- Cryo-focussing by blowing liquid nitrogen into the GC-oven was applied to reach the start temperature of -10 °C with the aim to obtain higher resolution for compounds with very high volatility.

- 64 individual aroma compounds have been identified in Vranec samples



HS-SPME-GC/MS chromatogram of the volatile compounds in Vranec wine

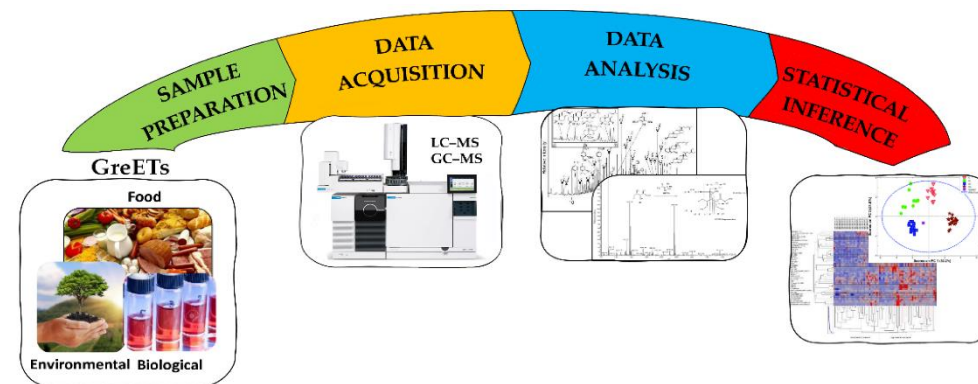
Ivanova Petropulos et al. *Food Chemistry*, 165 (15) 506-514, 2014

CONCLUSIONS



- The sample preparation, including the extraction process, is one of the **most important steps for any analysis**.
- It is the step that determines the quality of the measurements of the target analytes, and so it should be critically considered.
- Green chemistry approaches in the sample preparation techniques, as a sustainable and eco-friendly alternative to the classical techniques, are mandatory.

- In recent years, a rapid transition from solvent-based extractions and solid SBEs to miniaturized formats promoted a substantial reduction of the solvent consumption.
- At the same time, green sample preparation techniques are rapid, simple, generally solvent-free, sensitive, reliable, and cost effective.
- **Modern trends in sample preparation techniques are toward the simplification and miniaturization of sample preparation, and the minimization of sample size and organic solvent used.**



CEEPUS Network
RS-1816-03-2526

A top-down view of a spiral-bound notebook with a white cover, resting on a light-colored wooden desk. The notebook is open, and the text "THANK YOU FOR YOUR ATTENTION" is printed on the left page. The words "THANK YOU" and "FOR YOUR" are in black, while "ATTENTION" is in red. To the top left of the notebook are a pair of gold-rimmed glasses. To the right is a silver and black pen. In the bottom left corner, there is a small white pot containing a green succulent plant. A black circular object is partially visible in the bottom right corner.

**THANK YOU
FOR YOUR
ATTENTION**