UNIVERSITY OF CHEMISTRY AND TECHNOLOGY, PRAGUE
Faculty of Food and Biochemical Technology
Department of Food Analysis and Nutrition

Extraction methods

Isoolation and separation methods

Analytical method overview

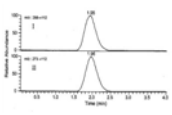
- Sample pre-treatment
- **Extraction**
- **Clean-up**
- Concentration
- Derivatisation
- Sample analysis

Sample

Analytical sample

Crude extract

Purified extract



	Solid sample	Liquid sample
Sample preparation	Cutting, Grinding Chemical/enzymatic digestion	Centrifugation Dilution Filtration
	Freeze-drying Hydrolysis of precursors/metabolite	
Sample extraction	*liquid/solid extraction (LSE)*	*liquid/liquid extraction (LLE)*
	Soxhlet extraction Accelerated solvent extraction (ASE) Supercritical fluid extraction (SFE) Solid phase microextraction (SPME) Matrix Solid phase dispersion (MSPD) Solid phase extraction (SPE) Molecular imprinting polymers (MIPs) Immunochemical methods Liquid chromatography	
Sample Clean-up		

Sample homogenisation

- **Solid matrices**

Grinding

https://www.epicurious.com/expert-advice/whats-the-best-mortar-and-pestle-review-chefn-mortar-and-pestle-article

Cutting

https://www.scientistlive.com/content/complete-homogenization-complex-samples

- **Liquid matrices**

Filtration, Centrifugation

https://www.restek.com/en/products/sample-preparation--air-sampling/sample-preparation-products/Sample-Filtration

Pre-treatment - freeze-drying

Advantages

- Sample conservation and storage (elimination of water prevents enzymatic and microbial activities)
- Sample homogenisation (grinding after freeze-drying lead to homogenous powder)
- Sample extraction efficiency (increased surface contact between matrix and solvent)

L imits

- Time consuming (24-48 h for freezing and freeze-drying)
- Risk of cross contamination (sample manipulation and grinding material)
- Compounds stability (risk for highly volatile substances)
- Price (equipment and energy)

Extraction - introduction

Aim of this step: extract the solutes from the matrix (solid, liquid, gas)

The 4 parameters to consider:

- Extraction efficiency (maximize the recovery)
- Quantity of co-extracted compounds from the matrix
- Stability of solutes (degradation,…)
- Introduction of eventual contamination

Extraction methods

- **Liquid-liquid extraction (LLE)**
- **Solid-liquid extraction (SLE)**
- **Accelerated solvent extraction (ASE) / Pressurised liquid extraction (PLE)**
- **Microwave assisted extraction (MASE)**
- **Supercritical fluid extraction (SFE)**
- Solid phase extraction (SPE)
- Solid phase microextraction (SPME)
- Adsorption chromatography
- Dispersive solid phase extraction (d-SPE)
- Gel permeation chromatography (GPC)

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Solvent extraction methods

Liquid-liquid extraction (LLE)

Solid-liquid extraction (SLE)

Solvent extraction

- The most applied isolation method
- Transfer all (or part of) the sample matrix to the solution

- *Classic methods:*
  - Large volumes of solvents (toxicity, costs), time consuming, formation of emulsion
- *Modern methods:*
  - Automation, use of hot and superheated solvents (pressure vessels)

Liquid-liquid extraction (LLE)

- Distribution of analytes and interferents between two immiscible liquids (mostly aqueous sample vs. organic solvent)
- The selection of extraction solvent affects the selectivity and efficiency of the extraction
- Required properties on solvent:
  - low solubility in water (<10%)
  - polarity ensuring good analyte yield
  - sufficient volatility (easy concentration)
  - compatibility with analytical suffix
- Other factors affecting balance:
  - pH adjustment (suppression of ionization - acidification for acids) also affects the yield of non-ionizable compounds due to the influence of the matrix
  - addition of salts (salting out effect) e.g.: QuEChERS extraction (addition of $MgSO_4$ and NaCl
  - addition of metal ions (formation of ion pairs)
  - addition of chelating or complexing agent (hydrophobic products)

LLE

- Good contact between phases must be ensured (mass transfer)

for example:

  - Shaking - separating funnel, shaker, **centrifugation, mixing**
- A risk of emulsion formation, especially for samples containing surfactants and fatty components
- Removal of emulsions:
  - addition of salt
  - heating and cooling the extraction funnel
  - filtration through glass wool or filter paper
  - centrifugation
  - addition of a small amount of another organic solvent

https://educalingo.com/en/dic-en/separating-funnel

LLE principle

- **Nernst distribution law**
  - when two immiscible solvents A and B taken in a beaker, they form separate layers.
  - if a solute X distributes itself between two immiscible solvents A and B at constant temperature and X is in the same molecular condition in both solvents."

$$K_D = \frac{c_0}{c_{aq}}$$

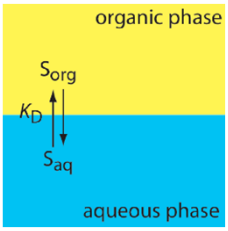
K_D … distribution constant
c_0 … concentration of compound in organic phase
c_{aq}… concentration of compounds in aqueous phase

- K_D describes the balance between analyte concentrations in both phases (characteristic of a given analyte in each system).
- To separate two substances, it is necessary that their K_D differ

LLE principle

- Extracted amount of analyte (E):

$$E = \frac{c_0.V_0}{c_0.V_0+c_{aq}.V_{aq}} = \frac{K_D.V}{(1+K_DV)}$$



K_D … distribution constant
V_0 … volume of organic phase
V_{aq}… volume of the aqueous phase
V … phase reatio V_0/V_{aq}

LLE principle

- Distribution constant (K_D):

$$K_D = \frac{c_0}{c_{aq}} = \frac{\frac{n_0}{V_0}}{\frac{n_{aq}}{V_{aq}}} = \frac{n_0}{n_{aq}} \cdot \frac{V_{aq}}{V_0} = \boldsymbol{\frac{n_0}{n_{aq}} \cdot \frac{1}{V}}$$

V … phase ratio V_0/V_{aq}

- Extracted amount of analyte (E):

$$K_D.V = \frac{n_0}{n_{aq}} = \frac{c_0.V_0}{c_{aq}.V_{aq}} \Rightarrow K_D.V.c_{aq}.V_{aq} = c_0.V_0$$

$$E = \frac{c_0.V_0}{c_0.V_0+c_{aq}.V_{aq}} = \frac{K_D.V.c_{aq}.V_{aq}}{K_D.V.c_{aq}.V_{aq}+c_{aq}.V_{aq}} = \frac{K_D.V}{(K_DV+1)}$$

LLE-principle

- One-step extraction
  - For quantitative yield, it must be K_D> 10, because it is practical for the phase ratio to be greater than 0.1 and at the same time less than 10 (0.1 < V < 10).

K_D = 10, V = 1: E = ((10*1)/(1 + 10*1))*100 = 91 %

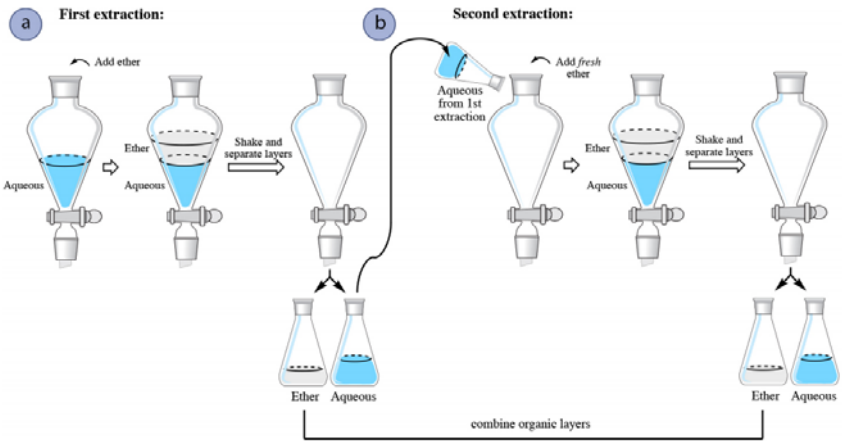
K_D = 10, V = 0,1: E = ((10*0,1)/(1 + 10*0,1))*100 = 50 %

$$E = 1 - \left[\frac{1}{1 + K_D V}\right]^n$$

- Multistep extraction
  - If the condition K_D> 10 is not met
  - Multiple extraction with a smaller volume of solvent is more efficient than extraction with a total volume.

LLE - multiple extractions

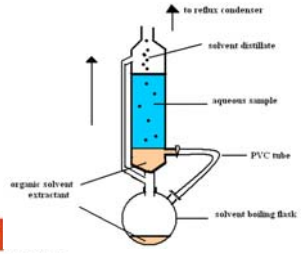
- An aqueous layer when the organic layer is on the top: a) First extraction, b) Second extraction.



https://chem.libretexts.org/Courses/SUNY_Oneonta/Chem_221%3A_Organic_Chemistry_I_(Bennett)/2%3ALab_Textbook_(Nichols)/04%3A_Extraction/4.05%3A_Extraction_Theory

LLE - continuous extraction

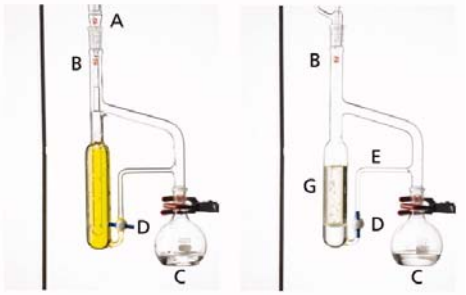
- K_D very small ⇒ multi-step extraction impractical (many repetitions, large extraction volume, slow equilibration).
- Continuous extractor - ***solvents lighter than water***
- Continuous extractor - ***solvents heavier than water***



https://www.researchgate.net/figure/Fig-1-Continuous-solvent-extraction-apparatus-for-solvent-heavier-than-water_fig1_316273189

Solvent lighter than water

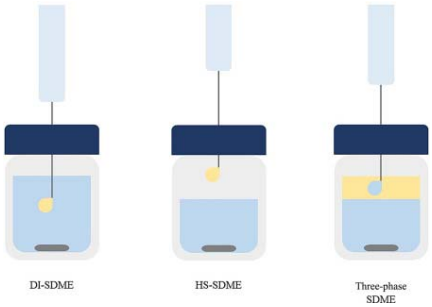
Solvent heavier than water



https://www.sigmaaldrich.com/CZ/en/product/aldrich/z562440

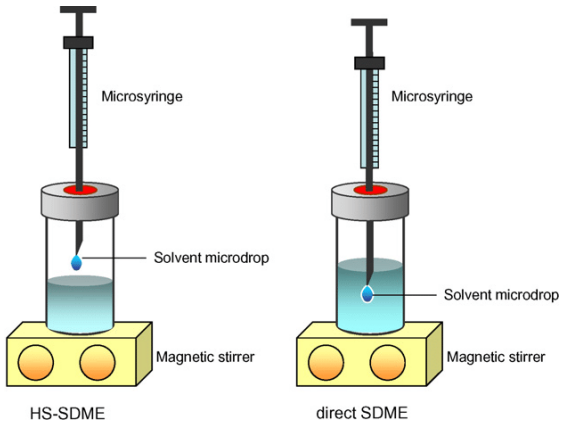
Solvent microextraction (SME)

- Simple procedure, requires only a manual syringe and a few microliters of solvent for extraction and concentration of analytes from liquids, solids, and headspace samples.



https://www.sciencedirect.com/topics/chemistry/microextraction

Single-drop microextraction (SDME)

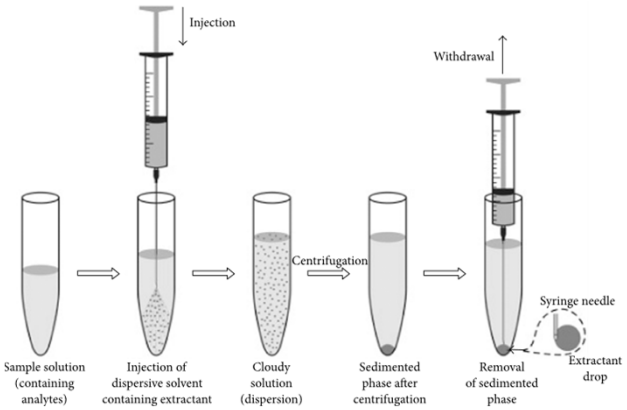


- During the extraction the sample solution is continuously stirred and heated.
- The intermediate phase can be the headspace above the sample solution, resulting in the headspace-single-drop microextraction (HS-SDME)
- The acceptor phase drop can be directly immersed into the donor phase, resulting in direct immersion-single-drop microextraction (DI-SDME).

https://www.researchgate.net/figure/Schematic-diagram-of-the-single-drop-microextraction-SDME-technique_fig1_23402927

Dispersive liquid-liquid microextraction (DLLME)

- Extraction technique which involves the dispersion of fine droplets of extraction solvent in an aqueous sample



https://www.hindawi.com/journals/jchem/2016/4040165/

Solvent extraction - solid samples

Solid- liquid extraction (SLE)

- Transfer analytes from solid sample to solution

Sample adjustment:

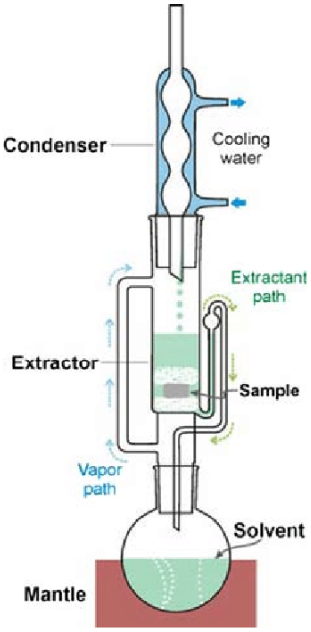
- The larger the total surface area of the particles (or the smaller the particles, the sample is finely ground) per unit mass, the better the extraction takes place = it is more efficient. (small particles = ease of extraction)
- *Solid samples*: slicing, crushing, grinding, sanding with sand, and other types of homogenization…
- *Viscous samples*: grinding, grinding or homogenisation with dry ice or liquid nitrogen (freezing mills) and other types of homogenisation…
- *Cryogenic grinding* = for solid and liquid samples, grinding in the presence of dry ice (solid CO2) or liquid nitrogen, special equipment, grinds the sample into a fine powder and prevents thermal degradation of the determined substances.
- Determination of moisture, possibly water removal

SLE - realization

- Ensuring good contact between phases (mass transfer)
- **Shaking** (and heating) - shaker (heated)
- **Homogenization** - homogenizers
- **Sonication** – ultrasound
- **Solvent reflux** (thermostable analytes)
- **Pressurized liquid extraction** (PLE) - an automated technique using elevated temperature and pressure to achieve the extraction of substances from solid matrices

Soxhlet extraction

- Solid sample placed in the **extraction thimble** - hardened filter paper/cellulose or fritted glass thimble instead of the bottom
- The thimble is placed in the apparatus, **the solvent, after heating to boiling point, condenses in the thimble, extracts out the analytes, returns them to the boiling flask, evaporates again…**
- Number of cycles per hour
- Max. temperature limited by the boiling point of the solvent (stability of analytes)
- The composition of the extraction mixture in the solution may not correspond to the composition in vapors (hexane: acetone (1: 1) x azeotrope (3: 1))
- High recovery x very slow/time consuming (6-8 hours)
- Well-established method, relatively undemanding and cheap



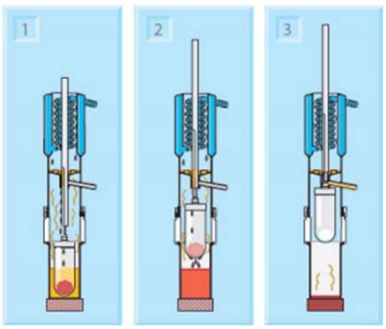
https://www.researchgate.net/figure/Schematic-of-a-Soxhlet-extraction-apparatus-indicating-the-vapor-and-liquid-extractant_fig4_251753895

Soxhlet extraction

Soxtec Extractor - a faster version of Soxhlet extraction

- Phase 1 - immersion of the sample in hot solvent (rapid extraction)
- Phase 2 - rinsing the sample with a condensing solvent
- Phase 3 - collection of pure solvent for further analyzes (65%)

- (semi) Automated extraction, 6-12 positions = extraction of 6-12 samples simultaneously
- Max. sample volume approx. 25 ml
- Extraction speed 30-60 min (Soxhlet 3-24 h)
- Heating rate from 20 ° C to 220 ° C in 20 min
- Use of common organic solvents



Advantages:

- Repeatability (± 1%)
- reduction of extraction time (vs Soxhlet)
  - + safety during heating
  - + solvent recycling
  - + simultaneous extraction of multiple samples

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Extraction methods

Accelerated solvent extraction (ASE) / Pressurised liquid extraction (PLE)

Microwave assisted extraction (MASE)

Supercritical fluid extraction (SFE)

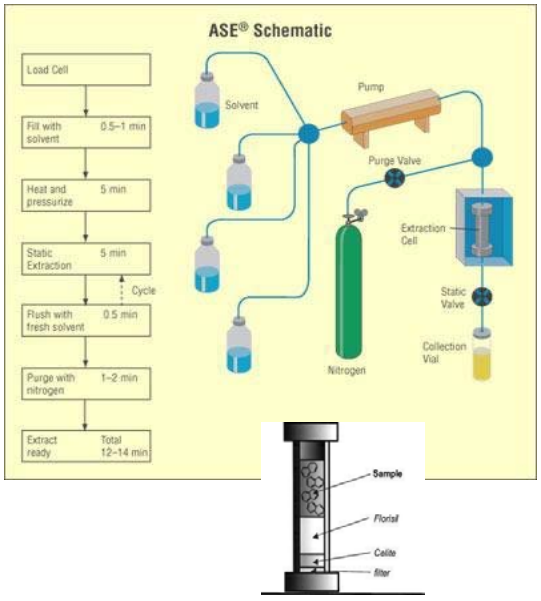
Pressurized liquid extraction (PLE)

- Also called pressurized solvent extraction (PSE) or accelerated solvent extraction (ASE)
- A steel cell is used (e.g. i.d. = 19.1 mm), the volume can be e.g. 11, 22 or 33 ml, up to 24 positions. The temperature is approx. Up to 200 ° C, the pressure up to approx. 20 MPa.
- Samples: dry, ground, possibly with the addition of sodium sulphate (desiccant)
- Solvent extraction of solid matrices at elevated temperature and pressure. Higher temperature means higher extraction kinetics, higher pressure keeps the solvent liquid.
- Obtaining a crude extract:
  - further purification necessary
  - compared to conventional methods: faster, lower solvent consumption, comparable yield, often better repeatability
- Selective extraction:
  - appropriate choice of solvent
  - by adding a sorbent to the sample (mixing + layer above the sample)

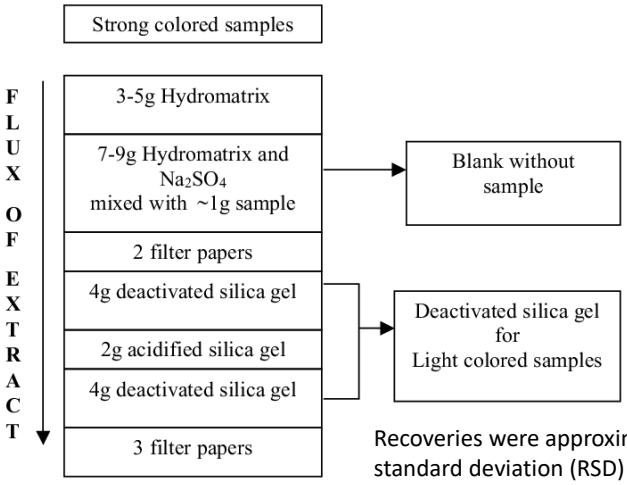
PLE

High temperature and high pressure : low solvent viscosity
- to enhance the **solubility** of the target analytes,
- assist in breaking down **analyte-matrix interactions**
- encourage the **diffusion** of the analyte to the matrix surface

Extraction
- Cell with sample under high T° and 100 bars (under N_2 with extractive solvent)
- n cycles in static mode
- Elution of the extract



PLE – example of method



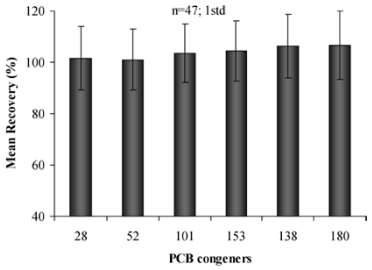


Fig. 2. Recovery of target PCBs from oil and oil-based products.

Recoveries were approximately 100% for all congeners with relative standard deviation (RSD) between 11–13%.

https://www.researchgate.net/figure/Packing-of-cells-for-ASE-extraction-of-PCBs-from-oils-and-oil-based-products-Modified_fig1_5278464

PLE

Advantages :

Extraction can be automated

Short extraction time compared to Soxhlet,

Moderate consumption of solvents,

Simplicity of sample preparation prior to analysis

Increasing of the extraction rates

Useful for organic pollutant (Kow>4)

Limits:

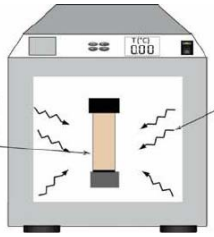
High costs of purchasing and maintaining apparatus,

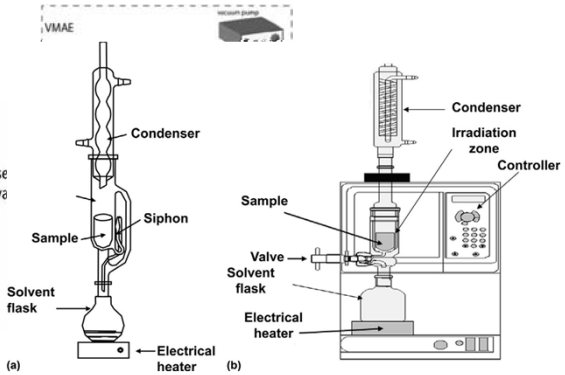
Low extraction selectivity,

Time-consuming cleanup of extracts and equipment

Microwave-Assisted Solvent Extraction (MASE)

- Different types of extraction, arrangement…
- Microwaves - electromagnetic waves, frequency 300 MHz - 300 GHz, used frequency 2450 MHz.



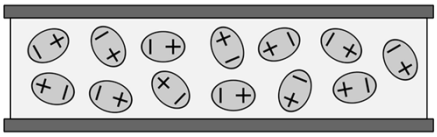


https://www.researchgate.net/figure/Similarities-and-differences-between-a-a-conventional-Soxhlet-extractor-and-b-a-first_fig1_288196194

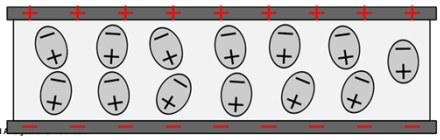
MASE

- Frequency - 2450 MHz:
- Heating - dipole rotation - molecules with a high dielectric constant try to orient themselves in an electric field, but this changes so fast that they start to vibrate and heat up due to friction (collisions of neighboring molecules).

non-polarized dipoles



polarized dipoles by an inserted electric field



Other frequencies will not induce heat
low frequency = molecules orient themselves
high frequency = molecules do not even begin to orient

MASE

Microwave energy absorbing solvent

- The solvent is heated above the boiling point in a closed vessel, accelerating the analyte extraction - high temperature and pressure (up to 200 °C and 175 psi)

Solvent not absorbing microwave energy

- The solvent is not heated, selective heating of certain substances in the sample → release of heated analytes into the cold liquid, closed or open vessel.
- Milder for thermolabile fabrics. The possibility of using liquid carbon dioxide, which does not absorb microwave energy - a substitute for supercritical fluid extraction (SFE). Lower pressure and temperature.

MASE

Boiling points and temperatures of the solvents in the extraction cell

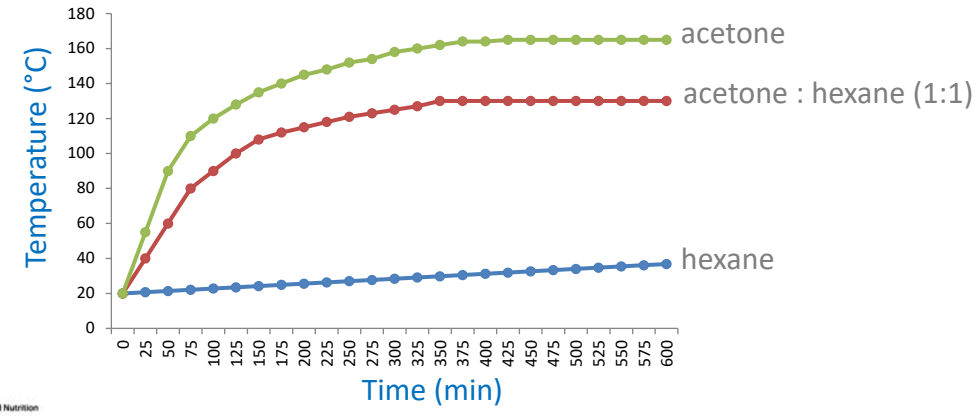
Table 2
Chemical formulas and properties of the solvents used in the experimental part of this study.

Solvent	Chemical Formula	Molecular weight (g/mol)	Boiling point (°C)	Dielectric constant	Dipole Moment (D)	Polarity
Alkanes						
Pentane	C_5H_{12}	72.15	36	1.84	0	Non-polar
Iso-hexane	C_6H_{14}	86.17	60	1.89	0.02	Non polar
Hexane	C_6H_{14}	86.17	69	1.88	0	Non-polar
Heptane	C_7H_{16}	100.2	98	1.924	0	Non-polar
Octane	C_8H_{18}	114.23	126	1.95	0	Non-polar
Alcohols						
Ethanol	C_2H_6O	46.07	79	24.26	1.69	Polar
Propanol	C_3H_8O	60.1	97.15	20.45	1.64	Polar
Butanol	$C_4H_{10}O$	74.12	116–118	17.8	1.74	Polar
Pentanol	$C_5H_{12}O$	88.15	138	13.9	1.91	Slightly polar
Hexanol	$C_6H_{14}O$	102.17	157	13.3	1.74	Non-polar
Others						
Dichloromethane	CH_2Cl_2	92.14	40	9.1	1.5	Slightly polar
Toluene	C_7H_8	84.93	111	2.38	0.36	Non-polar

Solvent	*Boiling point (°C)*	*Closed vessel temperature (°C) at 175 psi*
Dichloromethane	39.8	140
Acetone	56.2	164
Methanol	64.7	151
Ethanol	78.3	164
Acetonitrile	81.6	194
2-Propanol	82.4	145
Acetone–hexane (1:1, v/v)	52+	156
Acetone–cyclohexane (70:30, v/v)	52+	160
Acetone–petroleum ether (1:1, v/v)	39+	147
Dichloromethane–acetone (1:1, v/v)	[a]	160
Toluene–methanol (10:1, v/v)	[a]	110–112
Toluene–methanol (1:10, v/v)	[a]	146

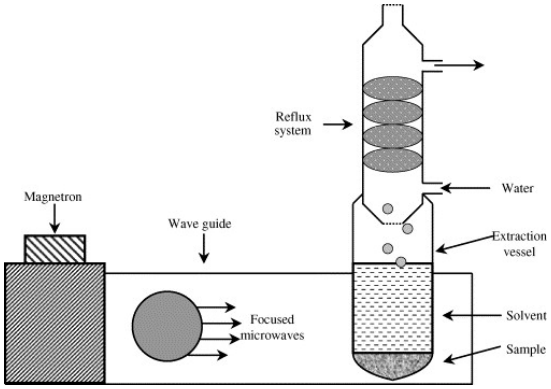
MASE

- Boiling points and temperatures of the solvents in the extraction cell



MASE - example

- MASE with focused field /focused microwaves
- Microwaves aimed at the bottom of the sample container, the neck is cold effective reflux (possible addition of reagents), magnetic stirrer microwave frequency:
- 2450 MHz, variable power 30 - 300 W
- 0.1 - 15 g of sample,
- approx. 30 - 50 ml of solvent,
- within 30 min



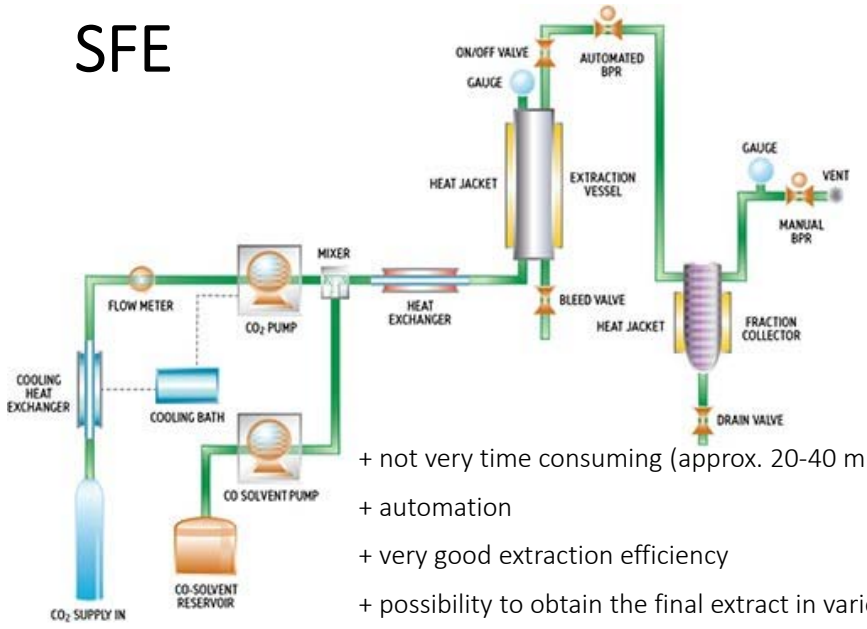
MASE - benefits

- Small amounts of solvents (30-50 ml)
- Speed (minutes x hours Soxhlet) - direct heating of the sample, not the vessel
- Efficiency, reproducibility
- Selectivity
  - can be influenced by the choice of solvent, the heating time
  - local heating and selective extraction and migration of certain substances from the matrix into the solvent
  - Soxhlet extraction - heating of the whole matrix and diffusion of the solvent into the matrix
- Simultaneous extraction of multiple samples

Supercritical fluid extraction (SFE)

- Liquid-solid extraction
- Alternative sample preparation method
- Allows setting of extraction selectivity (temperature, pressure)
- For analyte/matrix combinations, purification by the addition of a sorbent can be performed
- It allows the concentration of analytes in a small volume of solvent
- It requires relatively lengthy optimization for given analyte / matrix combinations
- Fast and easily automated extraction implementation
- Saving of solvents, laboratory utensils, laboratory work and low burden on the environment
- High acquisition costs, specialized service required

SFE



+ not very time consuming (approx. 20-40 min)

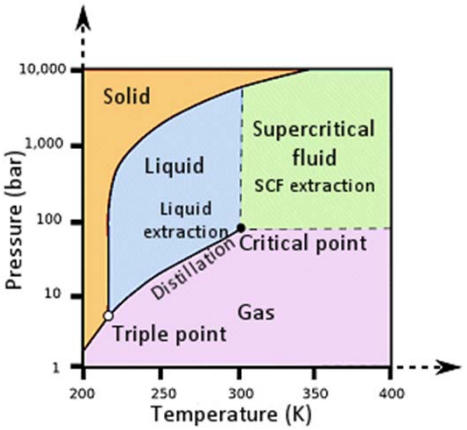
+ automation

+ very good extraction efficiency

+ possibility to obtain the final extract in various organic solvents (as needed)

https://www.labx.com/item/waters-sfe-5lx5l-optimized-for-cannabis-hemp-extractions/4343812

SFE



CO_2

State	Density (kg/m³)	viscosity (cP)	Diffusity (mm²/s)
gas	1	0,01	1-10
SCF	100 - 800	0,05-0,1	0,01 - 0,1
liguid	1000	0,5-1,0	0,001

SFC… supercritical fluid

Critical parameters of different fluids

Fluid	Molar weight (g/mol)	Critical temperature (K)	Critical pressure (MPa (atm))	Density (kg / m3)
carbon dioxide	44,01	304,1	7,38 (72,8)	0,496
nitrous oxide	44,01	309,1	7,29 (71,9)	0,453
freon22(CHClF2)	86,47	369,1	4,96 (49,0)	-
water	18,02	647,3	22,12 (218,3)	0,348
methane	16,04	190,4	4,60 (45,4)	0,162
ethan	30,7	305,3	4,87 (48,1)	0,203
propane	44,09	369,8	4,25 (41,9)	0,217
ethylene	28,05	282,4	5,04 (49,7)	0,215
propylene	42,08	364,9	4,60 (45,4)	0,232
methanol	32,04	512,6	8,09 (79,8)	0,272
ethanol	46,07	513,9	6,14 (60,6)	0,276
acetone	58,08	508,1	4,70 (46,4)	0,278

Supercritical CO_2 Extraction Process