EXTRACTION

- SOLVENT, i.e. LIQUID (LE)

- SOLID PHASE (SPE, SPME, SBSE)

- SUPERCRITICAL FLUID (SFE)

➢ LIQUID EXTRACTION

Liquid samples:

- Liquid-Liquid Extraction (**LLE)**
- Micro-Extraction (**ME)**

Solid samples:

- Liquid-Solid Extraction (**LSE**)
- Microwave Assisted Solvent Extraction (**MASE**)
- Accelerated Solvent Extraction (**ASE)**

  alternatively Pressurised Liquid Extraction (**PLE**)

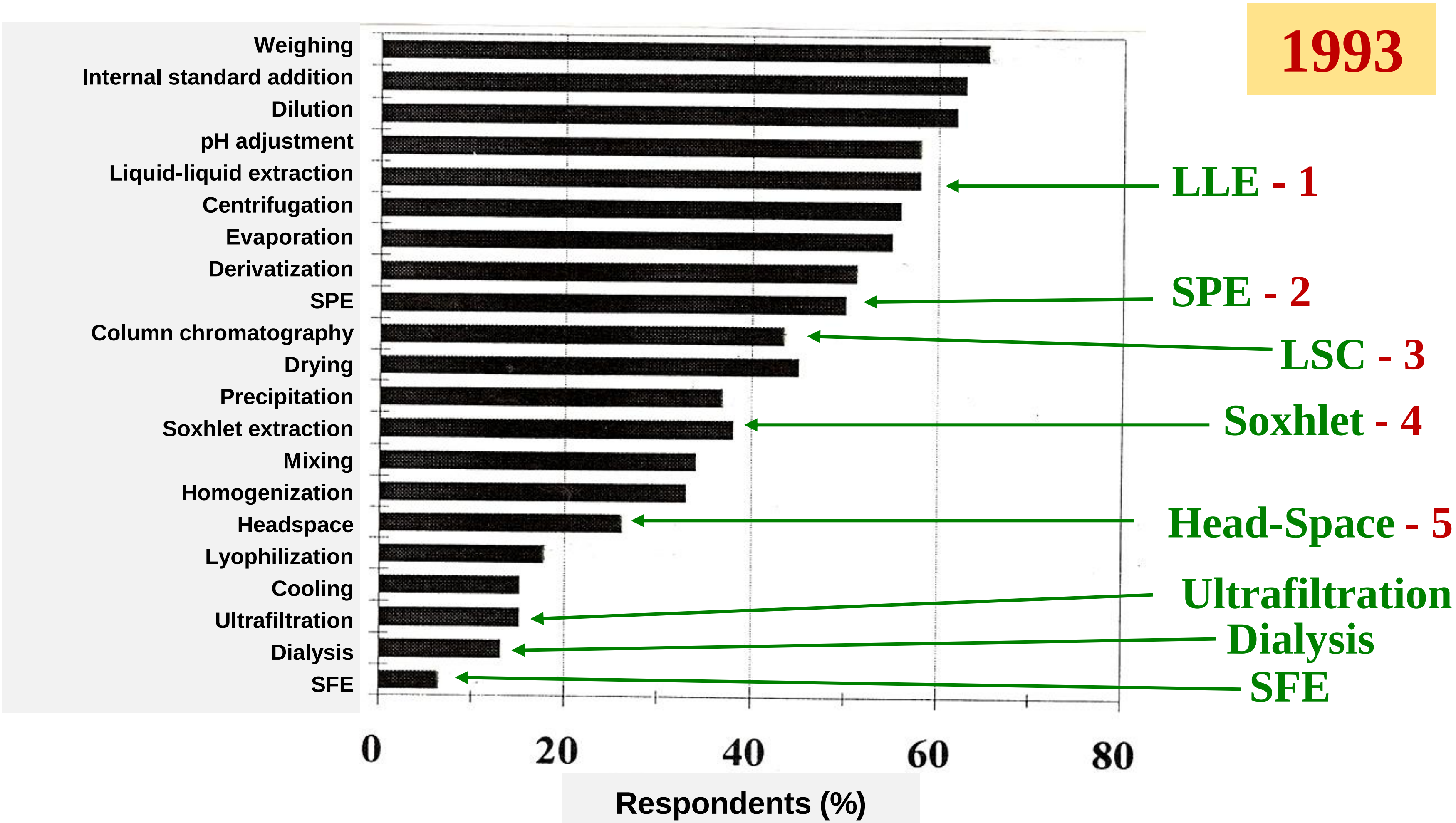
➢ SOLID PHASE EXTRACTION

Liquid samples: direct extraction

- **extraction mini-columns or extraction disks (SPE)**
- **solid phase microextraction (SPME, SBSE)**

Solid samples: after liquid extraction

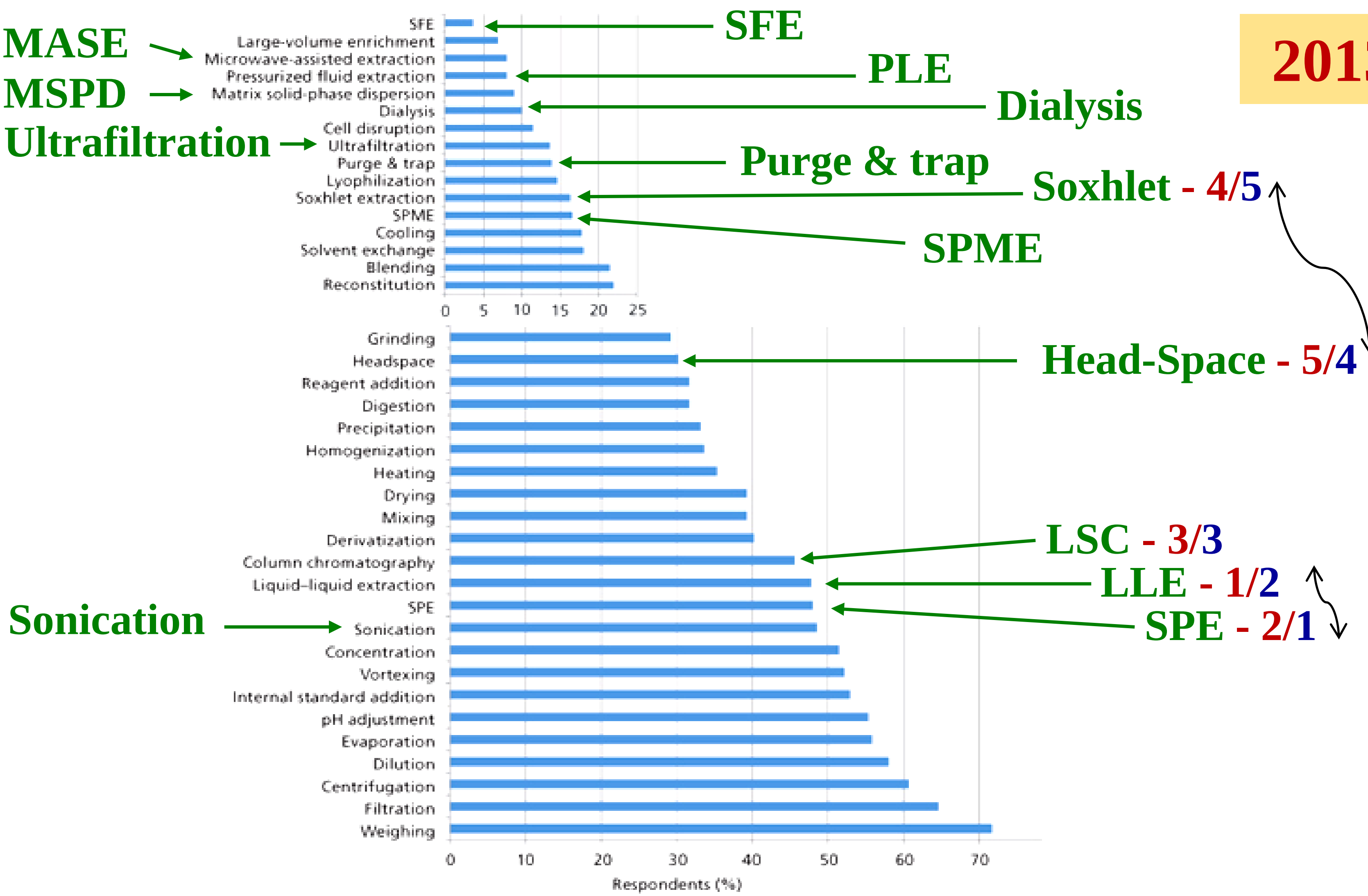
METHODS USED AT SAMPLE PREPARATION - I



R.E. Majors: A Comparative Study of European and American Trends in Sample Preparation, GC-LC INT., V6, no.3, 1993

METHODS USED AT SAMPLE PREPARATION - II

2013



R.E. Majors: Trends in Sample Preparation , LCGC North America, Vol. 31, Issue 3, pp. 190-203, Mar 1, 2013

LIQUID EXTRACTION

- the most commonly used isolation (and separation) method
- **complete or partial transfer of sample matrix to solution**
- **classic methods:**
  - big volumes of solvents - toxicity, cost
  - time demanding, emulsions forming
- **modernization:** automation;
  application of hot and overheated
  solvents (pressure vessels)

LIQUID EXTRACTION – LIQUID SAMPLES

Liquid – liquid extraction (LLE)

- **distribution of analytes and interfering compounds between two immiscible liquids** (mostly aqueous sample *and* extraction organic solvent)
- **extraction solvent influences selectivity and extraction efficiency**
  required parameters: low water solubility (< 10%)
  polarity suitable for good analytes extractability (yield)
  sufficient volatility (easy to concentrate analytes)
- **other factors influencing equilibrium:**
  pH adjustment (suppression of dissociation and ionization
  - acidification for acids)
  also affecting yield of non-ionisables
  - by means of matrix behaviour affecting
  salts addition (salting out effect)
  metal ions addition (ion-pairing)
  addition of chelating and complexing agents (hydrophobic products)

LIQUID EXTRACTION – LIQUID SAMPLES

Liquid – liquid extraction (LLE): REALIZATION

• necessary an **efficient contact of both phases** (mass transfer)

SHAKING – separation funnel, shaker
CENTRIFUGATION, MIXING

• **risk of an emulsion formation**, surfactants and fats presence

Emulsions breaking (reducing, removing):

- salt addition
- heating or cooling of separation funnel
- filtration over glass wool or filter paper
- centrifugation
- addition of a small amount of other organic solvent

LIQUID EXTRACTION – LIQUID SAMPLES

Liquid – liquid extraction (LLE): THEORY

Nernst distribution law – *Any solute distributes between two non-miscible solvents, that the ratio of the concentrations in these two solvents is constant* (at constant temperature and the same molecular condition of solute in the both solvents):

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

K_D *....distribution constant*

c_o ... *analyte concentration in organic phase*

c_{aq} *...analyte concentration in aqueous phase*

- K_D describes equilibrium between analyte concentrations in both phases
  - K_D is the characteristic value for the each analyte in given system
- separation of two substances is achieved, if their K_D values differ

LIQUID EXTRACTION – LIQUID SAMPLES

Liquid – liquid extraction (LLE): THEORY

Extracted amount of analyte (E) - fraction:

$$E = \frac{c_o V_o}{c_o V_o + c_{aq} V_{aq}} = \frac{K_D V}{(1 + K_D V)}$$

K_D ... *distribution constant*
V_o ... *organic phase volume*
V_{aq} …*aqueous phase volume*
V … *phase ratio **V_o/V_{aq}***

derivation of relations

LIQUID EXTRACTION – LIQUID SAMPLES

Liquid – liquid extraction (LLE): THEORY

Distribution constant (K_D):

$$K_D = \frac{c_o}{c_{aq}} = \frac{\frac{n_o}{V_o}}{\frac{n_{aq}}{V_{aq}}} = \frac{n_o}{n_{aq}} * \frac{V_{aq}}{V_o} = \frac{n_o}{n_{aq}} * \frac{1}{V}$$

V … phase ratio V_o/V_{aq}

Extracted amount of analyte (E):

$$K_D * V = \frac{n_o}{n_{aq}} = \frac{c_o * V_o}{c_{aq} * V_{aq}} \Rightarrow K_D * V * c_{aq} * V_{aq} = c_o * V_o$$

$$\mathbf{E} = \frac{c_o * V_o}{c_o * V_o + c_{aq} * V_{aq}} = \frac{K_D * c_{aq} * V_{aq} * V}{K_D * c_{aq} * V_{aq} * V + c_{aq} * V_{aq}} = \frac{\mathbf{K_D * V}}{\mathbf{K_D * V + 1}}$$

LIQUID EXTRACTION – LIQUID SAMPLES

ONE-STEP EXTRACTION

For the quantitative yield is necessary **K_D > 10**,
because in practice is **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%**

MULTI-STEP EXTRACTION

If it is not true, that **K_D > 10**

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

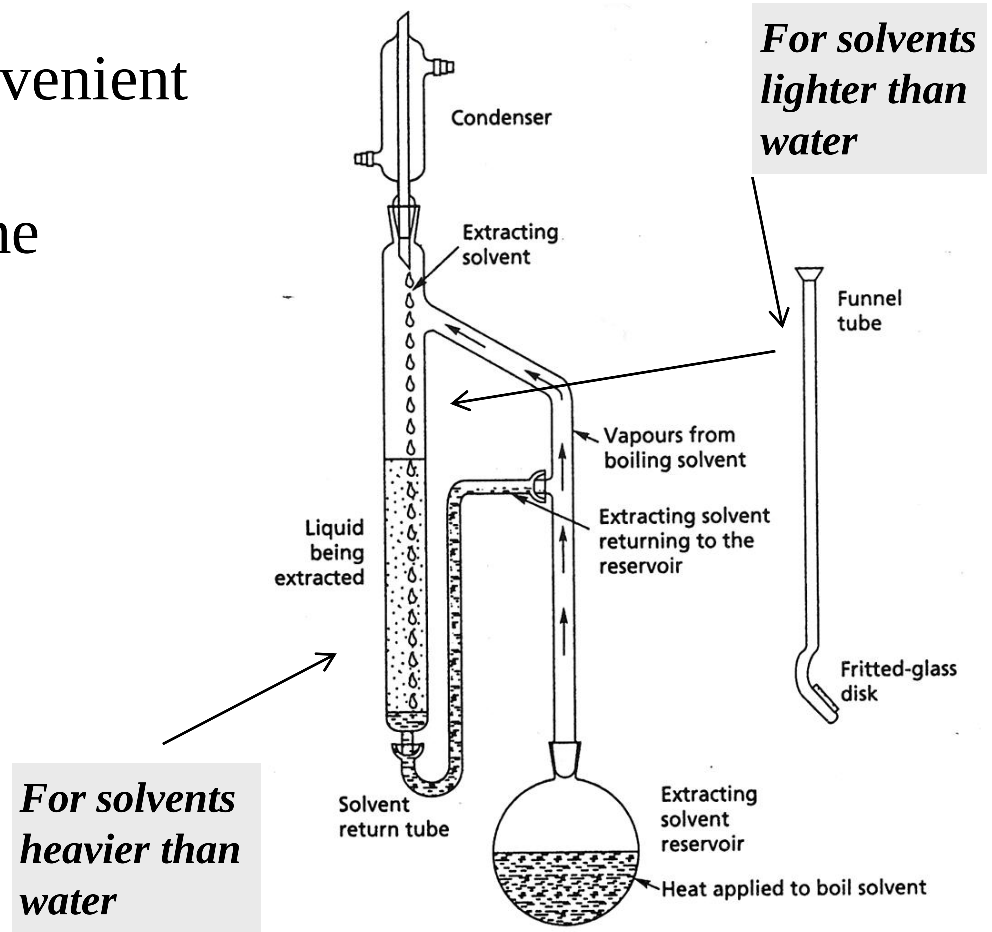
A multi-step extraction with several smaller portions of a solvent is more efficient than an one-step extraction with the total volume of all portions

LIQUID EXTRACTION – LIQUID SAMPLES

CONTINUOUS EXTRACTION

Very small K_D ⇒
multi-step extraction is inconvenient
- too many repetitions
- large total extraction volume
- slow equilibration

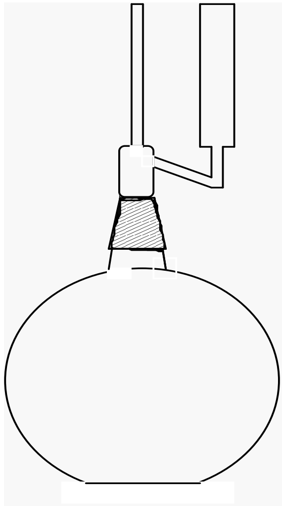
Continuous extractor



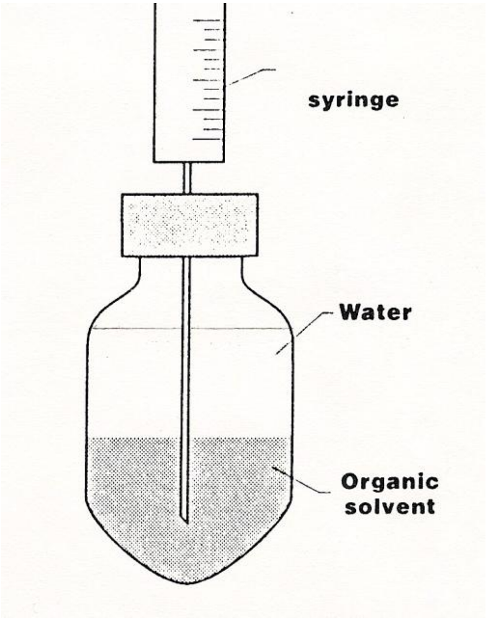
LIQUID EXTRACTION – LIQUID SAMPLES

MICROEXTRACTION (ME) X IN-VIAL EXTRACTION

- **miniaturization: V ≈ 0.001 – 0.01**
- small vol. of solv. lighter than water
- high concentration factor
- lower yield of analytes
- risk of emulsions (clean samples)
- suitable for non-polar analytes
- salting out application



V ≈ 1



LIQUID EXTRACTION – SOLID SAMPLES

Liquid – solid extraction (LSE)

Tranfer of analytes from solid sample to solution (extract)

Sample handling:

- creation of maximum surface per unit of mass

(for small particles = facilitation of extraction)

solid samples: cutting, crushing, grinding, milling,
i.e. disintegration + homogenization

slimy-sticky samples: addition of suitable material
– sand, sorbents (SiO_2), desiccators – Na_2SO_4)
or cryogenic milling using „dry ice“ - CO_2 or liquid N_2

- moisture determination, water removal or immobilization

LIQUID EXTRACTION – SOLID SAMPLES

Liquid – solid extraction (LSE): REALIZATION

Necessary an **efficient contact of both phases** (mass transfer)

SHAKING (+ heating) – shaker (heated)
HOMOGENIZATION – homogenizers
SONICATION – ultra sound bath
REFLUX – termostable analytes)

Forced-Flow Leaching

- sample placed into steel column eluted with a solvent at elevated pressure and temperature closed to its boiling point
- results comparable with Soxhlet extraction, faster realization

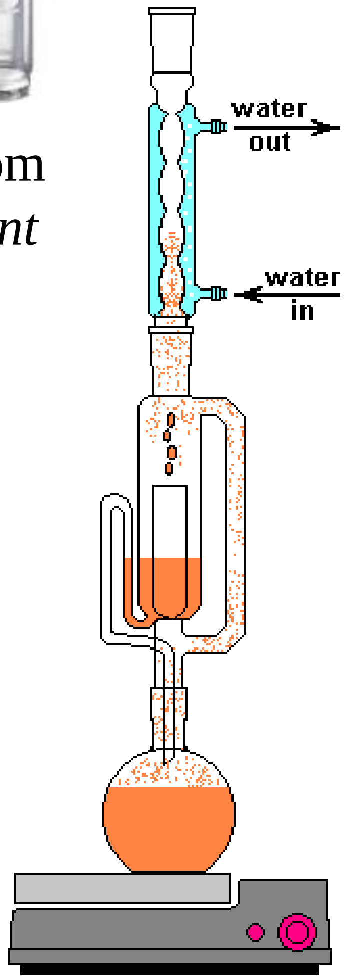
LIQUID EXTRACTION – SOLID SAMPLES

SOXHLET EXTRACTION

Solid sample placed into Soxhlet **extraction thimble**

- cellulose or glass (micro)fibers and/or glass with porous bottom

***Thimble** is placed into **Soxhlet apparatus**; an evaporated solvent condensates and drips to thimble, extracts analytes (and other components) to distillation flask, where the solvent is again evaporated and free of extracted compounds recycling …*



Parameters:

Number of cycles per hour (total number per sample)

Extraction temperature

- given by the boiling point of a solvent: efficiency X stability

Extraction mixture composition X vapours composition

- hexane : acetone (1:1) X azeotropic mixture (3:1)

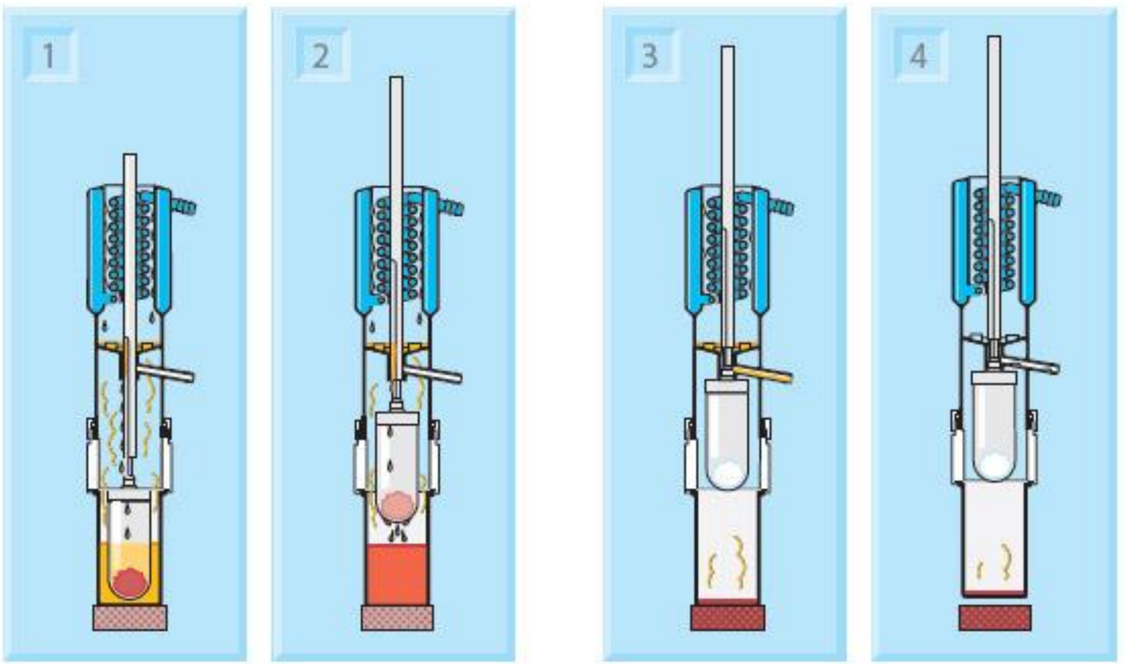
High efficiency (yield) and repeatability X slow process

Cheap and easy – a popular method

Innovations – ultrasound assisted apparatus (ultrasound probe)

LIQUID EXTRACTION – SOLID SAMPLES

SOXTEC® EXTRAKTOR (fa Tecator)



1 **Boiling**
Rapid solubilisation in boiling solvent.

2 **Rinsing**
Efficient removal of remaining soluble matter.

3 **Recovery**
Automatic collection of distilled solvent for re-use.

4 **Auto-shut down**
The system closes down and the cups are lifted from the hot plate.

LIQUID EXTRACTION – SOLID SAMPLES

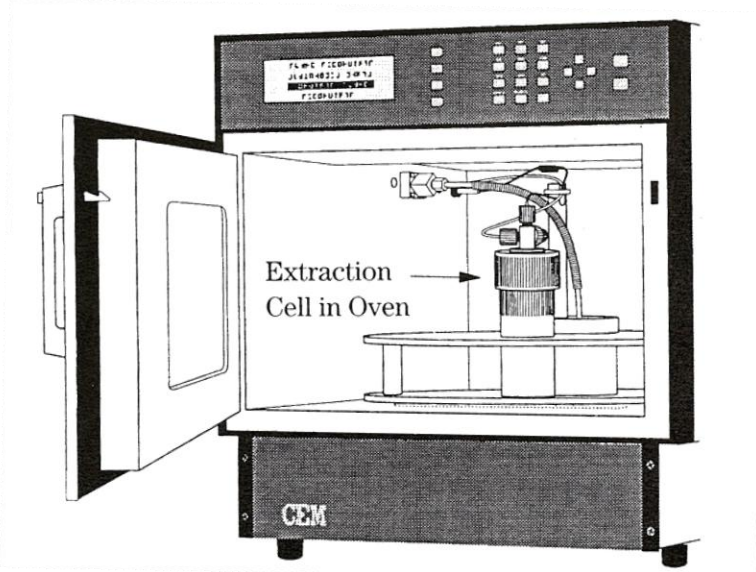
SOXTEC® EXTRACTOR (fa Tecator)

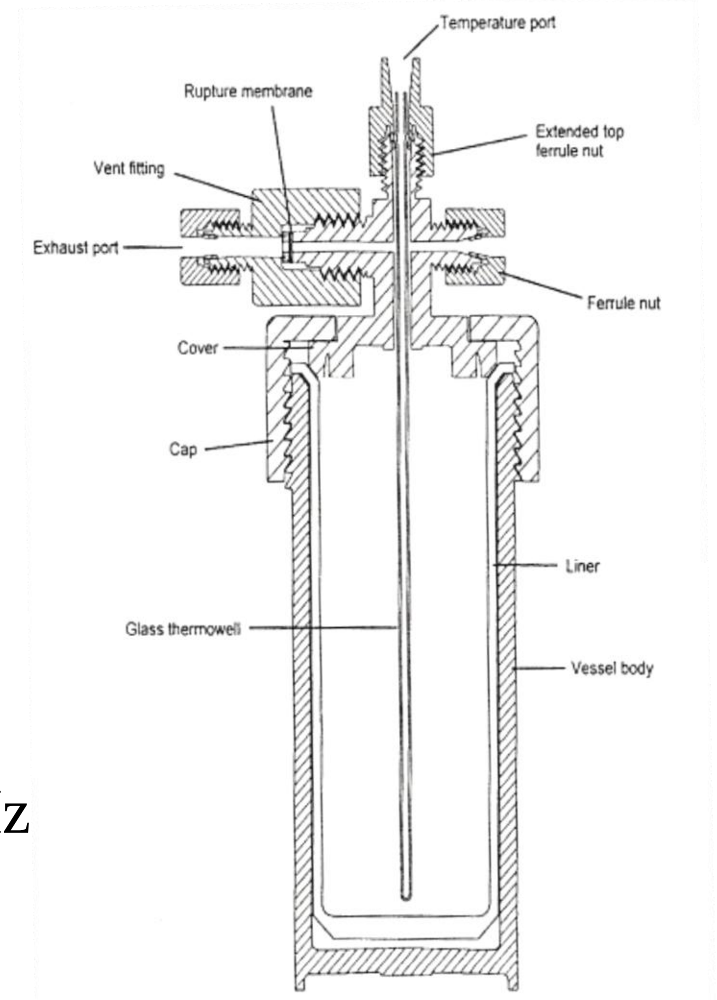
- model HT2, HT6 – up to **12 positions**
- max. sample volume **≈** 25 ml
- extraction speed **30 - 60 min** (Soxhlet 3 - 20 h)
- heating rate 20 - 220°C for 20 min
- suitable for common organic solvents

Advantages: **repeatability** ± 1%
time saving (compare to Soxhlet)
safety
solvents recycling (65%)
simultaneous extraction of several samples
easy operation

LIQUID EXTRACTION – SOLID SAMPLES

Microwave-Assisted Solvent Extraction (MASE)





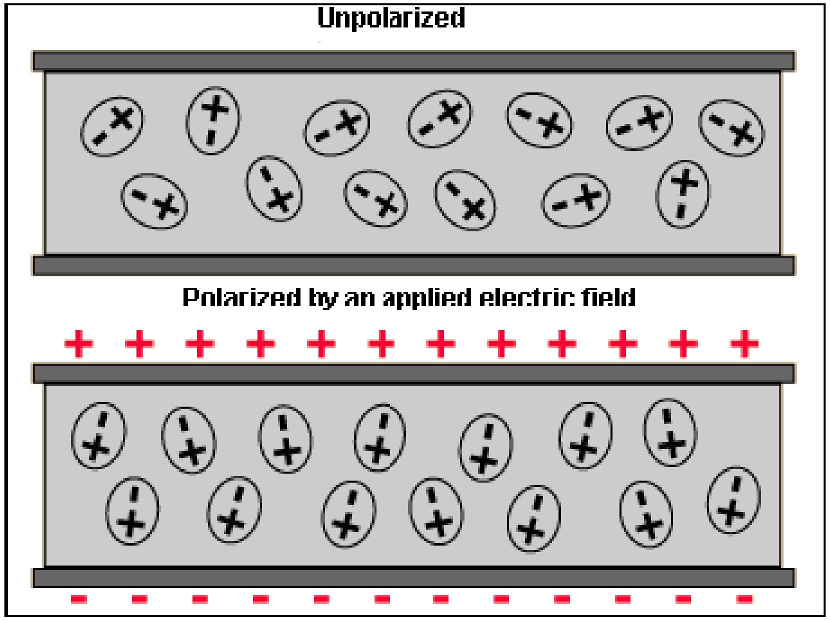
Microwaves

- electromagnetic waves
  with frequency 300 MHz – 300 GHz
- **used frequency 2450 MHz**

LIQUID EXTRACTION – SOLID SAMPLES

MASE (at 2450 MHz):

Heating – dipoles rotation – molecules having **a high dielectric constant** try to orient in electrical field, however, it is rapidly changeable, which induces vibration and friction (collisions of adjacent molecules) resulting in heating.



Different frequency does not induce heating:

↓ **frequency** ⇒ molecules reach stable orientation

↑ **frequency** ⇒ molecules do not try to orient

LIQUID EXTRACTION – SOLID SAMPLES

MASE

Solvent absorbing microwave energy:

a solvent is heated above its boiling point in closed vessel, acceleration of extraction – **high temperature and pressure** (up to 200^{o}C and 175 psi)

Solvent non-absorbing microwave energy:

a solvent is not heated, selective heating of some compounds in sample → **transfer of heated analytes into the cold solvent** in closed or open vessel

moderate conditions – suitable for thermolabile compounds

- use of liquid CO_2 – non-absorbing solvent
  → alternative to SFE at ↓ p, t

LIQUID EXTRACTION – SOLID SAMPLES

MASE – boiling points and extraction temperatures of selected solvents

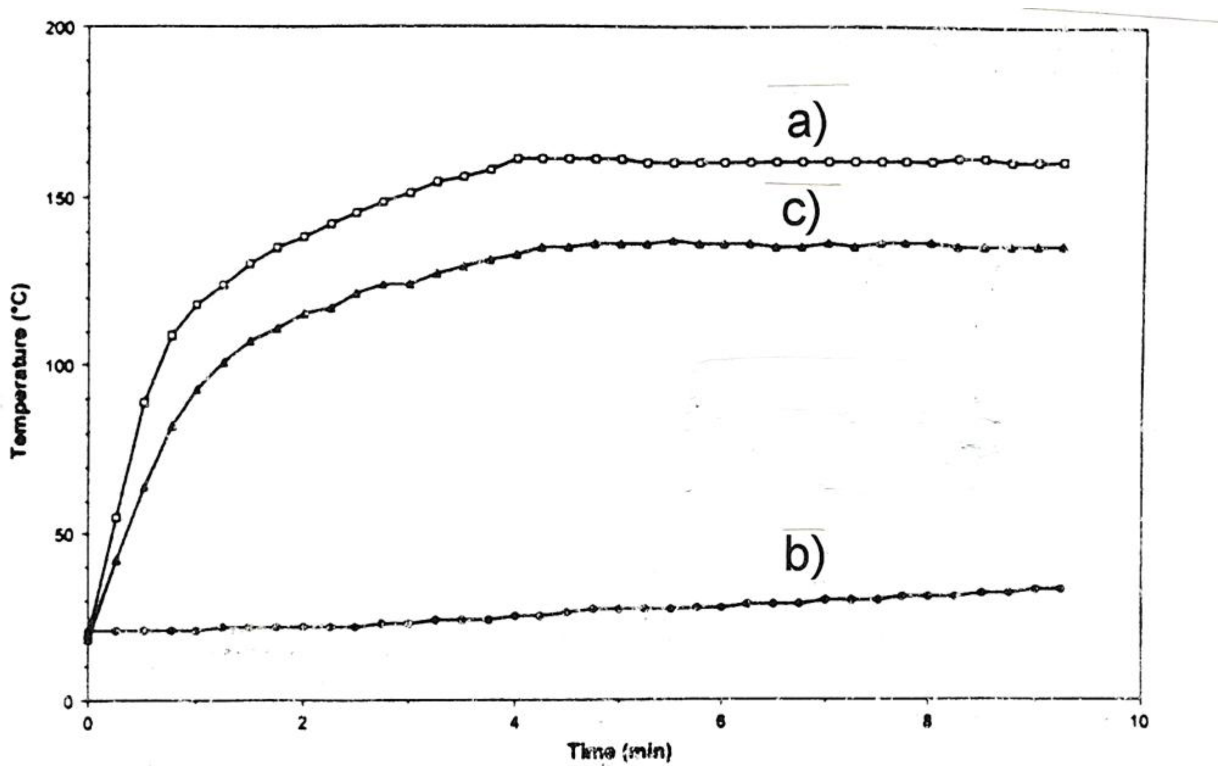
Solvent	Boiling point (°C)	Extraction temp. (°C)
dichloromethane	39.8	140
acetone	56.2	164
methanol	64.7	151
hexane	68.7	*
cyclohexane	80.7	*
acetone:hexane (1:1)	52.0	156

* ... a solvent is not heated by microwave energy

LIQUID EXTRACTION – SOLID SAMPLES

MASE – solvents heating in dependence on time

a) acetone b) hexane c) acetone:hexane (1:1)



LIQUID EXTRACTION – SOLID SAMPLES

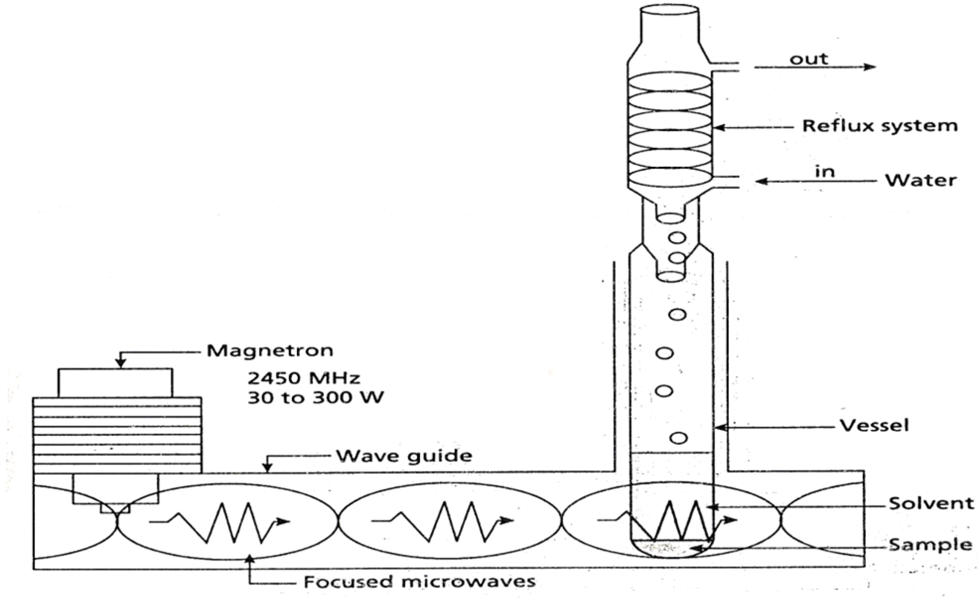
MASE – FOCUSED MICROWAVES

SOXWAVE 100 - PROLABO (France)

1 sample = 1 magnetron

= repeatability, efficiency, speed

(Classic apparatus: 1 magnetron for more samples = dispersed field , sample absorbs non-standard amount of μ-wave energy)



LIQUID EXTRACTION – SOLID SAMPLES

MASE – FOCUSED MICROWAVES

SOXWAVE 100 - PROLABO (France)

- μ-waves focused on bottom of vessel, neck is cool
- efficient reflux (modifiers addition), magnetic stirrer
- μ-wave frequency: **2450 MHz**, changeable power **30 - 300 W**
- **0.1 - 15 g** sample, **30 - 50 ml** solvent, up to **30 min**

SYNTHEWAVE 1000 *(organic synthesis)*
MICRODIGEST 3.6 *(mineralization)*

LIQUID EXTRACTION – SOLID SAMPLES

MASE – advantages

Small amounts of solvents: 30 - 50 ml

Speed (minutes X hours for Soxhlet extraction)

– direct heating of sample – not vessel

Efficiency, reproducibility

Selectivity - solvent selection, heating time

- local (over)heating, selective extraction and migration of certain substances from sample to solvent

X

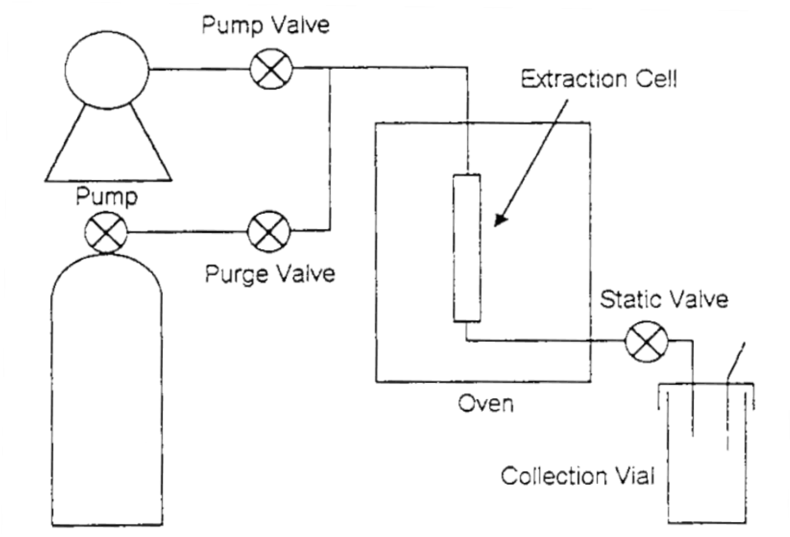
Soxhlet extraction – heating of the whole matrix and diffusion of solvent to matrix

Simultaneous extraction of more samples

LIQUID EXTRACTION – SOLID SAMPLES

ACCELERATED SOLVENT EXTRACTION - ASE
alternatively PRESSURISED LIQUID EXTRACTION – PLE

ASE Extractor™ 200 (Dionex)



Extraction cell

→ steel, i.d. = 19.1 mm

V = 11; 22; 33 ml 24 positions

T up to 200°C, **p** up to 20 Map

Samples: dry, ground

(e.g. + sodium sulphate anhyd.)

Extraction of solid samples – elevated temperature and pressure

↑ T ⇒ ↑ extraction kinetics; ↑ p ⇒ solvent in liquid phase

LIQUID EXTRACTION – SOLID SAMPLES

ASE, PLE – APPLICATION

➢ CRUDE EXTRACT OBTAINING

– necessary next clean-up

– compare to classic methods: *faster*

less solvents consumption

comparable yield

➢ SELECTIVE EXTRACTION

– selection of suitable solvents

– sorbent addition to sample (mixing + layer above sample)

LIQUID EXTRACTION – SOLID SAMPLES

ASE, PLE – extraction of PCBs from fish tissue

Sample: 3g homogenate, Na_2SO_4 + **Al_2O_3**

Solvent: hexane

Temperature: 100 ^{o}C

Pressure: 10 MPa (1500 psi)

Heating: 5 min

Extraction: 5 min (2x)

Total extraction time: 17 min

Yield (22-124 ppb):

PCB 52, 101, 105, 118 - 100 %,

PCB 138, 153 - 42 %

RSD (%): 1.7 – 4.3