

Microwave-Assisted Sample Preparation of Elemental Impurities in Pharmaceutical Products/food supplement and Cannabis plant/products



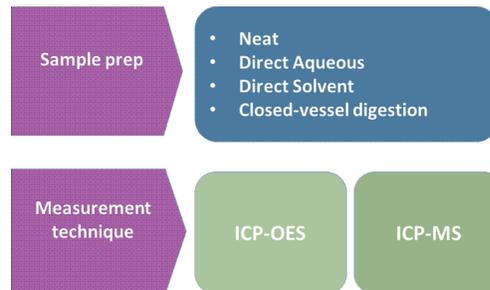
The new method for trace metal analysis in pharmaceutical samples

- The new USP improves the analytical way of pharmaceuticals.
- Better analytical technique improves the pharmaceutical productions.
- Metal determination chapters: USP <232>/<233>
- Implementation date: **1st January 2018.**
- ICH is working to harmonize pharmacopeias



USP<233> Elemental Impurities- Procedures

- New procedures required to measure at the levels described in USP 232.
- Choice of operating parameters and measurement technique is the responsibility of the user.



Users required to perform method validation to prove the applicability of the analytical technique used

Sample Preparation Methods

- Neat
 - Direct analysis of un-solvated samples
- Direct Aqueous Solution
 - Sample soluble in aqueous solvent
- Direct Solvent Solution
 - Sample soluble in organic solvent
- Closed-Vessel Digestion
 - Samples digested with a concentrated acid at elevated temperatures
 - Material is not directly soluble in an aqueous or organic solvent

Sample Digestion techniques



Sample prep for trace analysis

- Any analytical measurement is only as good as the sample prep
- Trace metal analysis puts more demand on the sample prep
- Sample prep is an important component of the analytical process

Organic samples

- Pharmaceutical, food, polymers... samples are organic samples
- Organic samples develop high pressure in the vessels



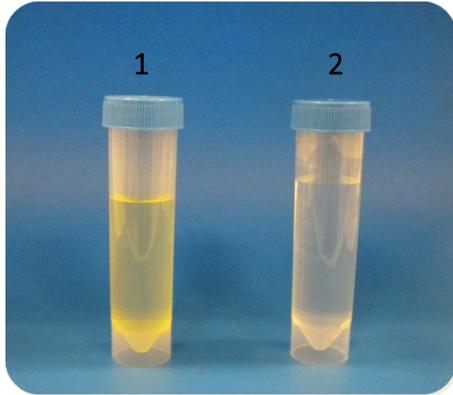
(Metals are in nitrates form)

- Complex organic samples (cyclic compounds, long carbon structure, etc...) requires high temperature conditions for a complete dissolution
- Rotors/ vessels of your microwave system, must be able to handle high T and P conditions

The Effect of the Temperature in a digestion process

Test conditions	Test 1	Test 2
Sample	Plant material	Plant material
Sample amount	0.5g	0.5g
Acid mixture	10mL of HNO ₃ 65%	10mL of HNO ₃ 65%
Vessel Volume	100mL	100mL
Digestion time	20 minutes	20 minutes
Set Temperature	180°C 32 bar	200°C 45 bar

The Effect of the Temperature in a digestion process

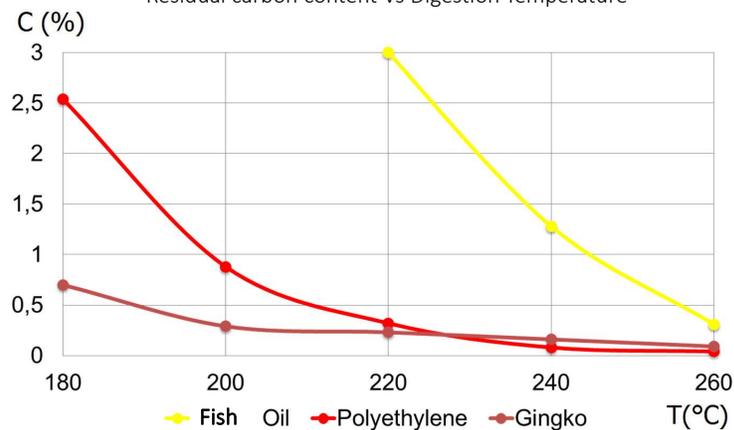


1. Incomplete digestion due to low T and P (180°C and 32 bar)

2. Complete digestion thanks to the high T and P (200°C and 45 bar)

Digestion Quality

Residual carbon content Vs Digestion Temperature



Lower is the carbon residue higher will be the digestion quality. High carbon content generates interferences and clogging in the ICP/ ICP-MS

Arsenic Recovery

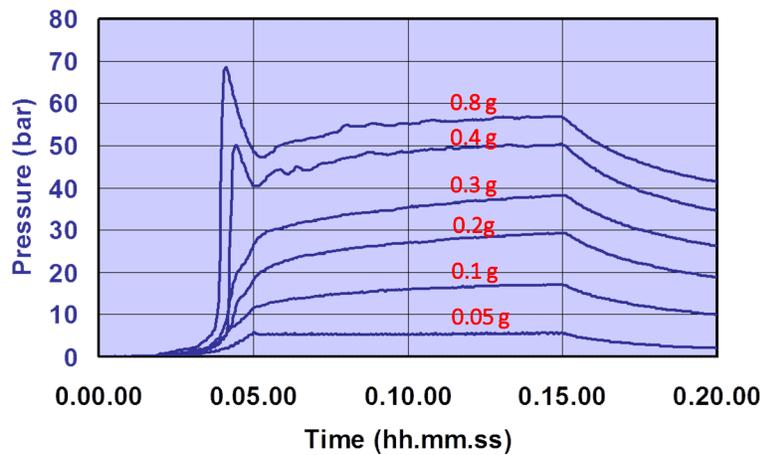
Oxidation of organic Arsenic (Arsenate) in 0.2g TORT 2 (Lobster sample) in 4 mL HNO₃ 69.5%

Temperature	TOC %	Arsenate recovery
220°C	8.3 ± 0.6 %	16%
240°C	2.1 ± 0.5 %	22%
260°C	1.2 ± 0.2 %	43%
280°C	0.4 ± 0.1 %	91%
300°C	<0.1 %	97%

Efficiency of oxidation in wet digestion procedures and influence from the residual organic carbon content on selected techniques for determination of trace elements.

M. Wasilewska,^a W. Goessler,^b M. Zischka,^c B. Maichinc and G. Knapp / JAAS

Effect of Sample Amount on Pressure



Effect of Sample Amount

- Higher pressure conditions
- More exothermic reactions
- Vessel and microwave system have to be capable of withstand or handle such over-pressurization
- Vessel design and materials are key factors for safe microwave digestions

Most common sample digestion techniques

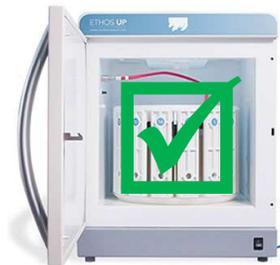
Hotplate systems



Closed Teflon Bomb



Microwave systems



Why “Microwave” Closed Vessel?

Will likely be the most common closed-vessel digestion technique due to:

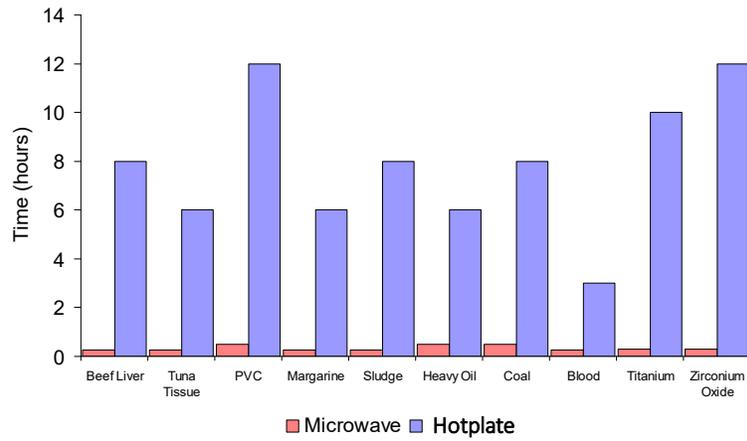
- Established technology in sample prep
- High Safety
- High throughput / productivity
- Easy of use

Advantages of the Microwave digestion system

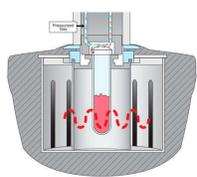
- Speed/ productivity
 - Microwave closed vessel digestion allow fast digestion of multiple samples simultaneously
- Digestion quality
 - Microwave closed-vessel allow to work at high T&P enhancing the digestion quality
 - Full recovery of volatiles elements
- Safety
 - Microwave hardware ensure high level of safety
- Ease of use
 - Built-in application libraries, easy handling
- Full control
 - Temperature and Pressure control



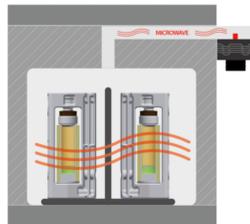
Microwaves save Sample Preparation Time



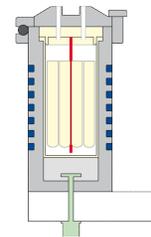
Available Microwave Technologies



Sequential systems



Rotor-based or Multimode



SRC
Single Reaction Chamber

Key parameters to select the right microwave digestion system

- **Sample type/amount** The choice of the right configuration strongly depends on your samples and your analysis technique.
- **Productivity** Number of samples per day/ week
- **Ease of use** Handling time, easy to work with
- **Full control of reaction** Control of temperature and pressure
- **Reliability** Robustness, hardware construction

Milestone SK-15 Milestone solution for the USP

- 15 high pressure positions segmented
- Digestion of large sample size
- Fully closed vessels
- Vent-and-reseal technology
- 100 bar and 300°C (working temp. 230°C)



Quality of Rotor construction

- Our vessels are made in PTFE since it is the best quality material with high chemical resistance.
- All other materials that compound SK-15 rotor, have been selected in order to resist at high T and P conditions reducing the overall consumable costs.
- Milestone is focused on quality since it is an important prerogative for trace metal analysis

Element	PFA	PTFE	Element	PFA	PTFE
B	7,5	1,8	Cr	0,4	0,1
Ba	0,9	< dl	Pb	67	0,03
Bi	0,5	< dl	Sb	0,6	< dl
Cd	4,7	0,1	Zn	0,6	0,2
Co	0,8	< dl	Zr	1	< dl

All values in mg/kg

Ref. Microwave Acid Digestion of Geological and Biological SRMs, T. Noltner – Spectroscopy

Recovery Study

- Sample amount up to 0.5g
- Digested with 10 mL HNO3 65%
- Digestion temperature up to 200 °C

		Sample without Multielement spike		Multielement* spike 25 ppb (**Hg is 50 ppb)		Multielement* spike 50 ppb (**Hg is 100ppb)	
		AVG µg/Kg	AVG %	AVG µg/Kg	AVG %	AVG µg/Kg	AVG %
Mg stearate	As	9.63	-	36.3	107%	52.7	86%
	Hg	<5	-	47.5	95%	91.6	92%
	Pb	<5	-	21.8	87%	46.3	93%
	Cd	<5	-	23.6	94%	47.9	96%
Capsule	As	8.7	-	22.9	91%	52.2	104%
	Hg	0	-	46.0	92%	89.8	90%
	Pb	0	-	25.6	102%	45.6	91%
	Cd	0	-	23.7	95%	46.9	94%
Dietary supplement	As	11.04	-	24.9	100%	53.4	107%
	Hg	0	-	48.5	97%	91.0	91%
	Pb	0	-	23.7	95%	47.3	95%
	Cd	0	-	24.3	90%	47.5	95%

High Temperature and Pressure performances



- Sample type: St John's Wort capsules
- Sample amount: 0.5 g
- Reagents: 8 mL HNO₃
- 15 positions rack (quartz vials)
- 250°C for 15min.
- Complete dissolution

	St John's Wort		
	Spike Conc. ug/g	Spike Result ug/g	Recovery %
As	5.57	5.75	100 %
Cd	1.89	1.88	94 %
Hg	5.57	5.6	101 %
Pb	3.78	3.72	93 %

High Temperature and Pressure performances



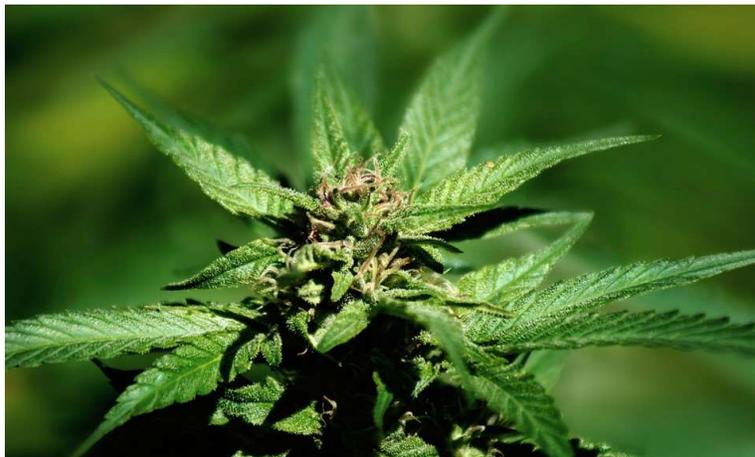
- Sample type: Fish Oil in softgel caps
- Standard injection: 50 ppb
- Sample amount: 1 g
- Reagents: 7 mL HNO₃ + 3 mL H₂O
- 5 positions rack (Quartz vials)
- 250°C for 15min.
- Complete dissolution

	Fish Oil	
	Recovery ppb	Recovery %
As	56.35	112.7 %
Se	56.70	113.4 %
Cd	50.55	101.1 %
Hg	49.36	98.7 %
Pb	50.40	100.8 %

Microwave sample preparation

for heavy metals determination in
cannabis plants and products

Cannabis plants



Cannabis concentrates and edible products



Heavy metal contaminate

source

- Environment
 - water, fertilizers, pesticides...
- Harvesting process
 - tools, machine, collections...
- Extraction process
 - Solvent, clean up, purify oils...
- Packaging



Heavy metal contamination

Elements

- Lead(Pb)
- Cadmium(Cd)
- Copper(Cu)
- Chromium(Cr)
- Arsenic(As)
- Mercury(Hg)
- Cobalt(Co)



Heavy metal contamination

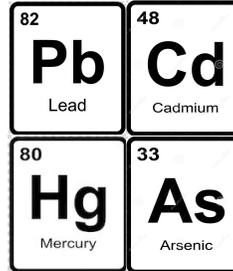
Elements

- Lead(Pb)
- Cadmium(Cd)
- Copper(Cu)
- Chromium(Cr)
- Arsenic(As)
- Mercury(Hg)
- Cobalt(Co)



Heavy metal determination in cannabis plants and products

- Sample preparation
 - Microwave digestion system
 - Single Reaction Chamber(SRC) Microwave digestion system
- Analytical
 - ICP-MS



Microwave sample preparation

Microwave digestion system



Milestone Ethos UP with SK-15 Rotor

Microwave sample preparation

Table I: Range of cannabis products tested, their sample weights, and digestion reagents using the rotor-based microwave system

Sample	Sample Weight Used in Ethos UP – SK15 8 mL Conc. HNO ₃ , 1 mL Conc. HCl, 1 mL H ₂ O ₂
Cannabis plant material (flowers)	1 g
CBD oil (cannabidiol)	0.5 g
Cannabis vape cartridge	0.5 g
Cannabis salve (ointment)	0.5 g
Cannabis flavored cookies	1 g
Cannabis flavored gummy bear	1 g

Table II: Rotor-based microwave program used to digest samples

Step	Time (min)	Temp.	Power
1	20:00	210 °C	1800 W
2	15:00	210 °C	1800 W

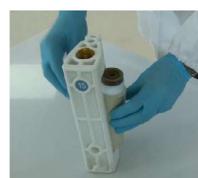
Microwave sample preparation



1. Weight sample



2. Add acid



3. Plate into segment rotor



4. Tighten with torque wrench

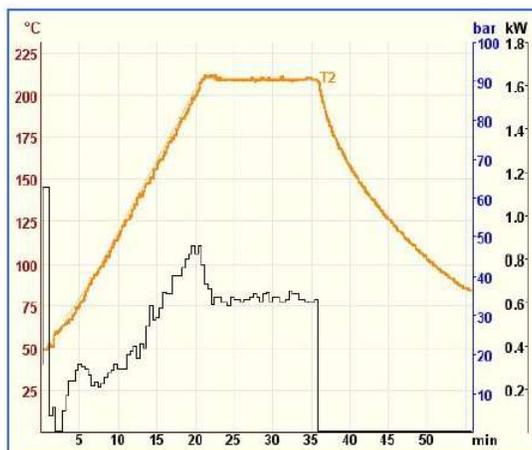


5. Place segment into the rotor plate



6. Close the door and run

Microwave sample preparation



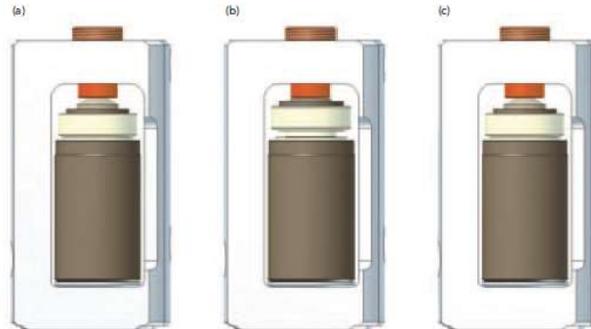
0.5 g to1g of Cannabis oil/Products + 8ml HNO3 + 1ml HCl + 1ml H2O2 @210 C

Analysis result

Table III: Recovery of 20 ppb Pb, As, Cd, and 10 ppb Hg spiked into cannabis products, digested by rotor-based microwave technology, and analyzed by ICP-MS

Cannabis Product	Analyte	As	Cd	Hg	Pb
Cannabis plant material	% Recovery	91.8	92.3	101.4	98.7
	% RSD (n = 3)	2.3	0.7	1.3	1.5
CBD oil	% Recovery	91.3	87.3	105.8	97.3
	% RSD (n = 3)	2.7	2.8	1.4	2.3
Cannabis vape cartridge	% Recovery	94.5	92.8	99.3	102.5
	% RSD (n = 3)	1.5	2.2	1.2	1.5
Cannabis salve	% Recovery	90.7	95.8	102.3	89.2
	% RSD (n = 3)	2.1	1.0	1.5	2.5
Cannabis flavored cookies	% Recovery	92.2	96.2	95.6	93.5
	% RSD (n = 3)	2.1	1.3	1.4	1.0
Cannabis flavored gummy bear	% Recovery	97.8	96.7	98.2	96.7
	% RSD (n = 3)	2.0	0.3	0.4	1.8

Microwave digestion system



Vent and reseal technology

Microwave sample preparation

Single Reaction Chamber(SRC) Microwave digestion system



Milestone ultraWAVE

Microwave sample preparation

Table IV: Range of cannabis products tested, their sample weights, and digestion reagents using the SRC microwave system

Sample	Sample Weights Used in the UltraWAVE 4 mL Conc. HNO ₃ , 1 mL Conc. HCl
Cannabis plant material (flowers)	1 g
CBD oil (cannabidiol)	1 g
Cannabis vape cartridge	1 g
Cannabis salve (ointment)	1 g
Cannabis flavored cookies	1 g
Cannabis flavored gummy bear	1 g

Table V: SRC microwave program used to digest samples

Step	Time (min)	Temp. 1	Temp. 2	Pressure	Power
1	20:00	240 °C	60 °C	110 bar	1500 W
2	10:00	240 °C	60 °C	110 bar	1500 W

Microwave sample preparation



1. Weight sample



2. Add acid into the vial



3. Close the vial



4. Prepare the TFM Liner



5. Place the sample rack



6. Close the chamber



7. Load pressure and run

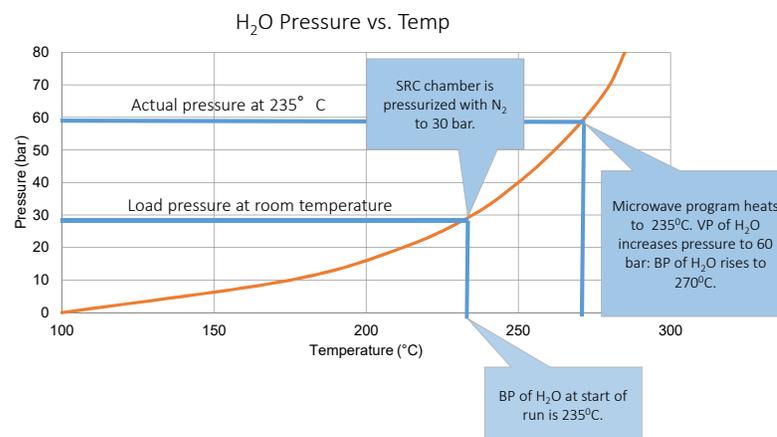
Analysis result

Table VI: Recovery of 20 ppb Pb, As, Cd, and 10 ppb Hg spiked into cannabis products, digested by SRC technology, and analyzed by ICP-MS

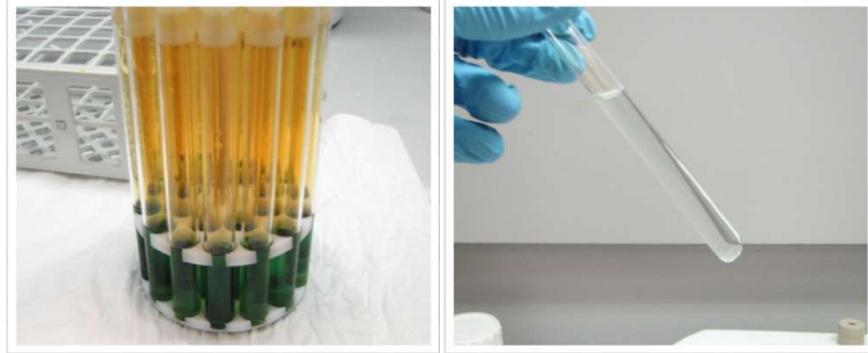
Cannabis Product	Analyte	As	Cd	Hg	Pb
Cannabis plant material	% Recovery	91.7	93.0	98.7	88.3
	% RSD (n = 3)	1.9	2.1	2.1	2.6
CBD oil	% Recovery	95.8	98.5	97.6	89.7
	% RSD (n = 3)	1.8	2.3	1.1	2.2
Cannabis vape cartridge	% Recovery	90.8	87.3	91.8	92.0
	% RSD (n = 3)	1.1	2.0	1.2	1.5
Cannabis salve	% Recovery	95.8	91.5	94.3	95.3
	% RSD (n = 3)	0.3	1.1	1.4	2.2
Cannabis flavored cookies	% Recovery	92.8	93.8	96.1	93.3
	% RSD (n = 3)	2.8	0.7	1.3	1.4
Cannabis flavored gummy bear	% Recovery	90.2	89.5	94.1	91.8
	% RSD (n = 3)	2.1	2.0	1.0	2.2
Soil (SRM 2711a)	Leachable Conc. (mg/kg)	89	47	7.4	1300
	% Recovery	90.4*	94.1*	98.7*	93.3*
	% RSD (n = 3)	2.1	1.9	1.6	1.1

* Note recovery based on leachable certificate values.

Single Reaction Chamber(SRC) Microwave digestion system



Digested Samples



Thank you for your attention

