APPLICATION REPORT

MICROWAVE DIGESTION FOR PHARMACEUTICAL SAMPLE PREPARATION IN ACCORDANCE WITH **USP <232>/<233>**

Introduction

The new USP chapters <232> and <233> for the measurement of inorganic contaminants in pharmaceutical samples are due to be implemented. While samples that are soluble in aqueous and organic solvents may be analyzed directly, a large proportion of samples will require digestion. Actually, digestion is expected to be the preferred technique for ICP-MS analysis even if the sample is soluble in organic solvent.

Closed-vessel digestion is stipulated by USP and it is expected that microwave digestion will be the predominant digestion technique to be used: its high pressure and temperature capability offer greater digestion potential than hot plate closed vessel digestion, for example.

Single reaction chamber (SRC) microwave digestion is a relatively new type of closed vessel microwave digestion technique that significantly differs from traditional closed vessel microwave digestion. A commercially available benchtop SRC digestion system can digest up to 15 samples simultaneously, at high temperature and pressure conditions. This high temperature and pressure capability enables the complete digestion of virtually every pharmaceutical sample type, producing digested solutions with a very low total organic carbon (TOC) content, which in turn is beneficial for ICP-MS analysis.

Three sample types, Dietary Supplement, Fish Oil Pill and Magnesium Stearate, were digested using an UltraWAVE (SRC) digestion system and analyzed for the toxic USP elements using ICP-OES.

Since all samples are digested together in a single chamber with SRC, duplicates and spike recoveries were performed to confirm the retention of volatile elements and the absence of cross contamination.

Instrumentations

The SRC features a large 1 liter pressurized stainless steel reaction chamber, which also serves as the microwave cavity.

This enables the intensity and distribution of the delivered microwave energy to be optimized to the shape of the reaction vessel, ensuring even heating without the need to rotate samples during the digestion program.

Samples are placed inside the SRC together and digested simultaneously. Because the samples are placed inside a pressurized vessel, individual

pressure vessels are not needed. Samples are weighed into auto sampler-type vials with the appropriate digestion acid and loaded into a rack.

The rack is loaded into the chamber, which is then sealed and pre-pressurized with nitrogen to **40 bar** prior to microwave heating. Pre-pressurization prevents splashing or boiling of the sample solutions, avoiding cross contamination or loss of volatiles.

SRC can operate at very high temperature and pressure – up to 300°C and 200 bar, enabling the complete digestion of every sample-type.
The higher pressure capability of a SRC system allows higher sample quantities to be digested – up to 1 gm organic in a 15 position rack.

With SRC, different sample types can be run simultaneously – there is no need to "batch" digestion runs into identical sample types as needed with traditional microwave digestion systems. For example, raw materials and finished products can all be digested together in the same run.

The SRC also requires less digestion acid (typically 2-4 mL), which lowers the reagent blank.
On completion of the program, the chamber is vented and the rack removed. Samples are diluted to volume in the vials, ready for aliquoting and measurement.

Analytical Procedure

Here are the details of the UltraWAVE microwave program and Pharmaceutical samples used for the test.

Sample	Weight	Multielement Spike added to samples	Reagents	Vials Configuration
Fish Oil	1.2 g	50 ppb	7 mL of HNO3 + 3 mL of H2O	Quartz vials - 35 mL volume
Magnesium Stearate	1.0 g	50 ppb	6 mL of HNO3 + 2 mL of H2O	Quartz vials - 35 mL volume
Dietary Supplement	1.0 g	50 ppb	6 mL of HNO3 + 2 mL of H2O	Quartz vials - 35 mL volume

Microwave program:

Step	Time	T1	Power
1	00:10:00	120°C	1500 W
2	00:03:00	120°C	1500 W
3	00:08:00	160°C	1500 W
4	00:05:00	160°C	1500 W
5	00:10:00	250°C	1500 W
6	00:10:00	250°C	1500 W

ICP-OES Parameters

Power	1.30 kW
Plasma Flow	15.0 L/min
Auxiliary Flow	1.50 L/min
Nebulizer Flow	0.75 L/min
Replicate read time	10 s
Instrument stabilization delay	15 s
Sample Uptake Delay	30 s
Pump Rate	15 rpm
Rinse Time	10 s
Replicates	3

ICP-OES Results

The samples were completely digested, forming a clear digestate, which was made up to 50 ml with DI water. The samples were analyzed for toxic elements like Lead (Pb), Cadmium (Cd), Mercury (Hg), Arsenic (As) and Selenium (Se).
Duplicate analysis is generally good for all samples and excellent recovery was obtained for the spiked samples and for the spiked reagent blanks.
Excellent recoveries for Hg demonstrate the effectiveness of pre-pressurization of the SRC prior to the digestion sequence. Volatile elements such as Hg have not been lost and no cross contamination of samples occurred caused by sample splashing or boiling.

	Blank	Fish Oil		Magnesium Stearate		Dietary supplement	
	ppb	ppb	recovery	ppb	recovery	ppb	recovery
As	<	56.35	112.7 %	52.75	105.5 %	56.20	112.4 %
Se	<	56.70	113.4 %	44.80	89.6 %	48.80	97.6 %
Cd	<	50.55	101.1 %	44.20	88.4 %	45.70	91.0 %
Hg	<	49.36	98.7 %	47.70	95.4 %	50.11	100.2 %
Pb	<	50.40	100.8 %	46.20	92.4 %	49.30	98.6 %

Conclusions

Milestone's SRC microwave digestion (UltraWAVE) offers multiple benefits for sample preparation for trace metals analysis over conventional benchtop microwave digestion systems.
Due to its higher sample capacity, use of disposable vials and faster cooling down time, sample processing throughput is 2x – 3x higher than conventional closed vessel digestion. The better digestion quality achieved at higher temperatures (and pressure) makes analysis by ICP-MS more accurate.
The data showed in this technical note demonstrates that the digestion of samples in a SRC, in loosely capped vials, does not negatively impact analytical data quality. While the ability to digest different sample types together and larger sample weights with minimum acid volume (1-4 mL per sample) makes it the suitable technique to perform pharmaceutical sample prep for trace metals analysis.