



PREPARATION OF PHARMACEUTICAL SAMPLES IN ACCORDANCE WITH USP <232>/<233>



Utilizing Single Reaction Chamber (SRC) Technology for trace metals analysis for pharmaceutical samples.

| INTRODUCTION

With stricter industry regulations now in place, demand for trace metals analysis at lower detection levels has reached an all-time high. ICP, once the standard for pharmaceutical metals analysis, is rapidly being replaced by ICP-MS, placing increased emphasis on sample preparation methodologies. Closed-vessel microwave digestion has proven to be an effective technique, offering fast, complete digestions, a clean environment, and effective recovery of volatile compounds. The single drawback has been the inability to run digestion on several matrix types simultaneously. Milestone's Single Reaction Chamber (SRC) microwave digestion is a revolutionary new approach, incorporating all of the benefits of closed vessel microwave digestion with new levels of convenience and effectiveness.

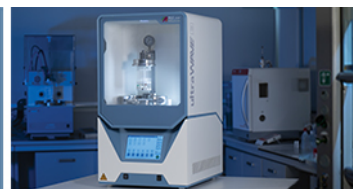
The Milestone ultraWAVE 3 is a benchtop instrument that operates at very high pressures and temperatures, capable of processing large, dissimilar and difficult samples quickly, easily—all without

batching. The data shown in this technical note demonstrates that the digestion of samples in the ultraWAVE 3 results in uniformly high analytical data quality, making it the ideal solution for trace metals detection in pharmaceutical samples.

This industry report describes how a variety of samples from the pharmaceutical industry were digested simultaneously using the Milestone's ultraWAVE 3, and this can be replicated in previous ultraWAVE generation, without sample-to-sample cross contamination.

| EXPERIMENTAL

Following the optimization of digestion methodology (vial type, digestion matrix and temperature program), dietary supplements were digested and analyzed for the "big four" toxic elements. Good QC data demonstrates the suitability of SRC microwave digestion for this application. New USP chapters <232> and <233> for the measurement of inorganic



contaminants in pharmaceutical samples have been implemented. While samples that are soluble in aqueous and organic solvents may be analyzed directly, a large portion of samples will require digestion, and in fact, digestion may be preferred 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 used: its high pressure and temperature capability offering greater digestion power than hot plate open vessel digestion for example.

SRC microwave digestion is a relatively new type of closed vessel digestion that differs significantly from traditional closed vessel digestion. A commercially available benchtop SRC digestion system can digest up to 15 samples simultaneously, at high temperature and pressure. This high temperature and pressure capability enables the complete digestion of virtually every pharmaceutical sample type, producing digest solutions with a very low total organic carbon (TOC) content which is beneficial for ICP-MS analysis. Two sample types, St. John's Wort and fish oil, typical of finished product pharmaceuticals, were digested using an SRC digestion system and analyzed for the four toxic USP elements using collision cell ICP-MS to evaluate the effectiveness of SRC digestion for this application. 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.

INSTRUMENT

- Milestone's ultraWAVE 3
- 20-positions rack with 15 mL glass vials
- Analytical balance
- ICP-MS

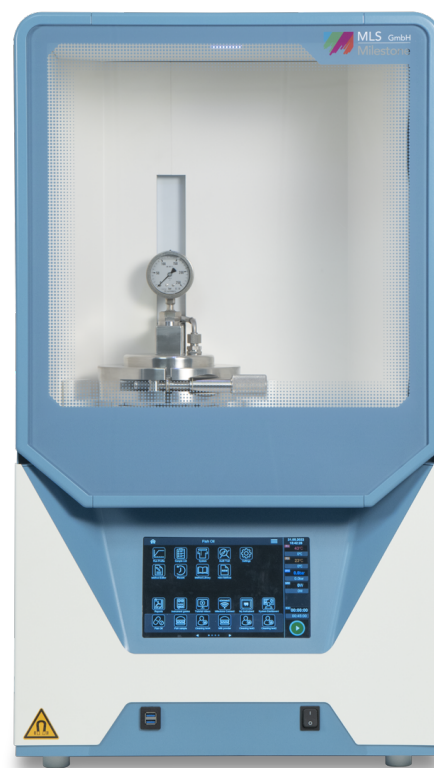


Figure 1 – Milestone's ultraWAVE 3

Developed and patented by Milestone, the ultraWAVE 3 represents another significant step forward for SRC technology and embrace Milestone 20+ years of experience. The stainless-steel reactor with a high-purity PTFE-TFM liner and cover, enables to achieve, higher pressures and temperatures regardless the sample type and acid mixture. The digestion process is continuously controlled by easyTEMP, an advance contactless sensor that measure the temperature directly of the reaction chamber. The simplified rack construction eliminates the time need to assemble and disassemble the vessels. Just as important, dissimilar samples can be processed simultaneously using disposable glass, quartz or PTFE-TFM vials, thus saving time and money. The ultraWAVE 3 addressed all the sample preparation challenges related to performance, time, workflow, and cost of ownership.



PROCEDURE AND METHOD OPTIMIZATION

Sample vials used in SRC instrumentation are typically available in quartz, TFM (a high temperature polymer) and borosilicate glass. The benefit of glass is very low cost which makes them disposable, eliminating vial cleaning procedures. The drawback of glass is elevated backgrounds (ppb level) for some elements - namely B, Na, Mg, Al, K and Ca.

However, since these elements are not stipulated in USP <232>, glass vials can be used. Fig. 3 shows the digestion blanks obtained from glass, quartz and TFM digestion vials for USP elements. In this data, Ru, Os and the Pt group elements were not measured. However, it can be assumed that the vial contribution for these elements is extremely low.

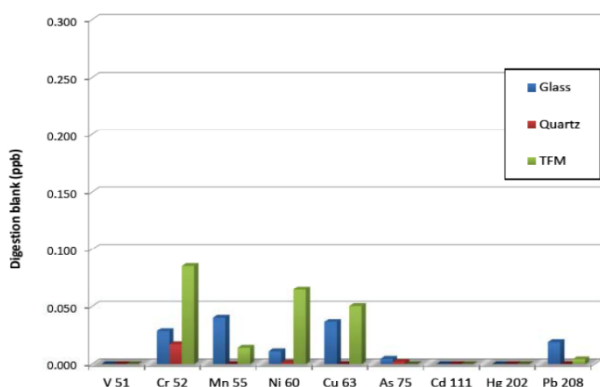


Figure 2 - Digestion blanks obtained from 2 different vial materials selected USP elements

Because the SRC system is capable of very high pressure, higher weights of high organic content sample can be digested, including whole gel caps, which is a benefit for pharmaceutical sample analysis. Also, the higher-pressure capability allows a higher temperature to be achieved, which gives a more complete digestion. Even with high organic content samples such as oils, virtually all the organic carbon is decomposed to CO₂ giving the sample digest and very low TOC content. This is a benefit for ICP-MS analysis, as the presence of carbon in the sample enhances on the sensitivity of poorly ionized elements, thus enhancing repeatability and reliability.

SAMPLES

Two set of samples were prepared for analysis by two different instruments: ICP-OES and ICP-MS.

The first set of samples analyzed by ICP-OES, includes fish oil capsules, magnesium stearate and a dietary supplement (Table 1). St. John's Wort has been analyzed by ICP-MS (Table 2) together with a laboratory fortified blank.

SAMPLE PREPARATION

Digest matrix depends on the sample type and weight. An amount of 0.5 g of each pharmaceutical sample were weighed inside a 15 mL glass vial and 4 mL of 65% HNO₃ + 1 mL of 37% HCl were added together with a 50 ppb of a multielement solution. For fish oil gel caps, an entire gel cap (1 g) was digested with 9 mL HNO₃ + 1 mL HCl.

The vial size used was 15 mL, allowing 15 samples to be digested simultaneously. Since all samples are digested together under the same pressure and temperature control, different sample weights and acid chemistries can be digested simultaneously. The only requirement is that the digestion temperature selected must be sufficient to digest the most difficult sample in the batch.

The rack with the 20 glass vials was positioned inside the SRC TFM liner inside the reaction chamber, which was previously filled with 120 mL of H₂O and 5 mL of HNO₃. The SRC system was pressurized up to 40 bar with Nitrogen gas to close the glass vials. The following microwave heating program was applied:

Step	Time (min)	Power (W)	T1 (°C)	T2 (°C)	P (bar)
1	20	1500	240	60	90
2	20	1500	240	60	90

Table 1 - Microwave Program

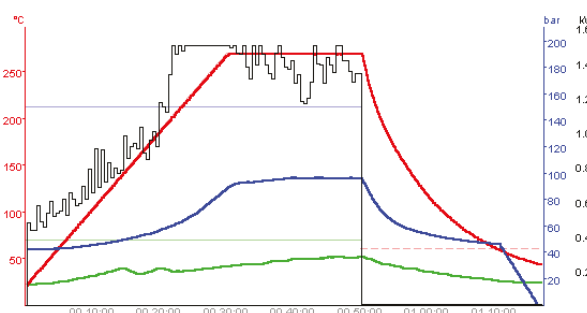


Figure 3 - Microwave program from simultaneous digestion of multiple pharmaceutical samples.

INDUSTRY REPORT

ultraWAVE 3 | PHARMACEUTICAL



ICP-OES AND ICP-MS RESULTS

Two set of samples were prepared for analysis by two different instruments: ICP-OES and ICP-MS. The first set of samples analyzed by ICP-OES, includes fish oil capsules, magnesium stearate and a dietary supplement (Table 1). St. John's Wort has been analyzed by ICP-MS (Table 2) together with a laboratory fortified blank.

Element	Blank	Spike	Fish Oil		Magnesium Stearate		Dietary Supplement	
	ppb	ppb	ppb	recovery	ppb	recovery	ppb	recovery
As	<	50	56.35	112.7 %	52.75	105.5 %	56.20	112.4 %
Cd	<	50	50.55	101.1 %	44.20	88.4 %	45.70	91.0 %
Pb	<	50	50.40	100.8 %	46.20	92.4 %	49.30	98.6 %
Hg	<	50	49.36	98.7 %	47.70	95.4 %	50.11	100.2 %

Table 1: ICP-OES analysis of the "Big Four" USP analytes on fish oils, magnesium stearate and dietary supplement following digestion in the ultraWAVE 3. Samples were spiked with 50 ppb of a multielement solution prior digestion.

St. John's Wort (µg/g)			Laboratory fortified blank (µg/g)			
Element	Detection limit	Sample result	Sample result	Spike conc	Spike result	Spike % recovery
As	0.008	0.184	ND	5.6	5.39	96
Cd	0.003	0.109	ND	1.9	1.86	98
Pb	0.03	0.24	ND	3.8	3.58	94
Hg	0.1	ND	ND	5.6	6.06	106

Table 2: ICP-MS analysis of the "Big Four" USP analytes in St. John's Wort & fish oil gelcaps following digestion in the ultraWAVE 3. The table shows the recovery study on St. John's Wort and on a laboratory fortified blank.

CONCLUSIONS

Milestone's Single Reaction Chamber technology offers multiple benefits for sample preparation for trace metals analysis over conventional 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 systems. The superior digestion quality achieved at higher temperatures (and pressure) makes analysis by ICP-OES and ICP-MS more accurate.

The data shown in this technical note demonstrates that the digestion of samples in a SRC, in vials with loose-fitting caps ensures complete recovery and full digestion. Furthermore, the ability to digest different sample types together and larger sample weights with minimum acid volume (1-4 mL per sample) makes it the optimal technique to perform pharmaceutical sample prep for trace metals analysis.



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