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

Utilizing Single Reaction Chamber (SRC) Technology for trace metals analysis of 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 system is a revolutionary new approach, incorporating all the benefits of closed-vessel microwave digestion with new levels of convenience and effectiveness.

The Milestone ultraWAVE is a benchtop instrument that operates at very high pressures and temperatures, capable of processing large, dissimilar and difficult samples quickly, and easily—all without batching. The data shown in this technical note demonstrates that the digestion of samples in the ultraWAVE results in uniformly high-quality analytical data, making it the ideal solution for trace metals detection in pharmaceutical samples.

| 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 an 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 offer 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 allow for 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 pharmaceuticals products were digested using an SRC digestion system. The samples were subsequently analyzed for the four toxic USP elements using collision cell ICP-MS. 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.

INSTRUMENTATION

The SRC features a large (typically 1 liter) pressurized reaction chamber, which is also the microwave cavity. This enables the intensity and distribution of the delivered microwave energy to be optimized to the shape of the reaction vessel. This ensures even heating and eliminates 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 autosampler-type vials with the appropriate digestion acid and loaded into a rack. Loose fitting vial caps prevent condensation from the roof of the chamber from dripping into the samples (the caps must be loose fitting to ensure pressure equalization within the chamber).

Figure 1 – Milestone's ultraWAVE

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 spatter or boiling of the sample solutions, which prevents cross contamination or loss of volatiles. Because the pressure in the chamber increases with sample temperature, boiling never occurs. The SRC can operate at very high temperature and pressure – up to 300 °C and 199 bar, which enables the complete digestion of virtually every pharmaceutical sample type, including oils and whole gel caps, a benefit for the pharmaceutical industry. Samples with high organic content such as oils generate pressure in the microwave vessel due to the generation of NO_x and CO_2: in traditional microwave digestion, high pressure vessels (typically 100 bar) must be used.

With SRC, because all the samples are digested together under the same conditions, different sample types can be digested together – there is no need to "batch" digestion runs into identical sample types as with traditional microwave digestion systems. For example, raw materials, excipients, API's and final 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.

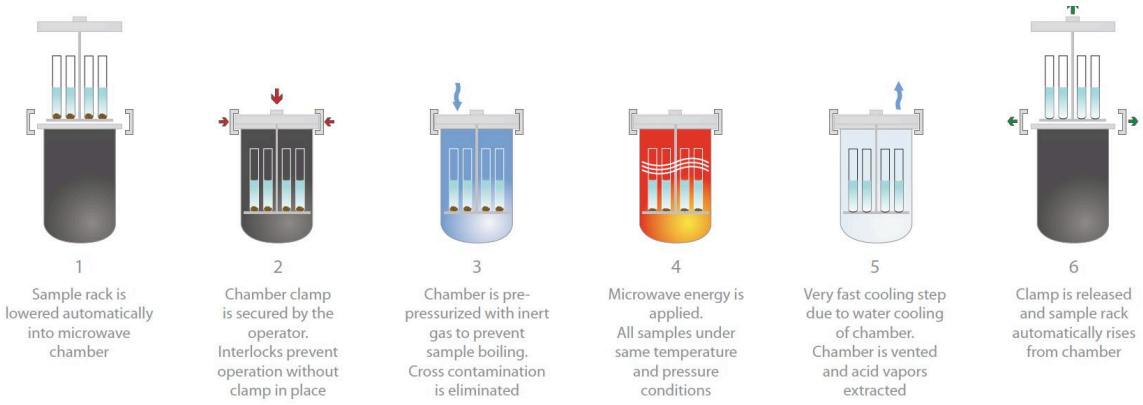

Figure 2 – Workflow of the ultraWAVE

PROCEDURE AND METHOD OPTIMIZATION

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

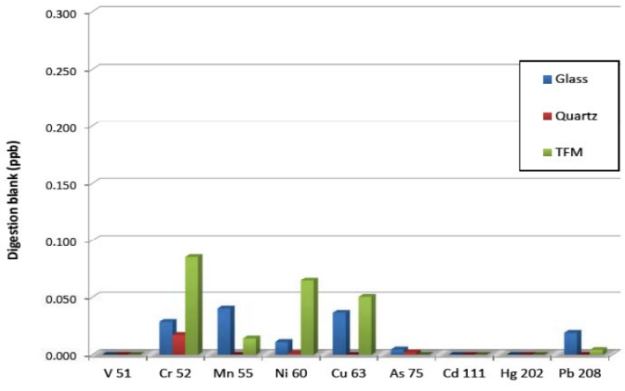

Figure 3 - Digestion blanks obtained from 2 different vial materials selected USP elements.

However, since these elements are not stipulated in USP <232>, glass vials can be used. Figure 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.

The same microwave program was used for all sample types: pre-pressurize with 40 bar nitrogen, ramp to 240 °C over 20 minutes with a 20 minute hold. Cool down time was approximately 15 minutes, yielding a total run time of 55 minutes. If all the samples in the run were easy to digest, the digestion temperature could be reduced to 200 °C, which reduces the cool down time. Figure 4 shows an actual SRC digestion run.

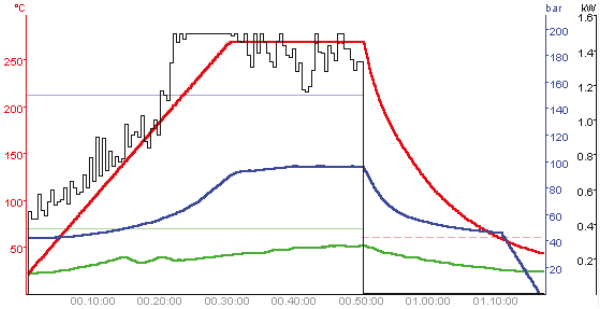

Figure 4 - Microwave program from simultaneous digestion of multiple pharmaceutical samples.

The red line shows the temperature program, with the actual temperature achieved during the run superimposed on it.

The black line shows the applied microwave power, which is automatically adjusted by the instrument software so that the actual digestion temperature follows the programmed temperature profile.

A PID (proportional integral derivative) controller adjusts the power automatically regardless of number of samples in the run, acid volume or sample weight. The blue line shows the pressure achieved during the run. Note that over 100 bar pressure is achieved, which is not possible with conventional closed-vessel digestion systems.

Because the SRC system is capable of very high pressure, higher weights of high organic content samples 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 yields a more complete digestion. Even with high organic content samples such as oils, virtually all the organic carbon is decomposed to CO_2 giving the sample digest a very low TOC content. This is a benefit for ICP-MS analysis, as the low presence of carbon in the sample enhances the sensitivity of poorly ionized elements, thus enhancing repeatability and reliability.

The digest matrix depends on the sample type and weight. Samples were weighed (0.5 g) and digested with 4 ml HNO_3 + 1ml HCl. For fish oil gel caps, an entire gel cap (1 g) was digested with 9 mL HNO_3 + 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.

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

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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 oil, magnesium stearate and a dietary supplement following digestion in the ultraWAVE. 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. The table shows the recovery study on St. John's Wort and on a laboratory fortified blank.

| CONCLUSION

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 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.