Tech Note

Preparation of Pharmaceutical Samples in Accordance with USP <232>/ <233>

Utilizing Single Reaction Chamber (SRC) Technology for Trace Metals Analysis for pharmaceutical samples.

Summary

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 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 results in uniformly high analytical data quality, making it the ideal solution for trace metals detection in pharmaceutical samples.

MILESTONE

Tech Note

SRC Microwave Digestion for Pharmaceutical Samples

SRC microwave digestion was used for the digestion of pharmaceutical samples prior to ICP-MS analysis according to draft USP chapters <232> and <233>.

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 are due to be implemented in 2014. 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 closed 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.

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

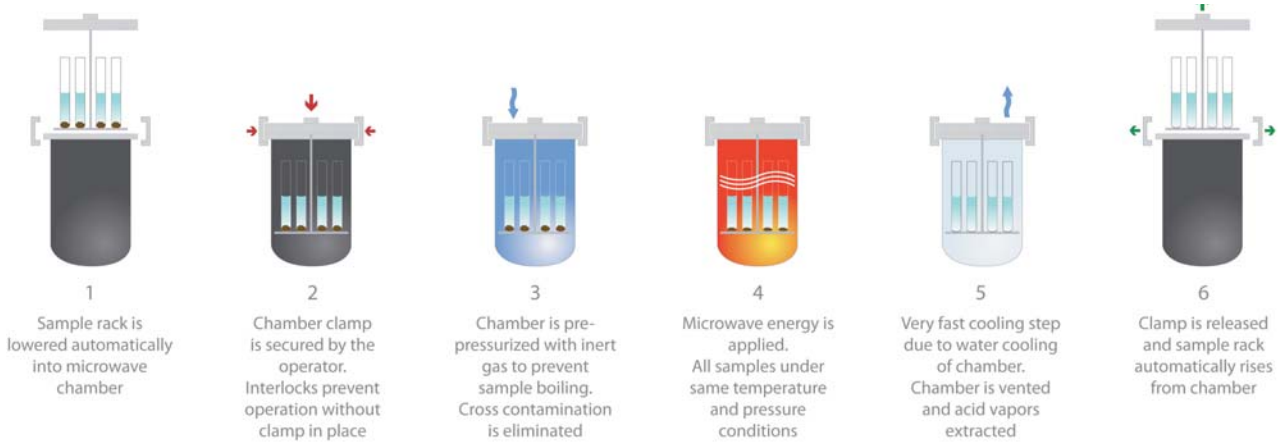

Figure 1. UltraWAVE microwave program.

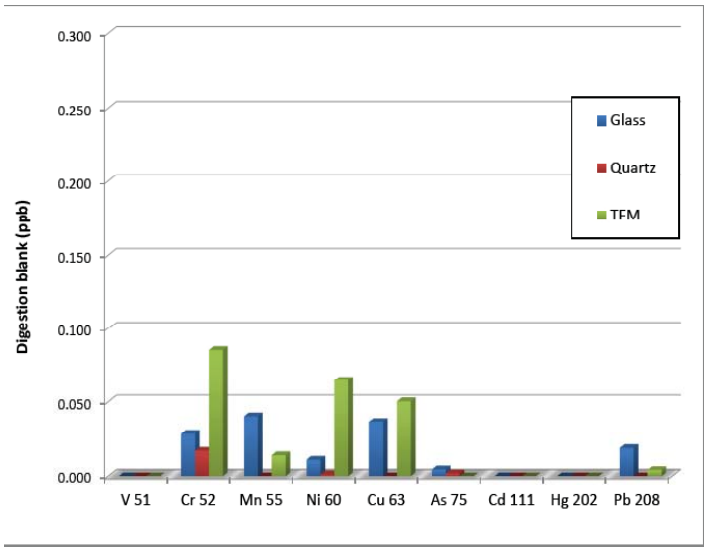

Figure 2. Digestion blanks obtained from 2 different vial materials selected USP elements.

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 dripping into the samples (the caps must be lose fitting to ensure pressure equalization within the chamber). 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 spitting 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. An 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. 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. The higher pressure capability of an SRC allows higher sample weights to be digested, including whole gel caps, which is a benefit for pharmaceutical samples analysis. 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.

So for example, raw materials, excipients, API and final product 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. The SRC microwave system used in this work was a benchtop UltraWAVE instrument (Milestone Inc. Shelton, CT).

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. 2 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.

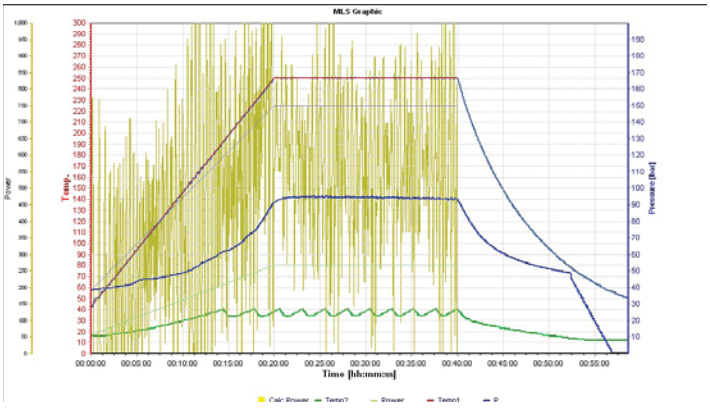

Figure 3. Need digestion run showing similar method described in text.

The microwave program used applied to virtually all sample types: pre-pressurize with 40 bar nitrogen, ramp to 240°C over 20 min and hold for 20 min, giving a total of 40 min. Cool down time was approx. 15 min, giving a total run time of 55 min. If all the samples in the run were easy to digest, the digestion temperature could be reduced to 200°C, which reduced the cool down time slightly. Fig. 3 shows an actual SRC digestion run.

Table 1. ICP-MS analysis of the "Big Four" USP analytes in St. John's Wort & fish oil gelcaps following SRC Digestion

Sample: St. John's Wort				
	Parts per million (µg/g)			
	Arsenic	Cadmium	Lead	Mercury
Sample Result	0.184	0.109	0.24	ND
Duplicate Result	0.195	0.115	0.19	ND
Detection Limit	0.008	0.003	0.03	0.1
Quality Control Summary				
	Parts per million (µg/g)			
Spike Conc	5.57	1.89	3.78	5.57
Spike Result	5.75	1.88	3.72	5.6
Spike % Recovery	100	94	93	101
Laboratory Fortified Blank (LFB)				
Sample Result	ND	ND	ND	0.144
Spike Conc	5.6	1.9	3.8	5.6
Spike Result	5.39	1.86	3.58	6.06
Spike & Recovery	96	98	94	106

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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 program temperature. 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 in conventional closed vessel digestion. 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 CO_2 giving the sample digest and very low TOC content. This is a benefit for ICP-MS analysis: the presence of carbon in the sample enhances on the sensitivity of poorly ionized elements. By removing the organic carbon, the analysis of poorly ionized elements becomes more predictable and reliable.

Digest matrix depends on the sample type and weight. Finished product capsules (St. John's Wort) were composited and 0.5 g sample digested with 4 ml HNO_3 + 1ml HCl. For fish oil gel caps, an entire gel cap (1 g) was digested with 10 mL HNO_3 + 1 mL HCl. The vial size used was 10 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.