CEM CORPORATION

APPLICATION NOTE

Microwave Digestion of Pharmaceutical Samples Followed by ICP-MS Analysis for USP Chapter <233>

Abstract

In order to ensure the safety and quality of pharmaceutical products, the United States Pharmacopeia (USP) will be replacing **USP chapter <231> — Heavy Metals**, which is acknowledged to be inadequate in terms of scope, accuracy, sensitivity, and specificity, with chapters <232> and <233> in May of 2014. The new methods will address the limitations of the current method, with respect to sample preparation, analytes of interest, and the use of ICP-OES or ICP-MS for the accurate recovery and determination of individual analyte concentrations. The upcoming **USP chapter <233> Elemental Impurities – Procedures** identifies fifteen elements for testing and limits for each, including the most pervasive heavy metals: arsenic, mercury, cadmium, and lead. Pharmaceutical ingredients and finished products that cannot be dissolved in aqueous or solvent solutions will require closed vessel digestion in order to prepare the sample for analysis. This paper reports the results of a 50 ppb spike recovery study of several pharmaceutical samples that were prepared for elemental analysis using CEM's MARS 6™ and Discover® SP-D microwave digestion systems. The samples were then diluted and analyzed with an inductively coupled plasma mass spectrometer (ICP-MS). Recovery data for the pharmaceutical samples in each system is presented.

Introduction

The absorption of even low concentrations of some metals can have adverse effects on the human body. This is particularly true of the heavy metal toxic elements: arsenic, mercury, cadmium, and lead. In lower concentrations, the symptoms of metals poisoning can include nausea and abdominal pain. In higher concentrations, metals contamination can lead to encephalopathy, cancer, and damage to the skeletal system and internal organs. Since 1905, USP chapter <231> has been used by laboratories for the determination of heavy metals in pharmaceutical products. Though there have been many changes in detection technology, there have been very few changes made to the chapter. The chapter details three sample preparation methods depending on the type of sample. USP chapter <231> Methods II and III use aggressive sample preparation techniques, which include ignition of the sample in a muffle furnace at 500 – 600 °C in Method II and open vessel acid digestion in Method III. These sample preparation techniques result in a colorless solution that is compared to lead standards.

Among the known issues for the aggressive sample preparation techniques in USP chapter <231> are the low spike recoveries of volatile metals, the need for large sample sizes in order to achieve low detection limits, and the inability to differentiate between metals and elemental species. These issues and other concerns have prompted the replacement of the method with **USP chapters <232> and <233>** in May of 2014.

USP chapter <232>
Elemental Impurities – Limits

outlines the maximum limits for heavy metals in active pharmaceutical ingredients and drug products based on three routes of administration which include oral, parenteral, and inhalation. There are several avenues by which these elements can find their way into drug products: they may be naturally occurring in the components, they may be added intentionally during the manufacturing process, or they can be transferred via contact with the processing equipment.

USP chapter <233>
Elemental Impurities – Procedures

discusses the sample preparation and analysis procedures for the determination of heavy metals in pharmaceutical raw materials and finished products. The chapter outlines four sample preparation techniques: Neat, Direct Aqueous Solution, Direct Organic Solution, and Indirect Solution. The optimal approach is the Neat technique, where samples are ready immediately for elemental analysis without preparation. The technique of preparing samples in Direct Aqueous Solution or Direct Organic Solution is a fairly simple approach, as the sample is prepared for analysis by mixing it with an aqueous or organic solvent; however, many samples cannot be completely dissolved using these techniques. Most pharmaceutical raw materials and finished products will require the Indirect Solution technique, a closed vessel digestion in a concentrated acid solution that solubilizes the sample for analysis via an inductively coupled plasma optical emission spectrometer (ICP-OES) or an inductively coupled plasma mass spectrometer (ICP-MS).

Microwave digestion instrumentation offers several advantages over other digestion techniques, such as hot

plates and hot blocks, including rapid heating in sealed containers to provide greater control of volatile elements, more complete destruction of background organics, and lower acid consumption. Microwave energy directly heats the acid and sample through the kinetic transfer of energy directly to the sample matrix. Thus, unlike conventional heating, there is no time lag conducting energy thermally from a heating element to a digestion bomb and finally to the acid and sample. Temperature and pressure control options available on microwave digestion systems allow for more reproducible digestion conditions, as well as rapid turnaround of digested samples. In addition, it allows for precise documentation of the preparation conditions of every sample.

CEM Corporation offers two microwave digestion systems, the **Discover® SP-D** and **MARS 6™,** both of which are well suited for sample preparation of pharmaceutical raw materials and finished products. The Discover® SP-D operates in a sequential format and, when combined with one of CEM's optional autosamplers, can run automatically overnight, while the MARS 6™ processes up to 40 samples simultaneously. Both systems are 21 CFR Part 11 compliant and are designed for R&D and quality control laboratories. In addition, we have developed a comprehensive solution to help make the transition as simple as possible for pharmaceutical manufacturers, including pre-programmed methods and intuitive software; expert applications assistance; and, CEM's ***Complete Guide to Implementing USP 232, 233, & 2232,*** a publication containing application notes, IQ/OQ/PQ and Standard Operating Procedures, training procedures, and documentation templates. This paper will include discussion of microwave digestion sample preparation in the MARS 6™ and Discover® SP-D systems and analyte recoveries for multiple elements of interest including arsenic, cadmium, and mercury in several pharmaceutical finished products.

MARS 6

Discover SP-D

Instrumentation

The first set of samples was digested in the MARS 6 with **One Touch™ Technology**, which uses sensors built into the floor of the cavity to automatically identify the number and type of vessels. The system then calculates the optimal microwave power output to ensure complete digestions. The samples were digested in the simple-to-assemble, high-temperature, high-pressure **EasyPrep™ Plus** vessels. The temperature was monitored and controlled using **DuoTemp™**, a proprietary feature that incorporates both a fiber optic probe and **Contactless All-Vessel IR Temperature Sensors.** DuoTemp automatically selects the control vessel based upon reaction conditions and will dynamically adjust during the run, so that the temperature is always controlled based on the most reactive vessel, providing reproducible, safe digestions every time by eliminating exothermic reactions.

The second set of samples was digested in the Discover SP-D, which was designed to maximize the amount of microwave power applied to the sample, reducing the digestion time of a pharmaceutical sample to less than 10 minutes with cool down. The compact Discover SP-D features temperature and pressure control for each vessel, ensuring reproducible digestions. The temperature of each sample is controlled via contactless IR sensor and the pressure is controlled using CEM's proprietary **ActiVent® Technology,** which monitors and automatically vents excess pressure, allowing higher temperatures to be achieved at moderate and safe pressures. The samples were digested in 35-mL Discover SP-D vessels with a simple to assemble snap-on cap.

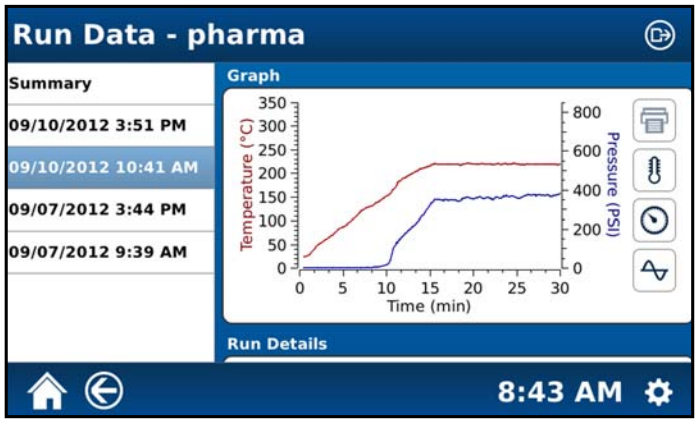
Digested and diluted samples were analyzed with a Thermo Scientific iCAP Q ICP-MS.

Analytical Procedure

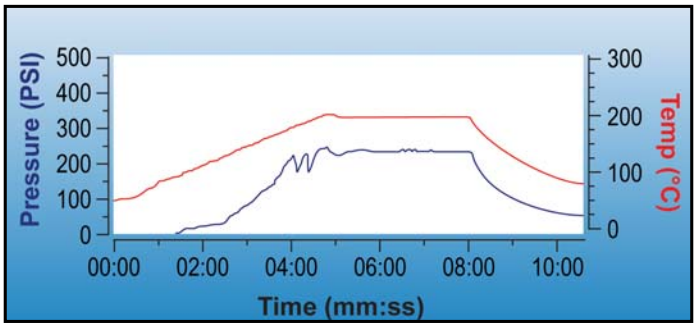
Three finished product samples were analyzed including a 0.1 g whole tablet containing the active ingredient acetylsalicylic acid, a 0.1 g whole tablet containing the active ingredient loratadine, and a 0.3 g whole liquid gel capsule containing the active ingredient diphenhydramine HCl. Each unprepared tablet or capsule was added to an EasyPrep Plus vessel or Discover SP-D 35-mL vessel. A total of 9.0 mL of concentrated ultra-pure nitric acid and 1.0 mL of a spike solution of deionized (DI) water containing 50.0 ppb of arsenic, mercury, and cadmium was added to all of the vessels. In addition, 1.0 mL of hydrogen peroxide was added to each Easy Prep vessel and 0.5 mL was added to each SP-D vessel. Each of the samples and blanks was prepared in triplicate using the digestion parameters shown in Table I.

Table I: Digestion Parameters for MARS 6 and Discover SP-D

	Sample Type	Ramp Time (minutes)	Hold Time (minutes)	Digestion Temperature (°C)
MARS 6 with EasyPrep Plus vessel	Organic	15	15	210
Discover SP-D with 35-mL vessel	Organic	5	3	210



Onboard temperature and pressure graph for a pharmaceutical sample digested in the MARS 6.



Temperature and pressure graph for Discover SP-D digestion of loratadine sample.

Samples were cooled to room temperature and diluted to 50.0 mL with DI water and transferred to autosampler vials. Calibration standards containing arsenic, mercury, and cadmium at concentrations of 0.10, 0.50, 1.00, 5.00, 10.00 ppb were prepared in 20% nitric acid. The samples were run on the Thermo Scientific iCAP Q ICP-MS using the following analysis conditions:

Forward Power	1500 W
Nebulizer Gas	1 L/min
Auxiliary Gas	0.8 L/min
Cool Gas Flow	14.0 L/min
Collision Cell Gas	He at 4.5 L/min
Sample Uptake/Wash Time	45 seconds each
Dwell Times	Optimized per analyte
Number of Points Per Peak	1
Replicates	3
Internal Standard	1.00 ppm yttrium

Results and Discussion

The results of the spike recovery study of pharmaceutical finished products are shown in the tables: MARS 6 Spike Recovery Results (ppb) of 50.0 ppb As, Cd, and Hg and Discover SP-D Spike Recovery Results (ppb) of 50.0 ppb As, Cd, and Hg.

Table II: Recovery Results of 50 ppb Spike of As, Hg, and Cd in Finished Pharmaceutical Products

MARS 6 Spike Recovery Results (ppb) of 50.0 ppb As, Cd, and Hg

	As	Hg	Cd
Acetylsalicylic Acid Finished Product			
Analysis 1	49.04	60.93	48.63
Analysis 2	58.79	46.87	46.21
Analysis 3	57.71	62.29	49.45
Average	55.18	56.70	48.10
Percent Recovery	110.36	113.40	96.19
RSD	9.68	15.05	3.50
Loratadine Finished Product			
Analysis 1	73.22	45.93	51.37
Analysis 2	67.40	50.02	58.88
Analysis 3	63.44	58.04	56.75
Average	68.02	51.33	55.67
Percent Recovery	136.04	102.66	111.34
RSD	7.23	12.00	6.95
Diphenhydramine HCl Finished Product			
Analysis 1	55.01	67.64	43.60
Analysis 2	62.65	51.09	51.80
Analysis 3	51.17	63.40	45.67
Average	56.28	60.71	47.02
Percent Recovery	112.55	121.42	94.04
RSD	10.38	14.16	9.07

Discover SP-D Spike Recovery Results (ppb) of 50.0 ppb As, Cd, and Hg

	As	Hg	Cd
Acetylsalicylic Acid Finished Product			
Analysis 1	56.16	52.53	53.50
Analysis 2	53.01	51.92	51.36
Analysis 3	55.53	46.90	50.65
Average	54.90	50.45	51.84
Percent Recovery	109.80	100.90	103.67
RSD	3.04	6.13	2.86
Loratadine Finished Product			
Analysis 1	58.08	55.76	51.07
Analysis 2	54.09	54.79	51.34
Analysis 3	49.65	53.24	50.85
Average	53.94	54.60	51.09
Percent Recovery	107.87	109.19	102.18
RSD	7.82	2.33	0.48
Diphenhydramine HCl Finished Product			
Analysis 1	66.23	53.78	61.64
Analysis 2	55.92	54.14	53.58
Analysis 3	55.99	53.19	56.12
Average	59.38	53.70	57.11
Percent Recovery	118.77	107.41	114.23
RSD	9.99	0.89	7.21

The accuracy of proposed USP Method <233> is 70 – 150% with a Relative Standard Deviation of not more than 20%. As shown in Table II, the MARS 6 achieved good recoveries of both the volatile and non-volatile elements with mercury at 101 – 122%, arsenic at 110 – 137%, and cadmium at 94 – 112%. Also, easily demonstrated by the data in Table II were the good results for volatile and non-volatile metals in the Discover SP-D with 100 – 110% mercury, 107 – 119% arsenic, and 102 – 115% cadmium recoveries achieved. The accuracy and precision results of this study show that the pharmaceutical samples prepared with the MARS 6 and Discover SP-D are well within the requirements of USP chapter <233>.

Conclusion

Microwave closed vessel digestion instrumentation allows for fast, simple, and safe sample preparation of pharmaceutical samples for metals analysis. Both the MARS 6 and the Discover SP-D are well suited to prepare pharmaceutical raw materials and finished products and offer a choice in instrumentation based upon laboratory workflow and sample throughput. Each system provides significant time savings and operates to a large degree unattended, freeing analysts to complete other tasks. Since both systems use completely sealed vessels as opposed to caps, the possibility of cross contamination is eliminated and the recovery of both non-volatile and volatile metals is possible. As demonstrated by the data above, the recovery results are well within the upcoming USP chapter <233> requirements.

APPLICATION NOTES

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