APPLICATION NOTE

Using graphite furnace atomic absorption to meet the requirements of elemental impurity analysis in pharmaceutical products for arsenic, cadmium and lead

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Key Words
Atomic Absorption, FDA, Pharmaceutical, USP 232, USP 233

Goal

Demonstrate that with GF-AA the required detection limits for arsenic, cadmium and lead as described in the USP Chapters 232 and 233 are achieved. Show that GF-AA is a cost effective alternative to ICP-AES / ICP-MS when only a small number of elements are to be considered, whilst meeting validation and data security requirements.

Introduction

The United States Pharmacopeia (USP) has introduced two new chapters (232 and 233) dealing with elemental impurities in pharmaceutical products, which will become official on January 1st, 2018.

Permissible limits for daily exposure to a range of trace elements are given in Chapter 232. These limits were revised in February 2016 to fully harmonize on an international level and the current values are shown in Table 1.

Table 1. Elemental impurities for drug products (from USP Chapter 232).

Element	Class	Oral Daily Dose PDE (µg/day)	Parenteral Daily Dose PDE (µg/day)	Inhalational Daily Dose PDE (µg/day)
Cd	1	5	2	2
Pb	1	5	5	5
As	1	15	15	2
Hg	1	30	3	1
Co	2A	50	5	3
V	2A	100	10	1
Ni	2A	200	20	5
Tl	2B	8	8	8
Au	2B	100	100	1
Pd	2B	100	10	1
Ir	2B	100	10	1
Os	2B	100	10	1
Rh	2B	100	10	1
Ru	2B	100	10	1
Se	2B	150	80	130
Ag	2B	150	10	7
Pt	2B	100	10	1
Li	3	550	250	25
Sb	3	1200	90	20
Ba	3	1400	700	300
Mo	3	3000	1500	10
Cu	3	3000	300	30
Sn	3	6000	600	60
Cr	3	11000	1100	3

PDE = Permissible daily exposure based on a 50-kg person

Chapter 233 defines two standard methods (ICP-AES and ICP-MS) but these do not have to be used, provided an alternative method meets the validation requirements specified. There are two levels of validation depending on whether the method only indicates that the samples are above or below the limit (Limit Procedure) or gives a concentration (Quantitative Procedure). Both procedures were carried out for cadmium, lead and arsenic in an oral electrolyte formulation.

Validation requirements

USP Chapter 233 details the two types of validation that can be performed to demonstrate that an alternative method is an acceptable replacement for the standard method. In these tests concentration referred to as J is used. This value is the concentration (w/w) of the element(s) of interest at the target limit (i.e. the permitted daily dose), which is specified in Table 1.

For limit procedures the tests and acceptance criteria are:

Detectability

Solutions needed:

- Standard solution – a preparation containing the elements of interest at the target concentration.
- Spiked sample solution 1 – a sample of the material under test spiked with the elements of interest at the target concentration.
- Spiked sample solution 2 – a sample of the material under test spiked with the elements of interest at 80% of the target concentration.
- Unspiked sample solution – a sample of the material under test.

Procedure: measure 3 replicates of each solution. Correct spiked solutions signals by subtracting the signal from the sample solution.

Acceptance criteria: the average value of spiked solution 1 should be within 15% of the standard solution value. The average value of spiked solution 2 should be less than the standard solution value.

Precision

Solutions needed:

- Six independent samples spiked with the elements of interest at the target concentration.

Procedure: Measure each solution.

Acceptance criteria: the 6 readings should have an RSD of not more than 20% for each target element.

Specificity

Acceptance criteria: the procedure must be able to unequivocally assess each target element in the presence of the matrix and other target elements.

For quantitative procedures the tests and acceptance criteria are:

Accuracy

Solutions needed:

- Standards at 50% to 150% of the target concentration J.
- Samples spiked with 50% to 150% of the target concentration J (made up in triplicate).

Procedure: Measure each of the standards and samples.

Acceptance criteria: 70%-150% recovery for the mean of replicate preparations at each concentration.

Repeatability

Solutions needed:

- Six independent samples spiked with the elements of interest at the target concentration.

Procedure: Measure each solution.

Acceptance criteria: the 6 readings should have an RSD of not more than 20% for each target element over the three independent events.

Ruggedness

Procedure: perform the repeatability analysis over three independent events using the following events or combinations thereof:

1. On different days or

2. With different instrumentation or

3. With different analysts.

Acceptance criteria: relative standard deviation less than 25% for each element over the three independent events.

Specificity

Acceptance criteria: the procedure must be able to unequivocally assess each target element in the presence of the matrix and other target elements.

Instrument and Software

The Thermo Scientific™ iCE™ 3500 Atomic Absorption Spectrometer with GFS35Z Zeeman Graphite Furnace and Autosampler were used for the analysis (Figure 1). This system was chosen because both Zeeman and Deuterium background correction can be utilized for graphite furnace analysis. This provides the capability to perform accurate analysis with almost any matrix.

The Thermo Scientific™ SOLAAR Software with Thermo Scientific SOLAARsecurity Software provides the tools to meet the requirements of the United States Food and Drug Administration (FDA) CFR 21 Part 11 regulations relating to the use, control and security of electronic records. The automatic optimization functions in the software simplify method development.

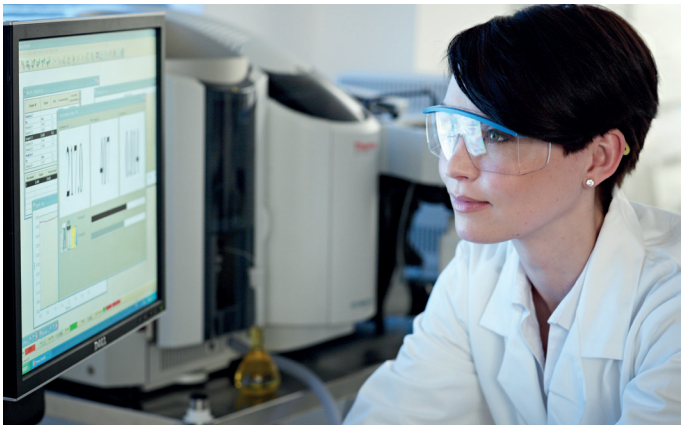

Figure 1. The Thermo Scientific iCE 3500 Atomic Absorption Spectrometer with GFS35Z Zeeman Graphite Furnace.

Sample and standard preparation

The maximum dose for the oral electrolyte formulation is 5 sachets a day (approximately 25 g). From Table 1 the daily limits are 15 µg arsenic, 5 µg cadmium and 5 µg lead. The stock sample solutions were made up of 12.5 g of the formulation in 0.5 litre 1% nitric acid and deionized water. The J value (target concentration) was 15 $\mu g \cdot kg^{-1}$ for arsenic, 5 $\mu g \cdot kg^{-1}$ for cadmium and 5 $\mu g \cdot kg^{-1}$ for Lead. The arsenic, cadmium and lead elemental standards were supplied by Fisher Scientific. All solutions were freshly prepared before each test. The regulations state that for a solid sample the volume of solvent can be chosen to ensure that the analyte concentration is in a range compatible with the sensitivity of the instrument.

Method development

Automatic Ash Atomize optimization plots were run for arsenic, cadmium and lead in the sample matrix to ensure an optimal furnace temperature program was achieved. Prior to carrying out the optimization the drying phase of the cycle was setup by watching the sample behavior using the graphite furnace television (GFTV) feature. The GFTV allows the inside of the graphite furnace cuvette to be viewed on screen via a camera and is a standard feature of the iCE 3500 AA.

The optimal furnace program was determined to be the same for cadmium and lead, the furnace parameters are shown in the table 2, for arsenic the optimal furnace program is shown in the table 3.

The primary wavelengths were used for each element, 193.7 nm for arsenic, 228.8 nm for cadmium and 217.0 nm for Lead together with D2 Quadline background correction. For arsenic analysis, 10 µl of sample was injected together with 10 µl of NiSO4 (10 µg) matrix modifier. For cadmium analysis, 10 µl of sample was injected together with 5 µl of Mg(NO3)2 (20 µg) matrix modifier. For lead analysis 20 µl of sample was injected together with 5 µl of Mg(NO3)2 (50 µg) matrix modifier. A standard electro-graphite cuvette was used for all analysis.

Table 2. Optimized Graphite Furnace program for cadmium and lead.

Temp (°C)	Time (s)	Ramp (°C/s)	Gas type	Gas flow (l/min)	Read	Temperature control
100	30	10	Argon	0.2		
350	20	150	Argon	0.2		
1100	3	0	Argon	Off	✓	✓
2500	3	0	Argon	0.2		✓

Table 3. Optimized Graphite Furnace program for arsenic.

Temp (°C)	Time (s)	Ramp (°C/s)	Gas type	Gas flow (l/min)	Read	Temperature control
100	30	10	Argon	0.2		
1250	20	150	Argon	0.2		
2550	3	0	Argon	Off	✓	✓
2700	3	0	Argon	0.2		✓

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Limit procedure results

Detectability

Table 3. Detectability test corrected data and as percentage of standard.

Element	Std (Abs)	Spike 1 (Abs)	Spike 2 (Abs)	Spike 1 (%Std)
Arsenic (As)	0.478	0.457	0.418	95.6%
Cadmium (Cd)	0.343	0.367	0.326	107%
Lead (Pb)	0.090	0.101	0.089	112%

Arsenic passes the test as the Spike 1 corrected value is within 15% of the standard value (95.6%) and Spike 2 is less than the standard value.
Cadmium passes the test as the Spike 1 corrected value is within 15% of the standard value (107%) and Spike 2 is less than the standard value.
Lead passes the test as the Spike 1 corrected value is within 15% of the standard value (112%) and Spike 2 is less than the standard value.

Precision

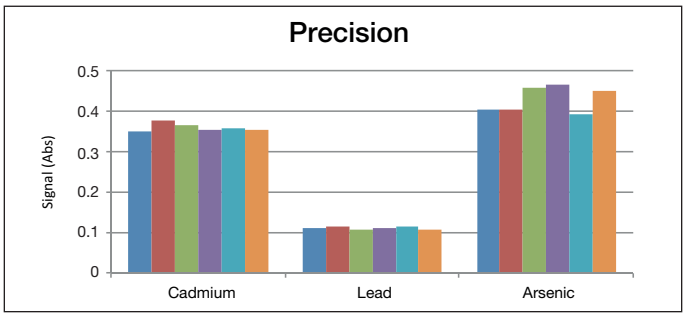

Figure 2. Precision test results.

All elements pass the test as their RSD is less than 20% (7.4% for arsenic, 2.7% for cadmium and 3.2% for lead). This is the same as the repeatability test for Quantitative Procedures.

Quantitative procedures results

Accuracy

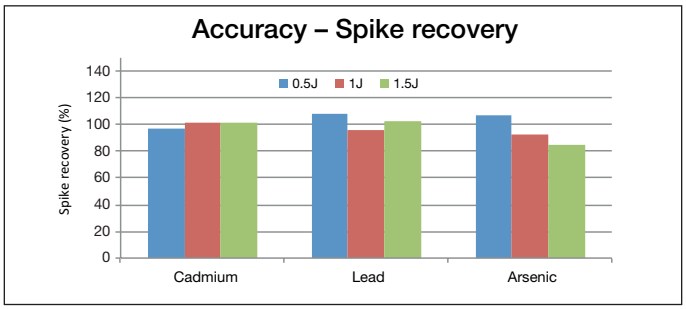

Figure 3. Spike recovery at 0.5, 1.0 and 1.5 times J (the target concentration).

All elements pass as the recoveries are in the range 70-150%. J for arsenic is 15 $\mu g \cdot kg^{-1}$, J for cadmium is $\mu g \cdot kg^{-1}$ and J for lead is $\mu g \cdot kg^{-1}$.

Ruggedness

Table 4. Raw data from a sample run on different days.

Element	Day 1 (Abs)	Day 2 (Abs)	Day 3 (Abs)	RSD
Arsenic (As)	0.478	0.421	0.435	6.7%
Cadmium (Cd)	0.375	0.378	0.372	0.8%
Lead (Pb)	0.112	0.106	0.100	5.6%

All elements pass having an RSD of less than 25% (arsenic 6.7%, cadmium 0.8% and lead 5.6%,).

Results of the validation tests

Validation tests specified in USP chapters 232 and 233 were successfully passed and hence demonstrates that Graphite Furnace AA can be used as an alternative method to ICP-AES and ICP-MS for the analysis of arsenic, cadmium and lead in this particular product.

Conclusions

The Thermo Scientific iCE3500 AA Spectrometer with GFS35Z Zeeman Graphite Furnace provides a simple, low cost means of complying with the requirements of USP chapters 232 and 233. The CFR 21 Part 11 compliance of the Thermo Scientific™ SOLAAR*security* Software enables the system to be used in regulated laboratories. The instrument has sufficient sensitivity to be used for all the target elements. For the particular product that was tested it has been demonstrated that arsenic, cadmium and lead meet the requirements of the alternative method validation procedure for both Limit and Quantitative Procedures.

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