APPLICATION NOTE

AN43381

Using cold vapor generation atomic absorption to determine mercury impurities in pharmaceutical products

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Key Words
Elemental Impurities, FDA, Pharmaceutical, USP 232, USP 233

Goal

To demonstrate the ability of the Thermo Scientific iCE 3000 Series AAS to meet the validation requirements for mercury as required by the United States Pharmacopeia (USP) Chapters <232> and <233>. To show that AAS is a cost effective alternative to ICP-OES and ICP-MS when only a small number of samples and elements needs to be determined.

Introduction

The United States Pharmacopeia (USP) has recently introduced two new chapters (<232> and <233>) dealing with elemental impurities in pharmaceutical products. Permissible limits were determined based on toxicological studies and are given in Chapter 232 for a number of elements. These limits were revised in February 2016 in order to harmonize on a global scale and values are shown in Table 1.

Chapter <233> defines two standard methods (ICP-OES 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 mercury in an oral electrolyte formulation.

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

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, the concentration corresponding to the permitted maximum amount, referred to as J, is used. This value is the concentration (w/w) of the element 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™ 3300 Atomic Absorption Spectrometer (AAS) was used during this analysis. The iCE 3300 AAS combines high-precision optics, state-of-the-art design and user-friendly software to provide unrivalled analytical performance.

A Thermo Scientific™ VP100 vapor generation accessory (figure 1) is also necessary to perform this analysis. The unique VP100 vapor generation accessory uses a continuous flow system to produce a steady-state signal and provides excellent analytical precision. The continuous flow of reagents ensures that the system is self-cleaning, reducing memory effects and increasing sample throughput. The VP100 vapor generation accessory is entirely controlled by the Thermo Scientific™ SOLAAR Software, meaning that developing a method and running an analysis is extremely simple.

A mercury cell (provided as standard with the VP100 vapor generation accessory and provides excellent) was also used. This cell provides an increased path-length compared to a normal vapor cell and gives exceptionally low detection limits.

Figure 1. Thermo Scientific VP100 Vapor generation accessory.

The SOLAAR Software with Thermo Scientific SOLAAR*security* 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.

Sample and standard preparation

The maximum dose for the oral electrolyte formulation is 5 sachets a day (corresponding to approximately 25 g in total). From Table 1 it can be seen that the maximum daily intake must not exceed 30 µg of mercury (Hg). The stock sample solutions were made up of 12.5 g of the formulation in 0.5 l 1% nitric acid and deionized water. The J value (target concentration) was thus 30 $\mu g \cdot kg^{-1}$ for mercury. All solutions were freshly prepared before each test within the mercury standard at the concentration of 1000 $mg \cdot kg^{-1}$ from Fisher Scientific. 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.

VP100 vapor generation accessory reagent preparation

The VP100 vapor generation accessory requires both a reductant and an acid solution to perform the reactions that form the gaseous mercury (figure 2). For this application the reductant was a solution of 0.5% NaBH4 stabilized in 0.1% NaOH. The acid solution was 20% HCl.

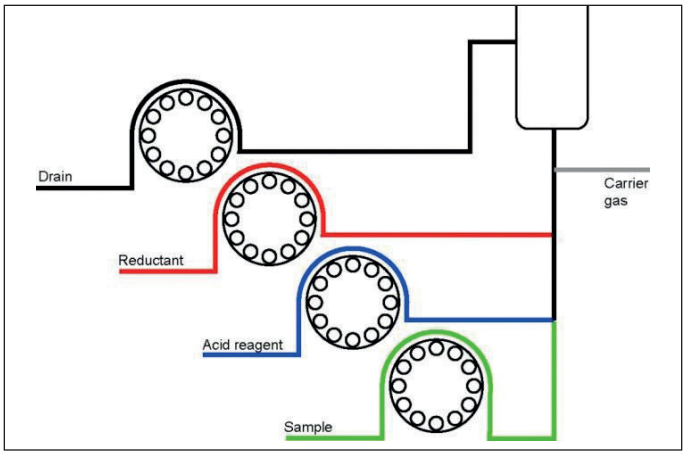

Figure 2. Schematic of the VP100 vapor generation accessory showing how the sample, acid and reductant are mixed.

Instrument conditions

The analysis was performed using the most sensitive absorption wavelength for mercury at 253.7 nm. Three resamples were used, with each resample taking four seconds. This was used to thoroughly assess the short-term stability of the instrument during the development of this method. Deuterium background correction was used throughout the analysis. The parameters used for both the VP100 vapor generation accessory and spectrometer are shown in Table 2. For further details on how to optimize the VP100 vapor generation accessory parameters for your analysis, please refer to the iCE 3000 Series Operator Manual.

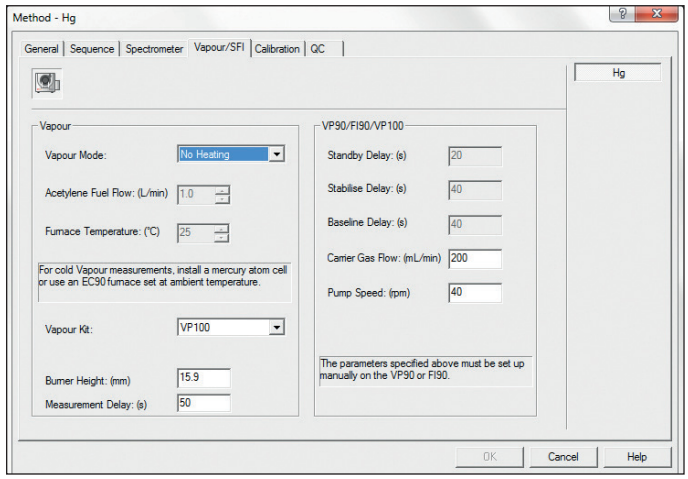

Figure 3. Settings with the SOLAAR software for vapor generation.

Table 2. Summary of the parameters used for the analysis.

Spectrometer Parameters	
Wavelength	253.7 nm
Lamp Current	75 %
Bandpass	0.5 nm
Background Correction	D_2 Quadline
Resamples	3
Measurement Time	4.0s
VP100 Vapor Generation Accessory Parameters	
Pump Speed	40 rpm
Gas Flow	200 $ml \cdot min^{-1}$
Acid Reagent	20% HCl
Reductant	0.5% $NaBH_4$ in 0.1% NaOH
Measurement Delay	70

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)
Mercury (Hg)	0.112	0.115	0.090	103%

Mercury passes the test as the Spike 1 corrected value is within 15% of the standard value (103%) and Spike 2 is less than the standard value.

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Precision

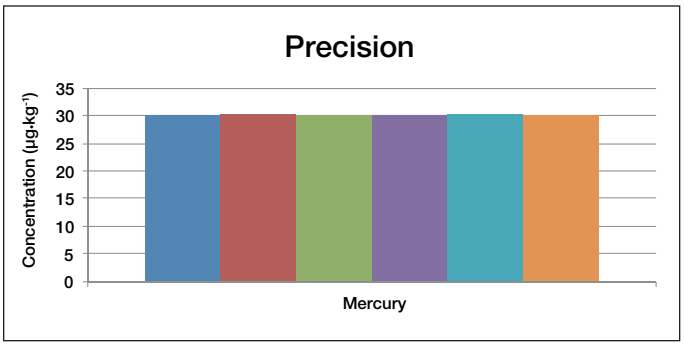

Figure 4. Precision test results.

All elements pass the test as their RSD is less than 20% (0.5% for mercury). This is the same as the repeatability test for Quantitative Procedures.

Quantitative procedures results

Accuracy

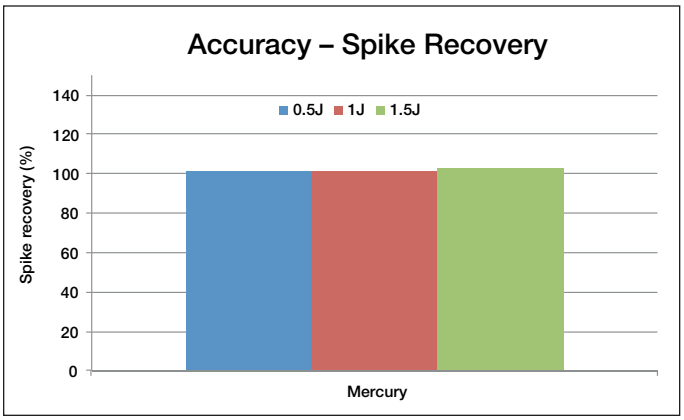

Figure 5. Spike recovery at 0.5, 1.0 and 1.5 times J (the target concentration).

Mercury passes as the recoveries are in the range 70-150%. J for Mercury is 30 µg·kg^{-1}.

Ruggedness

Table 7. Raw data from a sample run on different days.

Element	Day 1 (Abs)	Day 2 (Abs)	Day 3 (Abs)	RSD
Mercury (Hg)	0.112	0.115	0.111	1.85%

All elements pass having an RSD of less than 25% (mercury 1.85%).

Results of the validation tests

Validation tests specified in USP chapters 232 and 233 were successfully passed. It demonstrates that atomic absorption technique with vapor generation VP100 vapor generation accessory system to be used as an alternative method to ICP-OES and ICP-MS for the analysis of mercury in this particular product.

Conclusions

The Thermo Scientific iCE3300 AA Spectrometer with vapor generation VP100 vapor generation accessory system 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 the target elements. For the particular product that was tested it has been demonstrated that mercury meets the requirements of the alternative method validation procedure for both Limit and Quantitative Procedures.

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