

Spectroscopy

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PinAAcle™ 900T

Analysis of Class-I elements as per ICH-Q3D guidelines using Graphite Furnace AA and FIAS (Hydride) – Furnace Coupling Technique (FIFU)

Introduction

Heavy metal contamination is rightly a cause for concern in pharmaceutical products, and there are many ways in which a product might become contaminated. Heavy metals might be purposely used in the process as catalysts or occur naturally within the plant or

mineral sources that are used to produce active ingredients or excipients. They may also present as undetected contaminants from starting materials or reagents or come from the process e.g. leaching from pipes and other equipment.

With regard to set of toxic elements (Cd, Pb, As and Hg) which are mostly specified at much lower levels than the other metals in ICH-Q3D, it could be more challenging to determine them with good accuracy. Here the study is carried out to analyze Class-I elements in Oral drugs at very trace levels using Furnace and FIAS (hydride)-Furnace (FIFU) AA techniques. Graphite Furnace Atomic Absorption Spectrometry (GFAAS) has been widely applied to the determination of trace elements in pharmaceutical

compounds due to its selectivity, simplicity, high sensitivity, and its capability for accurate determinations in a wide variety of matrices. This unique proposition proved to be a cost effective solutions for labs with low to medium throughput sample load and cost effective setup on a existing AA investment to meet regulatory requirements.

Experimental Conditions

Instrumentation

The measurements were performed using the PerkinElmer® PinAAcle™ 900T AAS and FIAS 100 Hydride instruments equipped with Syngistix™ for AAS for measurement of all analyte wavelengths of interest. Mercury and Arsenic are the highly volatile elements were done using FIAS-Furnace coupling kit (Part No. N2010248) for determination at ultra-trace levels to achieve desire concentration. Lead and Cadmium were done directly by graphite furnace and Mercury, Arsenic which are volatile and less sensitive elements were done using FIAS-Furnace coupling (FIFU) to achieve target concentrations (J values) at very lower level.

What is FIFU:

The FI-Furnace Coupling Kit allows a PerkinElmer's FIAS Flow Injection System (Part No.B0508570) to be coupled

directly with a PerkinElmer graphite furnace, providing a fully integrated system. It is direct coupling of hydride atomic absorption with graphite furnace atomic absorption (GFAAS) automates online element preconcentration in the graphite tube. This improves graphite furnace detection limits for the hydride-forming elements and for mercury by 2-3 orders of magnitude.

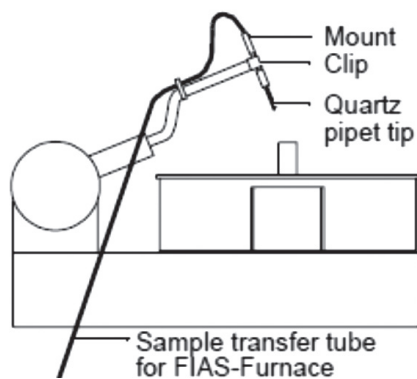


Figure 1. FIAS-Furnace coupling

Analyte	Pb	Cd	As	Hg
Wavelength (nm)	283.31	228.8	193.7	253.65
Slit (nm)	0.7	0.7	0.7	0.7
Lamp Type	EDL	EDL	EDL	EDL
Read time in sec	7	5	5	5
Injection temperature (°C)	20	20	20	20
Matrix modifier used	0.005 mg Pd + 0.003 mg Mg(NO ₃) ₂			
Modifier volume	5µL	5µL	5µL	5µL
Sample volume	30uL	20uL	20uL	20uL
Measurement type	Peak Area	Peak Area	Peak Area	Peak Area
Equation	Linear through zero			

Table 1: Instrument Parameters

Analyte	Pb	Cd	Hg	As	Internal gas flow (mL/min)
Drying 1	110°C	110°C	150°C	400°C	250
Drying 2	150°C	130°C	150°C	400°C	250
Pyrolysis	900°C	500°C	N.A.	N.A.	250
Atomization (Read Step)	1900°C	1500°C	1300°C	2100°C	0
Clean Out	2450°C	2450°C	2000°C	2300°C	250

N.A: Not Applicable.

Hg and As were done by FIFU and Pyrolysis step is not required.

Table 2. Temperature programs on the PinAAcle™ 900T

The high-efficiency optical system and solid-state detector used in this spectrometer provide outstanding signal-to-noise ratios. The solid-state detector is also highly efficient at low UV and longer wavelengths at the same time. The PinAAcle™ 900T instrument settings are listed in Table 1 and furnace program is listed in Table 2. The calibration equation was selected linear through zero.

FIAS setting:

Set a carrier solution flow of approximately 6 mL/minute and reductant solution flow approximately 3 mL/minute. Argon was used as a carrier gas and set flow to 120 mL/minute. FIAS program is mentioned in table 3.

Sample Volume: 500µL				
Step	Time, Sec	Pump 1 speed	Pump 2 speed	Valve position
Prefill	15	100	0	Fill
1	10	100	0	Fill
2	5	100	80	Fill
3	30	0	80	Inject

Table 3: FIAS Program for both Hg and As

Standards and Samples Preparation

Standard preparation:

Depending on the target values (J) for the analytes of interest and sample preparation technique used for the different drug compounds, the calibration solutions were prepared at half (0.5J) and twice (2J) of the target values. Table 4 shows the PDE values (µg/day) for the oral drugs and target concentration values in µg/L calculated based on a daily dose of 10 g/day and a final sample dilution (0.2 g/50 mL). The calibration graphs were plotted for 0.3J, 0.5J, 1J, 1.5J and 2J of the target values. Single-element PerkinElmer Pure Calibration Standards for atomic spectroscopy were used as the stock standards for preparing the working standards. Working standards were prepared by volume/volume (v/v) dilution in 50 mL conical free-standing polypropylene flasks. Five-point calibration curves were constructed for each individual metal ion and their calibration curve correlation coefficients (R²) were observed better than 0.999.

Lead and Cadmium were done by graphite furnace and matrix modifier were used to stabilize elements during ashing process. Matrix modifiers were prepared from 10% NH₄H₂PO₄ (Part No. N9303445), 1% Mg as Mg(NO₃)₂ (Part No. B0190634) and 1% Pd (Part No. B0190635) stock solutions by diluting with the 0.2% HNO₃ solution. Matrix modifiers were added automatically to each blank, standard, and sample by AS 900 auto-sampler which is an integral part of the PinAAcle™ 900T spectrometer.

Mercury and Arsenic were done by FIAS-Furnace technique. The preparation process followed for FIFU is as below:

First a layer of 0.1% iridium was deposited on the platform inside the graphite tube. This is done automatically by injecting an iridium solution into the graphite tube using a specified furnace program. A single iridium coating on a platform lasts for hundreds of determinations.

Reductant Solution: NaBH₄ was used as reductant whose concentration was 0.2% in 0.05% NaOH solution.

All calibration standards of Arsenic were prepared in 10% HCl (Merck suprapure). All standards and samples solution were reduced using 5% KI and 5% Ascorbic acid for Arsenic for half an hour and then diluted to 50mL. For mercury, added 2-3 drops of 5% KMnO₄ in all solutions to oxidized mercury and diluted to 50ml using 5%HCl acid.

Mercury and Arsenic were treated separately.

Sample preparation:

The complete sample digestion was carried out using the PerkinElmer's TITAN MPS microwave digestion system equipped with high pressure rotor. The TITAN MPS is a closed vessel technique which is easy to operate and allows for complete digestion of difficult substance. Approximately, 0.2 gm of samples were weighed into the sample vessels to which was added 5 mL HNO₃ and 1 mL H₂O₂. The digestion program is mentioned in table 5.

When the digestion was completed, the samples were transferred out of the digestion vessels by triple-rinsing with deionized (DI) water into sample vials and then brought up to the final solution volume to 50mL with DI water (18 MΩ-cm)

Element	Class	Oral Daily Dose PDEa (µg/day)	0.3J	0.5J	1J	1.5J	2J
			Concentrations in µg/L				
Based on Daily Dose of 10 g/day & final solution dilution 0.2g to 50mL							
Cd	1	5	0.6	1	2	3	4
Pb	1	5	0.6	1	2	3	4
As	1	15	1.8	3	6	9	12
Hg	1	30	3.6	6	12	18	24

Table 4: Calibration Standards and J Values

Step	Target Temp (°C)	Pressure Limit (bar)	Ramp Time (min)	Hold Time (min)	Power Limit (%)
1	170	30	8	5	90
2	200	30	2	20	100
3	50	30	1	20	0

Table 5: Titan MPS Digestion Method

Sample treatment for hydride forming element:

Sample solutions were prepared separately for Mercury and Arsenic and treated as mentioned in standard preparation procedure for hydride.

Results and discussion

The prepared sample solutions were analyzed on PerkinElmer® PinAAcle™ 900T AAS and FIAS 100 hydride instruments using Syngistix™ ES software. Syngistix™ software features is a unique icon-based design that simplifies navigation and walks the user through every analysis – from setting up to acquiring data to reporting results. Calibration curves were constructed for a blank and five point calibration standards at 0.3J, 0.5J, 1J, 1.5J and 2J concentrations as specified in ICH-Q3D. All calibration curves gave linear regressions >0.999. The accuracy of the method was assessed by determining pre-digestion spike recoveries of 0.5J and 1.5J, as specified in the method. Table 6 shows the recoveries in medicines, all well within the 70-150% window specified in the method. The <MDL values indicates that the element was less than the method detection limit.

Analyte	Sample value	Sample Spike -0.5J	% Spiked Recovery at 0.5J	Sample Spike -1.5J	% Spiked Recovery at 1.5J
Cd	<MDL	1.027	102.7	2.95	98.3
Pb	<MDL	0.949	94.9	2.983	99.4
As	<MDL	2.995	99.8	8.957	99.5
Hg	<MDL	6.120	102.0	16.92	94.0

Table 6: Sample results and spiked recoveries

The QC check standards were injected at 1J level before and after samples. Recoveries for all elements were observed satisfactory within expected range of 90% to 110%. The QC check recoveries are given in table 7.

QC Check at 1J before samples		QC Check at 1J after samples	
QC Conc in ppb	% recovery	QC Conc in ppb	% recovery
2.103	105.1	2.009	100.4
1.987	99.3	1.959	97.9
6.262	104.3	6.085	101.4
11.81	98.4	12.13	101.0

Table 7: QC Recoveries

Detection Limits:

All detection limits were determined using sample blank. These are calculated based on a 98% confidence level (3 standard deviations). The reported detection limits are given in ppm (wt/wt) in table 8.

Analyte	LOD in ppm (wt/wt)
Pb	0.071
Cd	0.002
As	0.023
Hg	0.029

Table 8: Detection Limits (MDLs)

Conclusions:

A complete method for the accurate determination of Class I elements as per ICH Q3D guidelines on PinAAcle™ 900T atomic absorption spectrometer is developed. This work has demonstrated that FIFU (FIAS-Furnace coupling) and PinAAcle™ 900T AAS from PerkinElmer can meet and exceed the requirements of ICHQ3D for Oral drug medications. The method detection limit (MDL) for all elements were determined to be well below the practical quantification limit (PQL) required by the method. This detection limit could be lowered even further by using a larger sample volume or through the use of end-capped THGA tubes (Part No. B3000655). Recoveries for spiked samples were all within the method's acceptable limits.

The PinAAcle 900Z (Longitudinal Zeeman Furnace only) spectrometer can also be used for this application. An Enhanced Security™ version meets the special needs of highly regulated labs such as those that must comply with the U.S. FDA's 21 CFR Part 11 regulations.

References:

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