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ANALYTICAL METHOD OF ANALYSIS: DETERMINATION OF TRACE METAL IMPURITIES BY ICP-MS IN TRIS AND TRIS HYDROCHLORIDE

TABLE OF CONTENTS

1. PURPOSE:

1.1. To provide a procedure for the assessment of Elemental Impurities via the NexION 350X S/N 85VN5093001 ICP-MS. This procedure was assessed as a full quantitative option-3 procedure as per validation report, BSI-RPT-0594 v1.0, and follows the validation parameters for quantitation procedures as outlined in USP <233>.

1.2. Elements under USP <232> validated for this test method are as follows:

1.2.1. Class 1: As, Cd and, Pb

1.2.2. Class 2A: Ni

1.2.3. Class 3: Cu

1.2.4. Class 4: Ca, Fe, Mg, Mn, and Zn

2. RESPONSIBILITIES:

2.1. The Laboratory Technology Manager, or other qualified designated individual, is responsible for the control, implementation, training, and maintenance of this Protocol.

2.2. The QC Staff is responsible for complying with the requirements of this Test Method.

2.3. If any abnormalities are determined during routine use of the ICP-MS or during calibration, the QC Managers shall be promptly notified. If necessary, the ICP-MS will be serviced and recalibrated by Perkin Elmer before being approved for use.

3. REFERENCES:

3.1. BSI-PRL-0352, Analytical Method Validation Protocol: Trace Metal Impurities: Tris and Tris HCl

3.2. BSI-RPT-0594, Determination of ICH Q3D Elemental Impurities by ICP-MS: Tris/THCl

3.3. BSI-SOP-0303, NexION 350X ICP-MS SOP

3.4. BSI-SOP-0304, NexION 350X ICP-MS Care and Maintenance SOP

3.5. BSI-SOP-0436, Analytical Methods Validation Master Plan

3.6. ICH Guideline for Elemental Impurities Q3D Current

3.7. NexION Operation with Syngistix Software Guide

3.8. USP <232>, <233>

3.9. USP <730> Plasma Spectrochemistry

3.10. USP <1730> Plasma Spectrochemistry—Theory and Practice

TABLE 1: LIMITS FOR TRIS AND TRIS HCL (50 GRAM/DAY PATIENT EXPOSURE)

Elements	ICH Class	Parenteral PDE Limits (µg/day)	0.3J LOQ (µg/g) in sample	0.5J Target(µg/g) in sample	1.0J Target (µg/g) in sample	1.5J Target(µg/g) in sample
As	1	15	0.09	0.15	0.30	0.45
Cd	1	2.0	0.012	0.02	0.04	0.06
Pb	1	5.0	0.03	0.05	0.10	0.15
Ni	2A	20	0.12	0.20	0.40	0.60
Cu	3	[1]100	0.60	1.0	2.0	3.0
Fe	4	[1]100	0.60	1.0	2.0	3.0
Mn	4	[1]100	0.60	1.0	2.0	3.0
Zn	4	[1]100	0.60	1.0	2.0	3.0
Ca	4	[1]100	0.60	1.0	2.0	3.0
Mg	4	[1]100	0.60	1.0	2.0	3.0

[1]PDE calculated based on customer specification

4. MATERIALS AND EQUIPMENT:

4.1. Equipment
- 4.1.1. Analytical Balance
- 4.1.2. NexION 350X ICP-MS S/N 85VN5093001, or qualified ICP-MS

4.2. Reagents
- 4.2.1. Nitric Acid, Trace metals grade or equivalent
- 4.2.2. Hydrochloric Acid, Trace metals grade or equivalent
- 4.2.3. Sulfuric Acid, Trace metals grade or equivalent
- 4.2.4. Deionized water (Type 1 Ultrapure)
- 4.2.5. Thiourea, Acros 99+ % grade
- 4.2.6. NexION Setup Solution
- 4.2.7. NexION KED Setup Solution, or equivalent

4.3. Consumable Supplies
- 4.3.1. SCP Digitubes® 15, 50, 100 mL
- 4.3.2. Pipette Tips of various sizes
- 4.3.3. Silicycle SiliaPrep Cation Solid Phase Extraction (SPE) cartridge

4.4. Personnel
- 4.4.1. All personnel that executed the protocol are trained on ICP-MS analysis or are considered Subject Matter Experts. The test method will be assigned a mark as read training to QC analysts involved with the execution.

TABLE 2: REFERENCE STANDARDS

Identification[1]	Manufacturer	Concentrations / Elements
1,000 µg/mL As Standard	SCP Science	As (1,000 µg/mL)
1,000 µg/mL Cd Standard	SCP Science	Cd (1,000 µg/mL)
1,000 µg/mL Pb Standard	SCP Science	Pb (1,000 µg/mL)
1,000 µg/mL Ni Standard	SCP Science	Ni (1,000 µg/mL)
1,000 µg/mL Cu Standard	SCP Science	Cu (1,000 µg/mL)
1,000 µg/mL Fe Standard	SCP Science	Fe (1,000 µg/mL)
1,000 µg/mL Mn Standard	SCP Science	Mn (1,000 µg/mL)
1,000 µg/mL Zn Standard	SCP Science	Zn (1,000 µg/mL)
1,000 µg/mL Ca Standard	SCP Science	Ca (1,000 µg/mL)
1,000 µg/mL Mg Standard	SCP Science	Mg (1,000 µg/mL)
Pharma-CAL Custom Standard AQ0-167-065 (Alternate Preparation Table 4)	SCP Science	As (30 µg/mL); Pb (10 µg/mL); Ni (40 µg/mL); Cu, Fe, Mn, Zn, Ca, Mg (200 µg/mL)
Pharma-CAL Custom Standard AQ0-086-125 (Internal Standard Stock)	SCP Science	Be, Sc, Y, Re (10 µg/mL); Te; 5 µg/mL Ge, Tb, Bi (25 µg/mL)

[1] Additional standards/custom standards can be used as long as the concentration remains the same in final preparations.

5. PROCEDURE:

5.1. All standards will be prepared volumetrically from stock solutions purchased from certified vendors. If the vendor supplied stock standard is within 2% of the nominal value as per the certificate of analysis, then the nominal value will be used to calculate the concentration of the standard. If the stock standard certificate of analysis value is greater than or less than 2% of the nominal value, then the certificate of analysis value will be used for the stock standard concentration.

5.2. **Stock Standards**

5.2.1. The stock standards listed in Table 2 will be used to prepare the calibration standards and spiked samples.

5.3. **Acid Digestion Mix**

[2:1] Nitric Acid (HNO_3) : Sulfuric Acid (H_2SO_4)

5.3.1. Add 50 mL of nitric acid to a 100 mL Digitube® and then slowly add 25 mL of sulfuric acid. Scale proportionally as needed for use.

5.3.2. Place solution in a cold-water bath to aid cooling. Prepare day of use.

5.4. **Internal Standard/Complexing Solution**

5.4.1. Weigh approximately 1.0 gram of Thiourea into a 50 mL Digitube®.

5.4.2. Add 20 mL of deionized water and dissolve.

5.4.3. Filter solution through a SiliaPrep Cation Solid Phase Extraction (SPE) cartridge into a separate 50 mL Digitube®.

5.4.4. Transfer 2.5 mL of Pharma CAL Custom Standard Stock (Internal Standard).

5.4.5. Add 25 mL of Hydrochloric Acid.

5.4.6. Dilute to a final volume of 50 mL with deionized water and mixed well.

5.4.7. Scale proportionally as needed for use

5.5. **Intermediate Standard**

5.5.1. Prepare a standard solution containing the elements listed in Table 3 or Table 4. Do not allow stock standards to contact concentrated acids while preparing solutions.

TABLE 3: INTERMEDIATE STANDARD

Identification	Element	Stock Identification	Amount added (mL)	Nitric Acid (mL)	Final Volume Deionized Water(mL)	Final Concentration (µg/mL)
Intermediate Standard	As	Pharma-CAL Custom Standard AQ0-167-065	5.0	1.0	50	3.0
	Pb					1.0
	Ni					4.0
	Cu					20
	Fe					20
	Mn					20
	Zn					20
	Ca					20
	Mg					20
Cd Stock	Cd	1,000 µg/mL Cd Std	0.02			0.40

TABLE 4: INTERMEDIATE STANDARD (ALTERNATE PREPARATION)

Identification	Element	Stock Identification	Amount added (mL)	Nitric Acid (mL)	Final Volume Deionized Water (mL)	Final Concentration (µg/mL)
Intermediate Standard	As	1,000 µg/mL As Std	0.15	1.0	50	3.0
	Cd	1,000 µg/mL Cd Std	0.02			0.40
	Pb	1,000 µg/mL Pb Std	0.05			1.0
	Ni	1,000 µg/mL Ni Std	0.20			4.0
	Cu	1,000 µg/mL Cu Std	1.0			20
	Fe	1,000 µg/mL Fe Std	1.0			20
	Mn	1,000 µg/mL Mn Std	1.0			20
	Zn	1,000 µg/mL Zn Std	1.0			20
	Ca	1,000 µg/mL Ca Std	1.0			20
	Mg	1,000 µg/mL Mg Std	1.0			20

5.6. **0.5J Calibration Standard Preparation**

5.6.1. Prepare a solution containing the elements listed in Table 5 below in 5.0% Nitric Acid, 2.5% Sulfuric Acid, 2.0% Hydrochloric Acid, and 0.04% thiourea with Be, Sc, Y, Re, Te, Ge, Tb, and Bi internal standards. Calibration standards are stable for 24 hours.

TABLE 5: 0.5J CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Acid Mix (mL)	HCl (mL)	Internal Standard/ Complexing Solution (mL)	Final Volume Deionized Water (mL)	Final Concentration (µg/L)
0.5J Calibration Standard	As	0.050	3.75	0.50	1.0	50	3.0
	Cd						0.40
	Pb						1.0
	Ni						4.0
	Cu						20
	Fe						20
	Mn						20
	Zn						20
	Ca						20
	Mg						20

5.7. **1.5J Calibration Standard Preparation**

5.7.1. Prepare a solution containing the elements listed in Table 6 below in 5.0% Nitric Acid, 2.5% Sulfuric Acid, 2.0% Hydrochloric Acid, and 0.04% thiourea with Be, Sc, Y, Re, Te, Ge, Tb, and Bi internal standards. Calibration standards are stable for 24 hours.

TABLE 6: 1.5J CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Acid Mix (mL)	HCl (mL)	Internal Standard/ Complexing Solution (mL)	Final Volume Deionized Water (mL)	Final Concentration (µg/L)
1.5J Calibration Standard	As	0.150	3.75	0.50	1.0	50	9.0
	Cd						1.2
	Pb						3.0
	Ni						12
	Cu						60
	Fe						60
	Mn						60
	Zn						60
	Ca						60
	Mg						60

5.8. **2.0J Calibration Standard Preparation**

5.8.1. Prepare a solution containing the elements listed in Table 7 below in 5.0% Nitric Acid, 2.5% Sulfuric Acid, 2.0% Hydrochloric Acid, and 0.04% thiourea with Be, Sc, Y, Re, Te, Ge, Tb, and Bi internal standards. Calibration standards are stable for 24 hours.

TABLE 7: 2.0J CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Acid Mix (mL)	HCl (mL)	Internal Standard/ Complexing Solution (mL)	Final Volume Deionized Water (mL)	Final Concentration (µg/L)
2.0J Calibration Standard	As	0.200	3.75	0.50	1.0	50	12
	Cd						1.6
	Pb						4.0
	Ni						16
	Cu						80
	Fe						80
	Mn						80
	Zn						80
	Ca						80
	Mg						80

5.9. **Calibration Blank**

5.9.1. Prepare a solution containing the elements listed in Table 8 below in 5.0% Nitric Acid, 2.5% Sulfuric Acid, 2.0% Hydrochloric Acid, and 0.04% thiourea with Be, Sc, Y, Re, Te, Ge, Tb, and Bi internal standards. Do not allow stock standards to contact concentrated acids while preparing solutions. Calibration blank is stable for 24 hours.

TABLE 8: CALIBRATION BLANK

Identification	Acid Mix (mL)	HCl (mL)	Internal Standard/ Complexing Solution (mL)	Final Volume Deionized Water (mL)
Calibration Blank	3.75	0.50	1.0	50

5.10. **Method Blank Preparation**

5.10.1. Refer to Calibration Blank Preparation.

5.11. **Sample Preparation**

5.11.1. Weigh approximately 1000 mg of the sample into a 50 mL Digitube®.

5.11.2. Transfer 2.5 mL of deionized water to the 50 mL Digitube®.

5.11.3. Add 0.50 mL of HCl and swirl to dissolve.

5.11.4. Transfer 3.75 mL of Acid Mix and again swirl solution to mix.

5.11.5. Allow the solution to digest at room temperature for approximately 5 minutes occasionally swirling the solution to aid with digestion.

Note: If reaction becomes too vigorous, add deionized water as per Section 5.11.6.

5.11.6. Add deionized water to approximately 45 mL mark and then transfer 1.0 mL of internal standard/complexing solution.

5.11.7. Dilute to a final volume of 50 mL with deionized water and mix thoroughly.

5.11.8. Samples are stable for 24 hours.

5.12. **Isobaric Overlap Correction**

5.12.1. An isobaric interference results from equal mass isotopes of different elements present in the sample solution. Analysis sequences that are processed utilizing multi-element standards will require the use of correction equations to compensate for known isobaric overlaps originating from the elemental standard and sample. The following correction equations should be used:

KED Mode:

$M_c(58) = M_u(58) \times 1 - M_{(rm)}(57) \times 0.13208$

The correction equations can be derived from the following equation:

$M_c = M_u - [M_{(rm)} \times (A_{(i.e.)}/A_{(rm)})]$

Where:

M_c = Corrected Count Rate for the analyte

M_u = Uncorrected count rate for the analyte

$M_{(rm)}$ = Count Rate of Reference Mass (rm) for the Interfering Element

$A_{(i.e.)}$ = Percent Abundance of Interfering Element (i.e.) at the analyte mass

$A_{(rm)}$ = Percent Abundance of Interfering Element at the Reference Mass (rm)

Example:

$M_c(58) = M_u(58) \times 1 - M_{(rm)}(57) \times (0.28 / 2.12)$

5.13. All correction coefficients were calculated based on the Agilent Technologies 2016 Relative Isotopic Abundance Table.

6. INSTRUMENT PROCEDURE:

6.1. Perform the ICP-MS daily performance check prior to beginning the analytical sequence. Refer to NexION 350X ICP-MS SOP, BSI-SOP-0303, for Daily Check procedures.

6.2. A calibration curve of no less than two standards and a blank must be used. The calibration correlation coefficient (R) must be ≥ 0.99.

6.3. Set up the sequence as per Table 9.

6.4. Confirm the calibration by analyzing the 1.5J standard after the calibration. The calibration check must recover ± 20% of the calculated theoretical concentration for multi-element analysis and ±10% for single element determinations.

6.5. The check standard must be verified after the calibration. A re-analysis of the check standard will be performed a minimum of once every 10 samples and at the end of the analytical run.

6.6. The sample concentration is calculated as:

$$\text{Conc. } (\mu g/g) = \frac{\text{Solution Conc. } (\mu g/L) \times \text{Solution vol. (L)} \times \text{Dilution Factor}}{\text{Sample Mass (g)}}$$

TABLE 9: EXAMPLE SEQUENCE

ID	Type	Level
Cal Blank	Cal Blank	Level 1
0.5J Cal Std	Cal Std	Level 2
1.5J Cal Std	Cal Std	Level 3
2.0J Cal Std	Cal Std	Level 4
Cal Blank Check	QC Check	Not Applicable
1.5J Check Std 1	QC Check	Not Applicable
Method Blank	Sample	Not Applicable
Sample(s) 10 or less	Sample	Not Applicable
1.5J Check Std 2	QC Check	Not Applicable

6.7. Instrument Setup and Parameters

6.7.1. Instrument settings are only listed as guidelines. Settings may be changed in order to accommodate changes in sample matrix or hardware configurations.

6.7.2. The AMS-II makeup gas system must be employed during analysis using a minimum argon dilution ratio of 20%.

6.7.3. The element arsenic is analyzed using hydrogen reaction gas in order to remove poly atomic interferences. A hydrogen DRC (Dynamic Reaction Cell) flow rate of approximately 4 mL/min should be used.

6.7.4. The instrument method is stored under the Approved Test Method Folder labelled as "Tris_THCl_TraceMetal.mth" for trace metal analysis.

TABLE 10: ICP-MS PARAMETERS

ICP-MS System	Perkin Elmer NexION350X Inductively Coupled Plasma Mass Spectrometry (ICP-MS) with Syngistix Software Version 2.4
Sweeps/reading	20
Replicates	3
Nebulizer Gas	Argon
Collision Cell Gas	Helium
Reaction Cell Gas	Hydrogen
Dilution Gas	Argon
Sample and Skimmer Cone	Platinum
Sample Rinses	Rinse-1: 60 sec at 45 rpm 5.0% Nitric Acid, 2.5% Hydrochloric acid with 0.04% Thiourea (prefiltered using SPE cartridge listed in Section 5.3) or as applicable to mitigate carry over

TABLE 11: LINEAR RANGE AND CORRESPONDING TUNING MODE

Isotope	Internal Standard	Mode	Linear Range (µg/L)
24Mg	72Ge	KED	12-80
44Ca	72Ge	KED	12-80
55Mn	72Ge	KED	12-80
57Fe	72Ge	KED	12-80
58Ni	72Ge	KED	2.4-16
60Ni	72Ge	KED	2.4-16
62Ni	72Ge	KED	2.4-16
63Cu	72Ge	KED	12-80
65Cu	72Ge	KED	12-80
67Zn	72Ge	KED	12-80
68Zn	72Ge	KED	12-80
75As	89Y	KED	1.8-12
111Cd	125Te	KED	0.24-1.6
113Cd	125Te	KED	0.24-1.6
206Pb	209Bi	KED	0.60-4.0
207Pb	209Bi	KED	0.60-4.0
208Pb	209Bi	KED	0.60-4.0

7. REPORTING:

7.1. Any result below the 0.3J target concentration will be reported as less than the corresponding LOQ value listed in Table 1. Results above the 0.3J Target Concentration will be reported in µg/g (ppm) according to Table 12 below. If there are multiple isotopes present in the method and the resultis above the LOQ target concentration, report the average result from the isotopes.

TABLE 12: RESULT REPORTING

Result	Reporting
If < LOQ	Report as < LOQ
If ≥ LOQ and < 1.0 ppm	Report to two (2) decimal places
If ≥ LOQ and ≥ 1.0 ppm	Report to whole number