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ANALYTICAL METHOD OF ANALYSIS: DETERMINATION OF ICH Q3D ELEMENTAL IMPURITIES BY INDUCTIVELY COUPLED PLASMA MASS SPECTOMETRY (ICP-MS) IN TRIS API

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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-0543 v2.0, 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: Hg, As, Cd, and Pb
  1.2.2. Class 2A: Co, V, and Ni
  1.2.3. Class 2B: Tl, Au, Pd, Ir, Os, Rh, Ru, Ag, Se, and Pt
  1.2.4. Class 3: Li, Sb, Ba, Mo, Cu, Sn, and Cr
  1.2.5. Class 4: Al, Fe, Mn, and Zn

2. SCOPE:

2.1. Applies to Tris products manufactured at BioSpectra.

2.2. Applies to the NexION 350X S/N 85VN5093001 ICP-MS located in the Quality Control (QC) Laboratory at the BioSpectra Bangor, PA facility.

3. RESPONSIBILITIES:

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

3.2. The QC Staff are responsible for complying with the requirements of this Protocol.

3.3. 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.

4. REFERENCES:

4.1. BSI-PRL-0457, Determination of Elemental Impurities by ICP-MS in Tris Method
4.2. BSI-RPT-0543, Determination of Elemental Impurities by ICP-MS in Tris High MDD Analysis
4.3. BSI-SOP-0303, NexION 350X ICP-MS SOP
4.4. BSI-SOP-0304, NexION 350X ICP-MS Care and Maintenance SOP
4.5. BSI-SOP-0436, Analytical Methods Validation Master Plan
4.6. ICH Guideline for Elemental Impurities Q3D
4.7. NexION Operation with Syngistix Software Guide
4.8. USP <232>, Elemental Impurities- Limits
4.9. USP <233>, Elemental Impurities- Procedures
4.10. USP <730> Plasma Spectrochemistry
4.11. USP <1730> Plasma Spectrochemistry—Theory and Practice

TABLE 1: LIMITED FOR TRIS AND TRIS HCL (100 GRAM/DAY PATIENT EXPOSURE)

Elements	ICH Class	Parenteral PDE Limits (µg/day)	0.1J LOQ (µg/g) in sample	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	0.015	0.045	0.075	0.15	0.225
Cd	1	2.0	0.002	0.006	0.01	0.02	0.03
Hg	1	3.0	0.003	0.009	0.015	0.03	0.045
Pb	1	5.0	0.005	0.015	0.025	0.05	0.075
Co	2A	5.0	0.005	0.015	0.025	0.05	0.075
Ni	2A	20	0.02	0.06	0.10	0.20	0.30
V	2A	10	0.01	0.03	0.05	0.10	0.15
Tl	2B	8.0	0.008	0.024	0.04	0.08	0.12
Se	2B	[1]50	0.05	0.15	0.25	0.50	0.75
Ag	2B	10	0.01	0.03	0.05	0.10	0.15
Au	2B	100	0.10	0.30	0.50	1.0	1.5
Pd	2B	10	0.01	0.03	0.05	0.10	0.15
Ir	2B	10	0.01	0.03	0.05	0.10	0.15
Os	2B	10	0.01	0.03	0.05	0.10	0.15
Pt	2B	10	0.01	0.03	0.05	0.10	0.15
Rh	2B	10	0.01	0.03	0.05	0.10	0.15
Ru	2B	10	0.01	0.03	0.05	0.10	0.15
Ba	2B	700	0.70	2.1	3.5	7.0	10.5
Sb	3	90	0.09	0.27	0.45	0.90	1.35
Li	3	250	0.25	0.75	1.25	2.5	3.75
Mo	3	[1]50	0.05	0.15	0.25	0.50	0.75
Cu	3	[1]25	0.025	0.075	0.125	0.25	0.375
Sn	3	600	0.60	1.8	3.0	6.0	9.0
Cr	3	[1]50	0.05	0.15	0.25	0.5	0.75
Al	4	[1]400	0.40	1.2	2.0	4.0	6.0
Fe	4	[1]200	0.20	0.60	1.0	2.0	3.0
Mn	4	[1]25	0.025	0.075	0.125	0.25	0.375
Zn	4	[1]200	0.20	0.60	1.0	2.0	3.0

[1] PDE calculated based on lower internal specification

5. MATERIALS AND EQUIPMENT:

- 5.1. Equipment
  - 5.1.1. Analytical Balance
  - 5.1.2. NexION 350X ICP-MS S/N 85VN5093001, or qualified ICP-MS
- 5.2. Reagents
  - 5.2.1. Nitric Acid, Trace metals grade or equivalent
  - 5.2.2. Hydrochloric Acid, Trace metals grade or equivalent
  - 5.2.3. Sulfuric Acid, Trace metals grade or equivalent
  - 5.2.4. Deionized water (Type 1 Ultrapure)
  - 5.2.5. Thiourea, 99+% grade
  - 5.2.6. SiliCycle: SiliaPrep MB SPE Cartridges, Silica-Based AMPA
  - 5.2.7. NexION Setup Solution
  - 5.2.8. NexION KED Setup Solution, or equivalent
- 5.3. Consumable Supplies
  - 5.3.1. SCP Digitubes® 15 mL, 50 mL, and 100 mL
  - 5.3.2. Pipette Tips of various sizes
- 5.4. Personnel
  - 5.4.1. 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
Pharma-CAL Custom Standard Parenteral STD# 1 IA AQ0-145-201	SCP Science	Ag (10 µg/mL), As (15 µg/mL), Cd (2 µg/mL), Co (5 µg/mL), Hg (3 µg/mL), Ni (20 µg/mL), Pb (5 µg/mL), Se (50 µg/mL), Tl (8 µg/mL), V (10 µg/mL)
USP232/ICH Q3D Parenteral STD# 2 IA 140-131-211	SCP Science	100 µg/mL; Au, 10 µg/mL; Ir, Os, Pd, Pt, Rh, & Ru
Pharma-CAL Custom Standard Parenteral STD# 3 IA AQ0-145-211	SCP Science	Al (400 µg/mL), Ba (700 µg/mL), Cr (50 µg/mL), Cu (25 µg/mL), Fe (200 µg/mL), Li (250 µg/mL), Mn (25 µg/mL), Mo (50 µg/mL), Sb (90 µg/mL), Sn (600 µg/mL), Zn (200 µg/mL)
Pharma-CAL Custom Standard AQ0-086-125 (Internal Standard)	SCP Science	10 µg/mL Be, Sc, Y, Re; 25 µg/mL Te;5 µg/mL Ge, Tb, Bi

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

6. PROCEDURE:

6.1. 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.

6.2. **Stock Standards**

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

6.3. **Acid Mix**

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

6.3.1. Caution: Combining nitric acid and sulfuric acid generates excessive heat. Never seal cap tightly before solution has completely cooled.

6.3.2. To prepare, add 50 mL of nitric acid to a 100 mL Digitube® and then slowly add 25 mL of sulfuric acid. Solution can be placed in a cold-water bath to aid cooling.

6.3.3. Scale proportionally as needed for use.(Prepare same day)

6.4. **Internal Standard/Complexing Solution**

6.4.1. Weigh approximately 2.0 grams of Thiourea into a 100 mL Digitube®.

6.4.2. Add approximately 20 mL of deionized water and mix to dissolve.

6.4.3. Filter solution through the SiliaPrep Cation Solid Phase Extraction (SPE) cartridge listed in Section 5.2 before proceeding.

6.4.4. Transfer 2.50 mL of Pharma CAL Custom Standard (Internal standard) Stock to the filtered solution and then add 75 mL of hydrochloric acid.

6.4.5. Dilute to a final volume of 100 mL with deionized water and mix well.

6.4.6. Scale proportionally as needed for use.

6.5. **Intermediate Standard Preparation**

6.5.1. Prepare a standard solution containing the elements listed in Table 3, using the standards STD#1 IA, STD#2 IA, and STD#3 IA. Do not allow stock standards to contact concentrated acids while preparing solutions.

TABLE 3: INTERMEDIATE STANDARD PREPARATION

Identification	Element	Stock Identification	Amount added (mL)	HCl (mL)	Final Volume Deionized Water (mL)	Final Concentration (µg/mL)
Intermediate Standard	As	STD# 1 IA AQ0-145-201	1.0	1.0	10.0	1.5
	Cd					0.20
	Hg					0.30
	Pb					0.50
	Co					0.50
	Ni					2.0
	V					1.0
	Se					5.0
	Ag					1.0
	Tl					0.80
	Au	STD# 2 IA 140-131-211	1.0			10
	Pd					1.0
	Ir					1.0
	Os					1.0
	Pt					1.0
	Rh					1.0
	Ru					1.0
	Ba	STD# 3 IA AQ0-145-211	1.0			70
	Sb					9.0
	Li					25
	Mo					5.0
	Cu					2.5
	Sn					60
	Cr					5.0
	Al					40
	Fe					20
	Mn					2.5
	Zn					20

6.6. 0.5J Calibration Standard Preparation

6.6.1. Prepare a solution containing the elements listed in Table 4 below in 5.0% HNO_3, 2.5% H_2SO_4, 3.0% HCl, and 0.04% (400 µg/mL) 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. Add deionized water to approximately 45 mL before adding Internal Standard/ Complexing Solution.

TABLE 4: 0.5J CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Acid Digestion Mix (mL)	Internal Standard/ Complexing Solution (mL)	Final Volume Deionized Water (mL)	Final Concentration (µg/L)
0.5J Calibration Standard	As	0.050	3.75	2.0	50	1.5
	Cd					0.20
	Hg					0.30
	Pb					0.50
	Co					0.50
	Ni					2.0
	V					1.0
	Se					5.0
	Ag					1.0
	Tl					0.80
	Au					10
	Pd					1.0
	Ir					1.0
	Os					1.0
	Pt					1.0
	Rh					1.0
	Ru					1.0
	Ba					70
	Sb					9.0
	Li					25
	Mo					5.0
	Cu					2.5
	Sn					60
	Cr					5.0
	Al					40
	Fe					20
	Mn					2.5
	Zn					20

6.7. 1.5J Calibration Standard Preparation

6.7.1. Prepare a solution containing the elements listed in Table 5 below in 5.0% HNO_3, 2.5% H_2SO_4, 3.0% HCl, and 0.04% (400 µg/mL) Thiourea with Be, Sc, Y, Re, Te, Ge, Tb, and Bi internal standards. Do not allow standards to contact concentrated acids while preparing solutions. Add deionized water to approximately 45 mL before adding Internal Standard/ Complexing Solution.

TABLE 5: 1.5J CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Acid Digestion Mix (mL)	Internal Standard/ Complexing Solution (mL)	Final Volume Deionized Water (mL)	Final Concentration (µg/L)
1.5J Calibration Standard	As	0.150	3.75	2.0	50	4.5
	Cd					0.60
	Hg					0.90
	Pb					1.5
	Co					1.5
	Ni					6.0
	V					3.0
	Se					15
	Ag					3.0
	Tl					2.4
	Au					30
	Pd					3.0
	Ir					3.0
	Os					3.0
	Pt					3.0
	Rh					3.0
	Ru					3.0
	Ba					210
	Sb					27
	Li					75
	Mo					15
	Cu					7.5
	Sn					180
	Cr					15
	Al					120
	Fe					60
	Mn					7.5
	Zn					60

6.8. 2.0J Calibration Standard Preparation

6.8.1. Prepare a solution containing the elements listed in Table 6 below in 5.0% HNO_3, 2.5% H_2SO_4, 3.0% HCl, and 0.04% (400 µg/mL) Thiourea with Be, Sc, Y, Re, Te, Ge, Tb, and Bi internal standards. Do not allow standards to contact concentrated acids while preparing solutions. Add deionized water to approximately 45 mL before adding Internal Standard/Complexing Solution. Expiration: 24 hours

TABLE 6: 2.0J CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Acid Digestion Mix (mL)	Internal Standard/ Complexing Solution (mL)	Final Volume Deionized Water (mL)	Final Concentration (µg/L)
2.0J Calibration Standard	As	0.200	3.75	2.0	50	6.0
	Cd					0.80
	Hg					1.2
	Pb					2.0
	Co					2.0
	Ni					8.0
	V					4.0
	Se					20
	Ag					4.0
	Tl					3.2
	Au					40
	Pd					4.0
	Ir					4.0
	Os					4.0
	Pt					4.0
	Rh					4.0
	Ru					4.0
	Ba					280
	Sb					36
	Li					100
	Mo					20
	Cu					10
	Sn					240
	Cr					20
	Al					160
	Fe					80
	Mn					10
	Zn					80

6.9. **Calibration Blank**

6.9.1. Prepare a solution containing 5.0% HNO_3, 2.5% H_2SO_4, 3.0% HCl, and 0.04% (400 µg/mL) Thiourea with Be, Sc, Y, Re, Te, Ge, Tb, and Bi internal standards as per Table 7 below. Do not allow Internal Standard/ Complexing Solution to contact concentrated acids. Add deionized water to approximately 45 mL before adding Internal Standard/ Complexing Solution.

TABLE 7: CALIBRATION BLANK

Description	Internal Standard/ Complexing Solution (mL)	Acid Digestion Mix (mL)	Final Volume Deionized Water (mL)
Cal Blank	2.0	3.75	50

6.10. **Method Blank Preparation**

6.10.1. Add 30 mL of deionized water and 3.75 mL of Acid mixture to a 50 mL Digitube®.

6.10.2. Add deionized water to approximately 45 mL and then transfer 2.0 mL of Internal Standard/Complexing Solution.

6.10.3. Dilute to a final volume of 50 mL with deionized water and mix well.

6.11. **Sample Preparation**

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

6.11.2. Add 30 mL of deionized water and swirl solution to mix.

6.11.3. Add 3.75 mL of Acid mixture.

6.11.4. Add deionized water to approximately 45 mL and then transfer 2.0 mL of Internal Standard/ Complexing Solution.

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

6.12. Isobaric Overlap Corrections

6.12.1. 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:

$M_c(58) = M_u(58) \times 1 - M_{(rm)}(57) \times 0.13208$
$M_c(98) = M_u(98) \times 1 - M_{(rm)}(99) \times 0.14655$
$M_c(106) = M_u(106) \times 1 - M_{(rm)}(111) \times 0.09766$
$M_c(108) = M_u(108) \times 1 - M_{(rm)}(111) \times 0.06953$
$M_c(120) = M_u(120) \times 1 - M_{(rm)}(125) \times 0.01273$
$M_c(123) = M_u(123) \times 1 - M_{(rm)}(125) \times 0.12588$
$M_c(190) = M_u(190) \times 1 - M_{(rm)}(195) \times 0.00036$
$M_c(192) = M_u(192) \times 1 - M_{(rm)}(195) \times 0.02315$
$M_c(196) = M_u(196) \times 1 - M_{(rm)}(202) \times 0.005023$

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:

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

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

6.12.3. Multiplier used in the correct equation may differ slightly from the multiplier used in the Syngistix instrument method due to rounding.

7. INSTRUMENT PROCEDURE:

7.1. 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.

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

7.3. Set up the sequence as per Table 8.

7.4. 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.

7.5. 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.

7.6. 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 8: EXAMPLE SAMPLE ANALYSIS 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

7.7. Instrument Setup and Parameters

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

7.7.2. The AMS-II makeup gas must be engaged during analysis using a minimum dilution gas ratio of 25%.

7.7.3. The elements arsenic and selenium are analyzed using hydrogen reaction gas in order to remove poly atomic interferences. A hydrogen DRC (Dynamic Reaction Cell) flow rate of approximately 4-5 mL/min should be used.

7.7.4. 7.7.1. The instrument method is stored under the Approved Test Method Folder labelled as"Tris API Elemental Impurities.mth" for elemental impurities testing.

TABLE 9: ICP-MS PARAMETERS

ICP-MS System	Perkin Elmer NexION350X Inductively Coupled Plasma Mass Spectrometry (ICP-MS) with Syngistix Software
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% HNO_3, 3.0% HCl with 0.04% Thiourea(or as applicable to mitigate carry over)

TABLE 10: LINEAR RANGE AND CORRESPONDING TUNING MODE

Isotope	Internal Standard	Mode	Linear Range (µg/L)	Isotope	Internal Standard	Mode	Linear Range (µg/L)
7Li	45Sc	STD	5.0-100	111Cd	89Y	KED	0.04-0.80
27Al	45Sc	KED	8.0-160	113Cd	89Y	KED	0.04-0.80
51V	45Sc	KED	0.20-4.0	118Sn	89Y	KED	12-240
52Cr	45Sc	KED	1.0-20	119Sn	89Y	KED	12-240
53Cr	45Sc	KED	1.0-20	120Sn	89Y	KED	12-240
55Mn	45Sc	KED	0.50-10	121Sb	89Y	KED	1.8-36
57Fe	45Sc	KED	4.0-80	123Sb	89Y	KED	1.8-36
58Ni	72Ge	KED	0.40-8.0	135Ba	159Tb	KED	14-280
59Co	72Ge	KED	0.10-2.0	136Ba	159Tb	KED	14-280
60Ni	72Ge	KED	0.40-8.0	137Ba	159Tb	KED	14-280
62Ni	72Ge	KED	0.40-8.0	138Ba	159Tb	KED	14-280
63Cu	72Ge	KED	0.50-10	188Os	185 Re	KED	0.20-4.0
65Cu	72Ge	KED	0.50-10	189Os	185 Re	KED	0.20-4.0
67Zn	89Y	KED	4.0-80	190Os	185 Re	KED	0.20-4.0
68Zn	89Y	KED	4.0-80	191Ir	185 Re	KED	0.20-4.0
75As	89Y	H_2 DRC	0.30-6.0	192Os	185 Re	KED	0.20-4.0
77Se	89Y	H_2 DRC	1.0-20	193Ir	185 Re	KED	0.20-4.0
78Se	89Y	H_2 DRC	1.0-20	194Pt	185 Re	KED	0.20-4.0
95Mo	89Y	KED	1.0-20	195Pt	185 Re	KED	0.20-4.0
97Mo	89Y	KED	1.0-20	196Pt	185 Re	KED	0.20-4.0
98Mo	89Y	KED	1.0-20	197Au	209Bi	KED	2.0-40
99Ru	89Y	KED	0.20-4.0	199Hg	185Re	KED	0.06-1.2
101Ru	89Y	KED	0.20-4.0	200Hg	185Re	KED	0.06-1.2
103Rh	89Y	KED	0.20-4.0	202Hg	185Re	KED	0.06-1.2
105Pd	89Y	KED	0.20-4.0	203Tl	209Bi	KED	0.16-3.2
106Pd	89Y	KED	0.20-4.0	205Tl	209Bi	KED	0.16-3.2
107Ag	89Y	KED	0.20-4.0	206Pb	209Bi	KED	0.10-2.0
108Pd	89Y	KED	0.20-4.0	207Pb	209Bi	KED	0.10-2.0
109Ag	89Y	KED	0.20-4.0	208Pb	209Bi	KED	0.10-2.0

8. REPORTING:

8.1. Any result below the 0.1J target concentration will be reported as less than the corresponding LOQ value listed in Table 1. Results above the 0.1J Target Concentration will be reported in µg/g (ppm) according to Table 11 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 11: 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