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

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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-0541 v1.0, and follows the validation parameters for quantitation procedures as outlined in USP <233>.

1.2. Elements under USP <232> will be considered and 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, Se, Ag, and Pt
  1.2.4. Class 3: Li, Sb, Ba, Mo, Cu, Sn, and Cr

2. SCOPE:

2.1. Applies to Trehalose 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 Protocol.

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-0509, Determination of ICH Q3D Elemental Impurities by ICP-MS in Trehalose
4.2. BSI-RPT-0541, Analytical Method Validation Report: Determination of Elemental Impurities by ICP-MS in Trehalose
4.3. BSI-SOP-0304, NexION 350X ICP-MS Care and Maintenance SOP
4.4. BSI-SOP-0303, NexION 350X ICP-MS SOP
4.5. BSI-SOP-0436, Analytical Methods Validation Master Plan
4.6. ICH Guideline for Elemental Impurities Q3D Current
4.7. USP <730> Plasma Spectrochemistry
4.8. USP <1730> Plasma Spectrochemistry—Theory and Practice
4.9. NexION Operation with Syngistix Software Guide
4.10. USP <232>, Elemental Impurities- Limits
4.11. USP <233>, Elemental Impurities- Procedures

TABLE 1: LIMITS FOR TREHALOSE (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	0.012	0.02	0.04	0.06
Hg	1	3	0.018	0.03	0.06	0.09
Pb	1	5	0.03	0.05	0.10	0.15
Co	2A	5	0.03	0.05	0.10	0.15
Ni	2A	20	0.12	0.20	0.40	0.60
V	2A	10	0.06	0.10	0.20	0.30
Se	2B	80	0.48	0.80	1.6	2.4
Ag	2B	10	0.06	0.10	0.20	0.30
Tl	2B	8	0.048	0.08	0.16	0.24
Au	2B	100	0.60	1.0	2.0	3.0
Pd	2B	10	0.06	0.10	0.20	0.30
Ir	2B	10	0.06	0.10	0.20	0.30
Os	2B	10	0.06	0.10	0.20	0.30
Pt	2B	10	0.06	0.10	0.20	0.30
Rh	2B	10	0.06	0.10	0.20	0.30
Ru	2B	10	0.06	0.10	0.20	0.30
Ba	2B	700	4.2	7.0	14	21
Sb	3	90	0.54	0.90	1.8	2.7
Li	3	250	1.5	2.5	5.0	7.5
Mo	3	1500	9.0	15	30	45
Cu	3	300	1.8	3.0	6.0	9.0
Sn	3	600	3.6	6.0	12	18
Cr	3	1100	6.6	11	22	33

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, Acros 99+ % grade
  - 5.2.6. NexION Setup Solution
  - 5.2.7. NexION KED Setup Solution, or equivalent
  - 5.2.8. SiliaPrep MB SPE Cartridges, Silica-Based AMPA
- 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 laboratory chemists or other testing personnel that are trained on ICP-MS are considered Subject Matter Experts.

TABLE 2: REFERENCE STANDARDS

Identification[1]	Manufacturer	Concentrations / Elements
Pharma-CAL Standard Parenteral STD# 1 IA; 140-131-201	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 (80 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 Standard Parenteral STD# 3 IA; 140-131-221	SCP Science	Ba (700 µg/mL), Cr (1,100 µg/mL), Cu (300 µg/mL), Li (250 µg/mL), Mo (1,500 µg/mL), Sb (90 µg/mL), Sn (600 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 1.0 gram of Thiourea into a 50 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 2.5 mL of Pharma-CAL Custom Standard (Internal standard) Stock to the filtered solution and add 25 mL of hydrochloric acid.

6.4.5. Dilute to a final volume of 50 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 140-131-201	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					8.0
	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 140-131-221	1.0			70
	Sb					9.0
	Li					25
	Mo					150
	Cu					30
	Sn					60
	Cr					110

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, 1.0% HCl, and 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. Expiration: 24 hours

TABLE 4: 0.5J CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Acid 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	1.0	50	1.5
	Cd					0.20
	Hg					0.30
	Pb					0.50
	Co					0.50
	Ni					2.0
	V					1.0
	Se					8.0
	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					150
	Cu					30
	Sn					60
	Cr					110

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, 1.0% HCl, and 400 µg/mL Thiourea matrix. 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 5: 1.5J CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	AcidMix (mL)	Internal Standard/ Complexing Solution (mL)	Final Volume Deionized Water (mL)	Final Concentration (µg/L)
1.5J Calibration Standard	As	0.150	3.75	1.0	50	4.5
	Cd					0.60
	Hg					0.90
	Pb					1.5
	Co					1.5
	Ni					6.0
	V					3.0
	Se					24
	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					450
	Cu					90
	Sn					180
	Cr					330

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, 1.0% HCl and 400 µg/mL Thiourea matrix. 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)	AcidMix (mL)	Internal Standard/ Complexing Solution (mL)	Final Volume Deionized Water (mL)	Final Concentration (µg/L)
2.0J Calibration Standard	As	0.200	3.75	1.0	50	6.0
	Cd					0.80
	Hg					1.2
	Pb					2.0
	Co					2.0
	Ni					8.0
	V					4.0
	Se					32
	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					600
	Cu					120
	Sn					240
	Cr					440

6.9. Calibration Blank

6.9.1. Prepare a solution containing 5.0% HNO_3, 2.5% H_2SO_4, 1.0% HCl, and 400 µg/mL Thiourea matrix 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 Mix (mL)	Final Volume Deionized Water (mL)
Cal Blank	1.0	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 1.0 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 500 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 Mix.

6.11.4. Add deionized water to approximately 45 mL and then transfer 1.0 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:

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

The correction equations can be derived from the following equation: $M_c = M_u - [M_{(rm)} \times (A_{(ie)}/A_{(rm)})]$

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

Example:

$M_c (98) = M_u (98) \times 1 - M_{(rm)} (99) \times (1.87 / 12.76)$

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

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 mL/min should be used.
- 7.7.4. The instrument method is stored under the Approved Test Method folder labelled as "Trehalose_EI_Profile.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, 2.5% HCl with 0.04% thiourea (or as applicable to mitigate carry over)

TABLE 10: LINEAR RANGE AND CORRESPONDING TUNING MODE

Isotope	Mode	Internal Standard	Linear Range (µg/L)	Isotope	Mode	Internal Standard	Linear Range (µg/L)
7Li	STD	45Sc	15-100	119Sn	KED	125Te	36-240
51V	KED	45Sc	0.60-4.0	120Sn	KED	125Te	36-240
52Cr	KED	45Sc	66-440	121Sb	KED	125Te	5.4-36
53Cr	KED	45Sc	66-440	123Sb	KED	125Te	5.4-36
59Co	KED	45Sc	0.30-2.0	135Ba	KED	159Tb	42-280
60Ni	KED	45Sc	1.2-8.0	137Ba	KED	159Tb	42-280
62Ni	KED	45Sc	1.2-8.0	138Ba	KED	159Tb	42-280
63Cu	KED	45Sc	18-120	188Os	KED	209Bi	0.60-4.0
65Cu	KED	45Sc	18-120	189Os	KED	209Bi	0.60-4.0
75As	H_2DRC	89Y	0.90-6.0	190Os	KED	209Bi	0.60-4.0
77Se	H_2DRC	89Y	4.8-32	191Ir	KED	209Bi	0.60-4.0
78Se	H_2DRC	89Y	4.8-32	192Os	KED	209Bi	0.60-4.0
95Mo	KED	89Y	90-600	193Ir	KED	209Bi	0.60-4.0
97Mo	KED	89Y	90-600	194Pt	KED	209Bi	0.60-4.0
98Mo	KED	89Y	90-600	195Pt	KED	209Bi	0.60-4.0
99Ru	KED	89Y	0.60-4.0	196Pt	KED	209Bi	0.60-4.0
101Ru	KED	89Y	0.60-4.0	197Au	KED	209Bi	6.0-40
103Rh	KED	89Y	0.60-4.0	199Hg	KED	209Bi	0.18-1.2
105Pd	KED	89Y	0.60-4.0	200Hg	KED	209Bi	0.18-1.2
106Pd	KED	89Y	0.60-4.0	202Hg	KED	209Bi	0.18-1.2
107Ag	KED	125Te	0.60-4.0	203Tl	KED	209Bi	0.48-3.2
108Pd	KED	89Y	0.60-4.0	205Tl	KED	209Bi	0.48-3.2
109Ag	KED	125Te	0.60-4.0	206Pb	KED	209Bi	0.30-2.0
111Cd	KED	125Te	0.12-0.80	207Pb	KED	209Bi	0.30-2.0
113Cd	KED	125Te	0.12-0.80	208Pb	KED	209Bi	0.30-2.0
118Sn	KED	125Te	36-240				

8. REPORTING:

8.1. 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 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