BIOSPECTRA
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ANALYTICAL METHOD VALIDATION REPORT: TRACE METAL ANALYSIS IN SODIUM HYDROXIDE

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1. PURPOSE:

1.1. The purpose of this validation report is to establish documented evidence that the validation protocol, BSI-PRL-0852 v. 1.0, for Trace Metals in Sodium Hydroxide products performs according to USP and BioSpectra requirements.

1.1.1. Elements under USP <232> will be considered and are as follows:

1.1.1.1. Class 1: Cd and Pb

1.1.1.2. Class 2A: Co and Ni

1.1.1.3. Class 3: Ba, Cr, Cu, and Mo

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

1.1.1.5. Other: Bi and Sr

2. SCOPE:

2.1. Applies to Sodium Hydroxide 10N and related products manufactured at BioSpectra.

2.2. Applies to the Perkin Elmer Avio 500 ICP-OES S/N 081S1905062 located in the Quality Control (QC) Laboratory at the BioSpectra Bangor, PA facility.

2.3. This report applies to the validation protocol for trace metals in Sodium Hydroxide by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) performed at BioSpectra Inc.

3. REFERENCES:

3.1. BSI-PRL-0852, Analytical Method Validation Protocol: Trace Metals in Sodium Hydroxide

3.2. BSI-SOP-0362, Operation and Maintenance of the Perkin Elmer Avio 500 ICP-OES

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

3.4. ICH Guideline for Elemental Impurities Q3D

3.5. USP <232> Elemental Impurities- Limits

3.6. USP <233> Elemental Impurities- Procedures

3.7. USP <730> Plasma Spectrochemistry

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

4. BACKGROUND:

4.1. The target concentration is set the same for all elements, so that the limit of quantitation is below the specification of each element. Sodium hydroxide is a strong corrosive base that impacts the ICP plasma negatively. Due to these negative effects, the limits for each element are kept close to the specification to reduce the amount of product introduced into the system.

4.2. The execution of the protocol was performed for the new BioTech sodium hydroxide 10N code. While the validation was performed on sodium hydroxide 10N, lower concentrations of sodium hydroxide that are manufactured could be run on the final testing method as sodium hydroxide 10N would be a worst-case scenario and highest concentration.

4.3. The test protocol validation report includes the following parameters:

4.3.1. Specificity

4.3.2. Linearity and Range

4.3.3. Limit of Quantification (LOQ)

4.3.4. Accuracy by “Spiked Recovery”

4.3.5. Precision (Repeatability)

4.3.6. Intermediate Precision (Ruggedness)

4.3.7. Standard and Sample Solution Stability

TABLE 1: LIMITS FOR SODIUM HYDROXIDE BIOTECH CODE					
Element	**ICH Class**	**30% LOQ (µg/g) in sample**	**50% Target (µg/g) in sample**	**100% Target (µg/g) in sample**	**150% Target (µg/g) in sample**
Cd	1	0.90	1.5	3.0	4.5
Pb	1	0.90	1.5	3.0	4.5
Co	2A	0.90	1.5	3.0	4.5
Ni	2A	0.90	1.5	3.0	4.5
Ba	3	0.90	1.5	3.0	4.5
Cr	3	0.90	1.5	3.0	4.5
Cu	3	0.90	1.5	3.0	4.5
Mo	3	0.90	1.5	3.0	4.5
Ca	4	0.90	1.5	3.0	4.5
Fe	4	0.90	1.5	3.0	4.5
Mg	4	0.90	1.5	3.0	4.5
Mn	4	0.90	1.5	3.0	4.5
Zn	4	0.90	1.5	3.0	4.5
Bi	Not Applicable	0.90	1.5	3.0	4.5
Sr	Not Applicable	0.90	1.5	3.0	4.5

5. MATERIALS AND EQUIPMENT:

TABLE 2: EQUIPMENT				
Type	**Supplier**	**Model**	**Serial Number**	**Cal. Due**
Analytical Balance	Sartorius	MSE224S	36707108	04/30/25
Automatic Pipette	Rainin	E4-XLS (20-200 µL)	C249353515	06/30/25
Automatic Pipette	Rainin	E4-XLS (100-1000 µL)	C244197408	06/30/25
Automatic Pipette	Rainin	E4-XLS (0.5-5 mL)	C238841856	06/30/25
ICP-OES	Perkin Elmer	Avio 500	081S1905062	09/2025
Deionized water system	Millipore	IQ-7005/ Element POD	F9SA14284H	05/17/25

TABLE 3: REAGENTS					
Type	**Grade**	**Supplier**	**Catalog Number**	**Lot Number**	**Expiration**
70% Nitric Acid	PlasmaPure	SCP Science	250-038-175	24010022	02/28/26
Deionized water	Type 1 Ultrapure	In-House	Not Applicable	Not Applicable	Not Applicable
Manganese Stock Standard	PlasmaCal	SCP Science	140-051-251	S231010001	04/30/25

5.1. Consumable Supplies
- 5.1.1. SCP Digitubes® 15 mL, 50 mL, and 100 mL
- 5.1.2. Pipette Tips of various sizes

5.2. Reagent Lots for validation analysis
- 5.2.1. Sodium Hydroxide sample used for validation is NAHY-M03-1224-0084

TABLE 4: REFERENCE STANDARDS					
Identification	**Part Number**	**Manufacturer**	**Lot Number**	**Expiration**	**Concentrations / Elements**
Cadmium Stock Standard	140-051-481	SCP Science	S240812001	03/02/26	Cd (1,000 µg/mL)
Lead Stock Standard	140-051-821	SCP Science	S230515002	05/2025	Pb (1,000 µg/mL)
Cobalt Stock Standard	140-051-271	SCP Science	S240408028	09/25/25	Co (1,000 µg/mL)
Nickel Stock Standard	140-051-281	SCP Science	S231102001	10/08/25	Ni (1,000 µg/mL)
Barium Stock Standard	140-051-561	SCP Science	S230623019	07/2025	Ba (1,000 µg/mL)
Chromium Stock Standard	140-052-241	SCP Science	S240306018	11/02/25	Cr (1,000 µg/mL)
Copper Stock Standard	140-051-291	SCP Science	S230721030	03/04/25	Cu (1,000 µg/mL)
Molybdenum Stock Standard	140-050-421	SCP Science	S240408002	03/30/26	Mo (1,000 µg/mL)
Calcium Stock Standard	140-051-201	SCP Science	S230427001	02/03/25	Ca (1,000 µg/mL)
Iron Stock Standard	140-051-261	SCP Science	S230504001	01/31/25	Fe (1,000 µg/mL)
Magnesium Stock Standard	140-051-121	SCP Science	S230607025	03/04/25	Mg (1,000 µg/mL)
Manganese Stock Standard	140-051-251	SCP Science	S231010001	04/30/25	Mn (1,000 µg/mL)
Zinc Stock Standard	140-051-301	SCP Science	S230619001	06/05/25	Zn (1,000 µg/mL)
Bismuth Stock Standard	140-051-831	SCP Science	S230523001	02/21/25	Bi (1,000 µg/mL)
Strontium Stock Standard	140-051-381	SCP Science	S240531005	02/01/26	Sr (1,000 µg/mL)
Scandium Stock Standard	140-051-211	SCP Science	S230824024	07/30/25	Sc (1,000 µg/mL)
Yttrium Stock Standard	140-051-391	SCP Science	S240404001	09/03/25	Y (1,000 µg/mL)

6. PROCEDURE:

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

6.2. Internal Standard Solution

6.2.1. Added 0.500 mL of Sc (1,000 µg/mL) and 0.500 mL of Y (1,000 µg/mL) to a 50 mL Digitube®.

6.2.2. Diluted to 50 mL final volume with deionized water.

6.2.3. Scaled proportionally as needed for use.

6.3. Intermediate Standard Preparation

6.3.1. Prepared a standard solution containing the elements listed in Table 5, using the individual single source 1,000 µg/mL stock standards. Prepared by adding stock standards to a 15 mL Digitube®. Diluted to approximately 8 mL using deionized water and added 1.0 mL of concentrated nitric acid. Diluted to final volume using DI Water.

TABLE 5: INTERMEDIATE STANDARD

Identification	Element	Stock Identification	Amount Added (mL)	Nitric Acid (mL)	Final Volume (mL)	Final Conc. (µg/mL)
Intermediate Standard	Cd	1,000 µg/mL Cd Std	0.150	1.0	10	15
	Pb	1,000 µg/mL Pb Std	0.150			15
	Co	1,000 µg/mL Co Std	0.150			15
	Ni	1,000 µg/mL Ni Std	0.150			15
	Ba	1,000 µg/mL Ba Std	0.150			15
	Cr	1,000 µg/mL Cr Std	0.150			15
	Cu	1,000 µg/mL Cu Std	0.150			15
	Mo	1,000 µg/mL Mo Std	0.150			15
	Ca	1,000 µg/mL Ca Std	0.150			15
	Fe	1,000 µg/mL Fe Std	0.150			15
	Mg	1,000 µg/mL Mg Std	0.150			15
	Mn	1,000 µg/mL Mn Std	0.150			15
	Zn	1,000 µg/mL Zn Std	0.150			15
	Bi	1,000 µg/mL Bi Std	0.150			15
	Sr	1,000 µg/mL Sr Std	0.150			15

6.4. 15 ppb Calibration Standard Preparation

6.4.1. Prepared a solution containing the elements listed in Table 6 below in 5.0% HNO_3. Intermediate standard was not allowed to contact concentrated acid while preparing solutions. Added intermediate standard to separate 50 mL Digitube® followed by addition of approximately 35 mL of DI Water. Added nitric acid then diluted to 45 mL using DI Water. Added internal standard solution and diluted to final volume using DI Water.

TABLE 6: 15 ppb CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Nitric Acid (mL)	Internal Standard Solution (mL)	Final Volume (mL)	Final Conc. (µg/L)
15 ppb Calibration Standard	Cd	0.050	2.50	1.0	50	15
	Pb					15
	Co					15
	Ni					15
	Ba					15
	Cr					15
	Cu					15
	Mo					15
	Ca					15
	Fe					15
	Mg					15
	Mn					15
	Zn					15
	Bi					15
	Sr					15

6.5. 45 ppb Calibration Standard Preparation

6.5.1. Prepared a solution containing the elements listed in Table 7 below in 5.0% HNO_3. Intermediate standard was not allowed contact concentrated acid while preparing solutions. Added intermediate standard to separate 50 mL Digitube® followed by addition of approximately 35 mL of DI Water. Added nitric acid then diluted to 45 mL using DI Water. Added internal standard solution and diluted to final volume using DI Water.

TABLE 7: 45 ppb CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Nitric Acid (mL)	Internal Standard Solution (mL)	Final Volume (mL)	Final Conc. (µg/L)
45 ppb Calibration Standard	Cd	0.150	2.50	1.0	50	45
	Pb					45
	Co					45
	Ni					45
	Ba					45
	Cr					45
	Cu					45
	Mo					45
	Ca					45
	Fe					45
	Mg					45
	Mn					45
	Zn					45
	Bi					45
	Sr					45

6.6. 60 ppb Calibration Standard Preparation

6.6.1. Prepared a solution containing the elements listed in Table 8 below in 5.0% HNO_3. Intermediate standard was not allowed contact concentrated acid while preparing solutions. Added intermediate standard to separate 50 mL Digitube® followed by addition of approximately 35 mL of DI Water. Added nitric acid then diluted to 45 mL using DI Water. Added internal standard solution and diluted to final volume using DI Water.

TABLE 8: 60 ppb CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Nitric Acid (mL)	Internal Standard Solution (mL)	Final Volume (mL)	Final Conc. (µg/L)
60 ppb Calibration Standard	Cd	0.200	2.50	1.0	50	60
	Pb					60
	Co					60
	Ni					60
	Ba					60
	Cr					60
	Cu					60
	Mo					60
	Ca					60
	Fe					60
	Mg					60
	Mn					60
	Zn					60
	Bi					60
	Sr					60

6.7. Calibration Blank

6.7.1. Prepared a solution containing 5.0% HNO_3 acid matrix as per Table 9 below. To a separate 50 mL Digitube®, added approximately 35 mL of DI Water. Added nitric acid then diluted to 45 mL using DI Water. Added Internal Standard and diluted to volume using DI Water.

TABLE 9: CALIBRATION BLANK

Description	Nitric Acid (mL)	Internal Standard (mL)	Final Volume (mL)
Cal Blank	2.50	1.0	50

6.8. Method Blank Preparation

6.8.1. Added approximately 35 mL of deionized water to a 50 mL Digitube®.

6.8.2. Added 2.50 mL of nitric acid and swirled to mix.

6.8.3. Added deionized water to approximately 45 mL and then transferred 1.0 mL of Internal Standard.

6.8.4. Diluted to a final volume of 50 mL using deionized water and mixed well.

6.9. Sample Preparation

6.9.1. Weighed approximately 500 mg of sample into a 50 mL Digitube®.

6.9.2. Transferred approximately 20 mL of deionized water and swirled the solution to mix thoroughly.

6.9.3. Added 2.50 mL of nitric acid and swirled solution to mix sample thoroughly.

6.9.4. Added deionized water to approximately 45 mL and then transferred 1.0 mL of Internal Standard.

6.9.5. Diluted to a final volume of 50 mL with deionized water and mixed thoroughly.

7. INSTRUMENT PROCEDURE:

7.1. Performed the ICP-OES daily performance check prior to beginning the analytical sequence. Refer to Avio 500 ICP-OES SOP, DCN BSI-SOP-0362, for Daily Check procedures.

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

7.3. Set up the sequence as per the example sequence in Table 10.

7.4. Confirmed the calibration by analyzing the 45 ppb standard after the calibration. The calibration check must recover ±20% of the calculated theoretical concentration for 45 ppb standard following calibration.

7.5. A re-analysis of the 45 ppb check standard was performed at a minimum of once every 10 samples and at the end of the analytical run.

7.6. Bracketing standard checks must recover ±20% of the calculated theoretical concentration for each bracketing standard. Additionally, the drift (calculated as absolute difference) between the bracketing standard checks must be NMT 20% for each element.

7.7. 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 10: EXAMPLE SAMPLE ANALYSIS SEQUENCE

ID	Type	Level
Cal Blank	Cal Blank	Level 1
15 ppb Cal Std	Cal Std	Level 2
45 ppb Cal Std	Cal Std	Level 3
60 ppb Cal Std	Cal Std	Level 4
Cal Blank Check	QC Check	Not Applicable
45 ppb Check Std 1	QC Check	Not Applicable
Method Blank	Sample	Not Applicable
Sample(s) 10 or less	Sample	Not Applicable
45 ppb Check Std 2	QC Check	Not Applicable

7.8. Instrument Setup and Parameters

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

7.8.2. The gas glows for Plasma, Auxiliary, and Nebulizer can be set at 12 mL/min, 0.20 mL/min, and 0.70 mL/min, respectively.

TABLE 11: ICP-OES PARAMETERS

ICP-OES System	Perkin Elmer Avio 500 Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)
Points per Peak	4
Replicates	3
Viewing Distance	15.0
Nebulizer Gas	Argon
Shear Gas	Compressed Air
Sample Rinses	Rinse-1: 30 sec at 1.0 mL/min 5.0% HNO_3 (or as applicable to mitigate carry over)

TABLE 12: LINEAR RANGE			
Element	**Mode**	**Wavelength (nm)**	**Linear Range (µg/L)**
Cd	Axial	214.440	9-60
Pb	Axial	220.353	9-60
Co	Axial	230.786	9-60
Ni	Axial	227.022	9-60
Ba	Axial	233.527 455.403 493.408	9-60
Cr	Axial	267.716 205.560	9-60
Cu	Axial	324.752	9-60
Mo	Axial	202.031 203.845 204.597	9-60
Ca	Axial	396.847	9-60
Fe	Axial	259.939	9-60
Mg	Axial	279.553	9-60
Mn	Axial	257.610	9-60
Zn	Axial	202.548	9-60
Bi	Axial	206.170	9-60
Sr	Axial	407.771	9-60

7.9. Linearity and Range

7.9.1. The ICP-OES linearity study included standards equivalent to the concentrations shown in Table 14 and encompassed the following standards: 9 ppb, 15 ppb, 30 ppb, 45 ppb, and 60 ppb. Each standard was prepared in triplicate and analyzed against the calibration curve described in Section 6.4 to Section 6.7. The average standard recovery for each level of the three replicates was then determined.

7.9.2. For all replicates of the linearity standards, intensity was plotted against concentration and correlation coefficients were determined for each wavelength. The data is uploaded as supporting information with the report.

7.9.3. The preparation for linearity standards is described in Table 13 below. The concentrations of each element analyzed is listed in Table 14. Percent recovery values for each wavelength were tabulated in Table 15.

7.9.3.1. Acceptance Criteria:

7.9.3.1.1. The mean standard recovery for each element at each of the spike levels, as per USP <233> requirement, must be in the range of 70% - 150%.

TABLE 13: LINEARITY STANDARD PREPARATION

Description	Intermediate Standard (mL)	Nitric Acid (mL)	Internal Standard (mL)	Final Volume Deionized Water (mL)
Cal Blank Reference	Not Applicable	2.50	1.0	50
9 ppb Standard	0.030	2.50	1.0	50
15 ppb Standard	0.050	2.50	1.0	50
30 ppb Standard	0.100	2.50	1.0	50
45 ppb Standard	0.150	2.50	1.0	50
60 ppb Standard	0.200	2.50	1.0	50

TABLE 14: LINEARITY STANDARD CONCENTRATIONS					
Element	**Standard 1 (µg/L)**	**Standard 2 (µg/L)**	**Standard 3 (µg/L)**	**Standard 4 (µg/L)**	**Standard 5 (µg/L)**
Cd	9	15	30	45	60
Pb	9	15	30	45	60
Co	9	15	30	45	60
Ni	9	15	30	45	60
Ba	9	15	30	45	60
Cr	9	15	30	45	60
Cu	9	15	30	45	60
Mo	9	15	30	45	60
Ca	9	15	30	45	60
Fe	9	15	30	45	60
Mg	9	15	30	45	60
Mn	9	15	30	45	60
Zn	9	15	30	45	60
Bi	9	15	30	45	60
Sr	9	15	30	45	60

TABLE 15: LINEARITY PERCENT RECOVERY RESULTS											
Wavelength	**9 ppb Mean**	**15 ppb Mean**	**30 ppb Mean**	**45 ppb Mean**	**60 ppb Mean**	**Wavelength**	**9 ppb Mean**	**15 ppb Mean**	**30 ppb Mean**	**45 ppb Mean**	**60 ppb Mean**
Cd 214.440	103%	101%	103%	103%	102%	Ba 233.527	103%	103%	103%	103%	103%
Pb 220.353	117%	102%	106%	101%	102%	Ba 455.403	102%	102%	102%	102%	102%
Fe 259.939	110%	118%	100%	100%	101%	Ba 493.408	103%	103%	103%	103%	104%
Mn 257.610	102%	100%	101%	101%	100%	Mo 203.845	70%	82%	92%	99%	100%
Zn 202.548	102%	101%	101%	101%	101%	Mo 204.597	92%	91%	95%	100%	102%
Ca 396.847	104%	103%	103%	102%	103%	Cu 324.752	101%	106%	102%	102%	101%
Mg 279.353	103%	102%	102%	103%	103%	Ni 227.022	104%	105%	106%	106%	105%
Cr 267.716	105%	102%	104%	103%	104%	Bi 206.170	105%	102%	105%	105%	105%
Cr 205.560	104%	103%	103%	103%	103%	Sr 407.771	105%	104%	104%	105%	106%
Co 230.876	100%	99%	101%	103%	103%						

All analytes meet Linearity acceptance criteria of 70% - 150%.

7.10. Accuracy

7.10.1. Three (N=3) unspiked samples were prepared for analysis. The unspiked sample preparations were used for spike recovery calculations. Samples were prepared in triplicate at three spiking levels (50%, 100%, and 150% of the 100% Target Concentration) as shown in Table 1. The solutions were analyzed by ICP-OES, as per the method, by a single analyst. Results are shown in Table 21 for spike recovery.

$$\% \text{ Recovery} = \frac{(\text{Conc. of spiked replicate} - \text{Average Conc. of 3 unspiked samples}) \times 100}{\text{Expected spiked concentration}}$$

7.10.1.1. Acceptance Criteria

7.10.1.1.1. The mean spike recovery for each element at each of the three spike levels, as per USP <233> requirement, must be in the range of 70% - 150%.

7.10.2. Spiked Reference (Unspiked) Solution Preparation

7.10.2.1. Prepared as per Section 6.9.

7.10.3. Spike Recovery Sample Preparation

7.10.3.1. Weighed approximately 500 mg of sample as per Table 16 into a 50 mL Digitube®.

7.10.3.2. Pipetted appropriate intermediate standard spike amount as per Table 16.

7.10.3.3. Added approximately 20 mL of deionized water and swirled to mix sample thoroughly.

7.10.3.4. Pipetted 2.50 mL of nitric acid then allowed to react. Swirled solution to mix.

7.10.3.5. Added deionized water to 45 mL and transferred 1.0 mL Internal Standard Solution.

7.10.3.6. Diluted to final volume of 50 mL using deionized water and mixed well.

7.10.3.7. Prepared spiked sample solutions in triplicate and three preparations of unspiked sample solutions.

TABLE 16: ACCURACY/LOQ SAMPLE SPIKES

Description	Sample Amount (mg)	Intermediate Standard Spike (mL)	Nitric Acid (mL)	Internal Standard (mL)	Final Volume (mL)
Method Blank	None	None	2.50	1.0	50
Unspiked	500	None	2.50	1.0	50
30% Spiked Sample	500	0.030	2.50	1.0	50
50% Spiked Sample	500	0.050	2.50	1.0	50
100% Spiked Sample	500	0.100	2.50	1.0	50
150% Spiked Sample	500	0.150	2.50	1.0	50

TABLE 17: ACCURACY RESULTS FOR SODIUM HYDROXIDE
(Mean percent recovery of triplicate preparations)

Wavelength	50% Mean	100% Mean	150% Mean
Cd 214.440	100%	101%	100%
Pb 220.353	99%	95%	93%
Fe 259.939	87%	88%	87%
Mn 257.610	91%	89%	90%
Zn 202.548	89%	94%	94%
Ca 396.847	93%	94%	94%
Mg 279.353	96%	97%	97%
Cr 267.716	102%	102%	102%
Cr 205.560	99%	99%	99%
Co 230.876	93%	93%	93%
Ba 233.527	98%	98%	98%
Ba 455.403	89%	89%	88%
Ba 493.408	109%	110%	109%
Mo 203.845	88%	96%	94%
Mo 204.597	93%	99%	99%
Cu 324.752	111%	109%	108%
Ni 227.022	104%	97%	96%
Bi 206.170	103%	102%	102%
Sr 407.771	98%	99%	99%

All elements meet Accuracy acceptance criteria of 70% - 150%.

7.11. Specificity

7.11.1. Specificity was demonstrated by using a calibration blank and spiked calibration blank for ICP-OES analysis. The calibration blank was prepared as per the analytical method protocol. A separate blank was spiked with a mixed standard solution which produced a spiked solution at a concentration equivalent to the 60 ppb calibration standard.

7.11.2. The solutions were analyzed as per the analytical method and the intensities for the calibration blank, 60 ppb calibration standard, and method blank are reported in Table 18 below.

7.11.2.1. Acceptance Criteria:

7.11.2.1.1. The lack of a significant interference (as demonstrated by the spike recovery of 70% to 150%, as per the Accuracy requirement from USP <233>) or by any other element in the spiked blank solution or the solution matrix itself will indicate the specificity of the method.

TABLE 18: SPECIFICITY RESULTS

Wavelength	Blank (CPS)	60 ppb STD (CPS)	Method Blank (CPS)
Cd 214.440	-56	4700	28
Pb 220.353	4	330	2
Fe 259.939	48	8218	-16
Mn 257.610	567	38809	-40
Zn 202.548	37	2636	-5
Ca 396.847	3045	333646	21
Mg 279.353	162	166737	-33
Cr 267.716	-28	3718	-23
Cr 205.560	-35	1646	-1
Co 230.876	-122	2122	-6
Ba 233.527	-66	6087	-16
Ba 455.403	82	330613	-33
Ba 493.408	-718	386724	61
Mo 203.845	266	413	16
Mo 204.597	242	569	34
Cu 324.752	1099	9358	-26
Ni 227.022	185	881	-7
Bi 206.170	49	1411	-18
Sr 407.771	-427	917504	-84

7.12. Precision

7.12.1. All solutions for the Precision (Repeatability) experiments were prepared by a single analyst for Sodium Hydroxide samples and reported in Table 19.

7.12.2. The value of the unspiked sample preparations from Section 7.10, "Accuracy," was used for spike recovery calculations. Six sample solutions were prepared at the 100% Target Concentration as shown in Table 1. For ICP-OES analysis, the Target Concentration spiked samples and the unspiked samples were used for the accuracy experiment.

7.12.3. Precision results are reported to three decimal places and %RSD results are reported to the nearest whole number, but values are calculated from data that contains nine decimal places.

7.12.3.1. Acceptance Criteria:

7.12.3.1.1. The %RSD for the spike recovery concentration must be NMT 20% for each element in each sample.

TABLE 19: PRECISION RESULTS FOR SODIUM HYDROXIDE
(Mean recovery concentration of 6 preparations)

Wavelength	100% Mean Recovery Conc. N=6 (ppm)	%RSD N=6
Cd 214.440	3.011	0%
Pb 220.353	2.985	3%
Fe 259.939	2.883	3%
Mn 257.610	2.680	0%
Zn 202.548	3.074	1%
Ca 396.847	2.827	1%
Mg 279.353	2.898	0%
Cr 267.716	3.098	1%
Cr 205.560	3.001	1%
Co 230.876	2.699	1%
Ba 233.527	3.266	0%
Ba 455.403	2.961	0%
Ba 493.408	3.655	0%
Mo 203.845	3.486	4%
Mo 204.597	3.434	2%
Cu 324.752	3.318	1%
Ni 227.022	2.863	4%
Bi 206.170	3.046	2%
Sr 407.771	2.989	0%

All analytes meet Precision RSD% acceptance criteria of NMT 20%.

7.13. Intermediate Precision (Ruggedness)

7.13.1. A second analyst, on a different day from the performance of the Repeatability experiments, prepared and analyzed the Intermediate Precision solutions. Six sample solutions were prepared at the 100% Target Concentration level found in Table 1 for ICP-OES analysis (this fulfilled two events as "different day" and "different analyst").

7.13.2. Ruggedness results are reported to three decimal places and %RSD results are reported to the nearest whole number, but values are calculated from data that contains nine decimal places. Results are reported in Table 20.

7.13.2.1. Acceptance Criteria:

7.13.2.1.1. The %RSD for the spike recovery concentration from both analysts (N=12) must be NMT 25% for each element.

TABLE 20: RUGGEDNESS RESULTS FOR SODIUM HYDROXIDE
(Mean recovery concentration of 12 preparations)

Wavelength	100% Mean Recovery Conc. N=12 (ppm)	%RSD N=12
Cd 214.440	3.010	0%
Pb 220.353	3.022	5%
Fe 259.939	2.918	3%
Mn 257.610	2.718	2%
Zn 202.548	3.106	5%
Ca 396.847	2.825	1%
Mg 279.353	2.907	0%
Cr 267.716	3.092	1%
Cr 205.560	3.040	2%
Co 230.876	2.748	2%
Ba 233.527	3.278	0%
Ba 455.403	2.982	1%
Ba 493.408	3.658	0%
Mo 203.845	3.518	3%
Mo 204.597	3.446	2%
Cu 324.752	3.387	2%
Ni 227.022	2.956	5%
Bi 206.170	3.059	1%
Sr 407.771	2.977	1%

All analytes meet the Ruggedness %RSD acceptance criteria of NMT 25%.

7.14. Limit of Quantitation (LOQ)

7.14.1. The limit of quantitation (LOQ) is demonstrated from spike recovery performed at the 30% Target Concentration spiking levels as shown in Table 1.

7.14.2. Samples were prepared in triplicate following Section 7.10.3 and 7.10.4. Results for Sodium Hydroxide are reported in Table 21 below.

7.14.2.1. Acceptance Criteria:

7.14.2.1.1. The mean percent spike recovery for each wavelength at the 30% Target Concentration spiking levels, as per the USP <233> accuracy guideline, must be in the range of 70% - 150%.

TABLE 21: LIMIT OF QUANTITATION RESULTS
(Mean percent recovery of 3 preparations)

Wavelength	30% Mean %Recovery
Cd 214.440	104%
Pb 220.353	85%
Fe 259.939	90%
Mn 257.610	92%
Zn 202.548	84%
Ca 396.847	93%
Mg 279.353	97%
Cr 267.716	103%
Cr 205.560	97%
Co 230.876	93%
Ba 233.527	98%
Ba 455.403	89%
Ba 493.408	110%
Mo 203.845	82%
Mo 204.597	87%
Cu 324.752	106%
Ni 227.022	90%
Bi 206.170	107%
Sr 407.771	98%

All analytes meet LOQ acceptance criteria of 70% - 150%.

7.15. Sample and Standard Stability

7.15.1. The 15 ppb and 60 ppb calibration standards were analyzed as samples against calibration curves constructed from freshly prepared calibration standards at T=1 day and T=7 days from the date of preparation.

7.15.2. A spiked sample solution prepared at the 100% Target Concentration level in Table 1 from the Precision experiment was used for sample stability. The spiked sample solution was analyzed against calibration curves constructed from freshly prepared calibration standards at time points T=0 (day of preparation) and T=1 (1 day from the date of preparation).

7.15.3. Both sample and standard solution stability results are reported in Table 22.

7.15.3.1. Acceptance Criteria:

7.15.3.1.1. The recovery of each element must be within the range of 80% to 120% recovery of the T = 0 results for the calibration standard.

7.15.3.1.2. The recovery of each element must be within the range of 80% to 120% recovery of the T = 0 results for the spiked sample solution.

TABLE 22: SAMPLE AND STANDARD STABILITY (% RECOVERY)

Wavelength	15 ppb Std T=1 (%)	60 ppb Std T=1 (%)	15 ppb Std T=7 (%)	60 ppb Std T=7 (%)	100% Spike T=1 (%)
Cd 214.440	102%	103%	102%	104%	101%
Pb 220.353	101%	102%	123%	108%	93%
Fe 259.939	114%	112%	115%	122%	106%
Mn 257.610	102%	102%	103%	103%	105%
Zn 202.548	104%	102%	105%	104%	102%
Ca 396.847	104%	104%	106%	106%	103%
Mg 279.353	102%	103%	105%	105%	103%
Cr 267.716	101%	103%	105%	104%	103%
Cr 205.560	102%	103%	104%	103%	104%
Co 230.876	101%	104%	100%	105%	101%
Ba 233.527	101%	103%	102%	104%	102%
Ba 455.403	102%	102%	103%	105%	103%
Ba 493.408	102%	103%	102%	104%	102%
Mo 203.845	125%	98%	124%	106%	98%
Mo 204.597	118%	104%	119%	105%	94%
Cu 324.752	101%	103%	102%	105%	107%
Ni 227.022	105%	105%	112%	105%	103%
Bi 206.170	102%	104%	116%	113%	103%
Sr 407.771	102%	103%	108%	112%	100%

All analytes, except Mo 203.845 nm, meet stability acceptance criteria of 80% - 120% for sample and standard of 1 day. There were multiple failures for stability of T=7 days.

8. DEVIATIONS:

8.1. Due to contamination, one additional sample at the 30% spike level was re-prepared. This is justified as the percent recoveries for iron were inflated and due to salted roads during winter months.

8.2. During the validation, Mo 202.031 nm was listed as a wavelength to be analyzed. While this wavelength was analyzed during each analysis, the calibration standard correlation coefficient was never above 0.99 and thus results were not included for this wavelength. This is deemed acceptable as two other wavelengths were included during execution of the protocol.

8.3. Mo 203.845 nm and Cr 205.560 nm were not apart of the initial protocol and were included in the instrument method. This is deemed acceptable as these wavelengths were held to the same validation parameters set forth in the protocol and were included as additional wavelengths in the event that the listed wavelengths did not met all parameters.

9. CONCLUSION:

9.1. The test method for Trace Metals in Sodium Hydroxide products has been validated on the ICP-OES. The Method was found to be:

9.1.1. Specific: The method blank did not show any significant interference for all analyzed masses.

9.1.2. Linear: 9 ppb to 60 ppb of working standard solution. Mean percent recovery ranged from 70% to 118% and met acceptance criteria for all wavelengths.

9.1.3. Sensitive: LOQ recoveries were within 82% to 110% for Sodium Hydroxide. All analytes met acceptance criteria established at the 30% level, thus the LOQ will be 30% in the final method.

9.1.4. Accurate: From 50% to 150% of working standard concentration level with mean percent recoveries ranging from 87% to 111% for Sodium Hydroxide. All wavelengths analyzed met acceptance criteria within the specified range.

9.1.5. Precise: Closeness of agreement demonstrated between six sample preparations by percent RSD's ranging from 0% to 4% for Sodium Hydroxide.

9.1.6. Rugged: Satisfactory precision was demonstrated between two sets of six sample preparations performed on different days and by different analysts. The percent RSDs ranged from 0% to 5% for Sodium Hydroxide.

9.1.7. Stable: With respect to stability of solutions, the sample solutions are shown to be stable for 1 day for all elements analyzed using this protocol. The working standard preparations were shown to be stable for all analytes, with exception of Mo 203.845. Multiple wavelengths were above the 80-120% acceptance criteria for T=7 days. The samples are to be noted as stable for 1 day and the standards will be noted as stable for 1 day in the final analytical testing method since Mo 204.597 will be the reported wavelength.

9.1.8. All wavelengths met all validation parameters set forth in the validation protocol. In order to simplify the method and reduce the number of total wavelengths, the final method will incorporate one wavelength per element as shown below in Table 23. For elements having multiple wavelengths, the wavelength chosen was selected based on factors including intensity, baseline resolution, and possible sample interference. Wavelengths could be reintroduced at later date if needed.

TABLE 23: FINAL METHOD WAVELENGTHS			
Element	**Wavelength (nm)**	**Element**	**Wavelength (nm)**
Cd	214.440	Ca	396.847
Pb	220.353	Fe	259.939
Co	230.786	Mg	279.553
Ni	227.022	Mn	257.610
Ba	493.408	Zn	202.548
Cr	267.716	Bi	206.170
Cu	324.752	Sr	407.771
Mo	204.597		

10. NOTEBOOK REFERENCE:

TABLE 24: NOTEBOOK REFERENCE		
STUDY		**NOTEBOOK REFERENCE**
Specificity		BEIV01/ pages 65-68
Linearity and Range		BEIV01/ pages 69-72
LOQ by "Spiked" recovery		BEIV01/ pages 65-68
Accuracy/ Precision by "Spiked" recovery		BEIV01/ pages 65-68
Intermediate Precision (Ruggedness)		BEIV01/ pages 69-72
Standard Solution Stability	Day-0	BEIV01/ pages 65-68
	Day-1	BEIV01/ pages 69-72
	Day-7	BEIV01/ pages 73-75
Sample Solution Stability	Day-0	BEIV01/ pages 65-68
	Day-1	BEIV01/ pages 69-72