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ANALYTICAL METHOD VALIDATION REPORT: L-HISTIDINE MONOCHLORIDE MONOHYDRATE ASSAY BY POTENTIOMETRIC TITRATION WITH 0.1N SODIUM HYDROXIDE

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1. PURPOSE:

1.1. The purpose of this Report is to:
- 1.1.1. Provide performance data demonstrating that the L-Histidine Monochloride Monohydrate assay procedure via Potentiometric Titration with 0.1N Sodium Hydroxide is adequately evaluated and validated.
- 1.1.2. Provide proof that the procedure for determining the Assay value of L-Histidine Monochloride Monohydrate at the set specification meets all requirements for System Suitability, Accuracy, Precision, Linearity, Range, Specificity, and Intermediate Precision.
- 1.1.3. To ensure that the proper reagents and testing materials were used and the correct documentation was provided for evaluation.

2. SCOPE:

2.1. This Analytical Method Validation Report applies to the L-Histidine Monochloride Monohydrate Assay method by Potentiometric Titration with standardized 0.1N Sodium Hydroxide.
2.2. This method validation followed Category I (Quantitative) guidelines.
2.3. The scope of this method validation protocol covered L-Histidine Monochloride Monohydrate content for a 500 mg sample size from a range of 25.0%-120.0%.
2.4. The approximate standardization volume of the titrant determined the 100% target sample size.
- 2.4.1. Standardization of the 0.1N Sodium Hydroxide was performed with 0.6 g of traceable Potassium Hydrogen Phthalate (204.22 g/mol) material; equivalent to 3 mmol.
- 2.4.2. 100% Assay level is 0.500 g of L-Histidine Monochloride Monohydrate (209.64 g/mol) Sample; equivalent to 2.5 mmol.

3. RESPONSIBILITIES:

3.1. The Laboratory Manager was responsible for the control, implementation, and maintenance of this procedure.
3.2. The Laboratory Analysts or qualified designees were responsible for performing the testing stated in this Protocol and for performing the Validation.
3.3. The Analysts performing the test, with help from the Laboratory Manager if necessary, were responsible for completing the Method Validation Report using conclusions made from the results obtained from testing.

4. REFERENCE:

4.1. BSI-PRL-0769, Analytical Method Validation Protocol: L-Histidine Monochloride Monohydrate Assay by Potentiometric Titration with 0.1N Sodium Hydroxide
4.2. BSI-SOP-0098, Balance SOP
4.3. BSI-SOP-0126, Laboratory Notebooks
4.4. BSI-SOP-0134, Pipette SOP
4.5. BSI-SOP-0140, Standardization of Titrants
4.6. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
4.7. BSI-SOP-0436, Analytical Methods Validation Master Plan

5. PRE-VALIDATION REQUIREMENTS:

5.1. Equipment
- 5.1.1. All equipment used in this Validation was in proper working order and with current calibrations. This is included in the Materials and Equipment portion of the Analytical Method Validation Report.

5.2. Personnel
- 5.2.1. All personnel performing this Validation were properly trained on the analysis technique.

5.3. Supplies
5.3.1. Any supplies used in the Validation were clean and appropriate for the intended use. A list of supplies used is included in the Materials and Equipment section of the Analytical Method Validation Report and is identified with the supplier and description.
5.4. Reagents
5.4.1. All reagents were current, met required specifications, and were suitable for the intended use. A list of reagents used is included in the Analytical Method Validation Report and laboratory documentation. This includes: Reagent name, lot number, manufacturer, date of opening (if applicable), part number, and expiration date (if applicable).
5.5. Reference Standards
5.5.1. Any standards used in this Validation are listed in the Materials and Equipment section of the Analytical Method Validation Report. Details regarding the storage conditions and usage of the reference standard (expiration date) are detailed in this list. The name of the reference standard, lot number, manufacture, date of opening, date of expiration, and part number are provided in the Validation Report and were recorded during validation testing.

6. MATERIALS AND EQUIPMENT:

6.1. All materials and equipment utilized in this Validation are outlined in this section. This is a list of the anticipated materials and equipment required. As part of the Analytical Method Validation Report, all materials and equipment used is documented in this section including the Instrument Name, Model Number, Manufacturer, Serial Number, and Calibration Information. Any specifications on materials or equipment is listed in the method validation report.
6.2. Equipment
6.2.1. Analytical Balance
6.2.2. Calibrated Pipettes
6.2.3. Titrando 907 Auto-titrator equipped with pH electrode and 50 mL burette
6.3. Reagents
6.3.1. **0.1N Sodium Hydroxide –** Purchased Commercially.
6.3.2. **L-Histidine Monochloride Monohydrate -** Purchased Commercially.
6.3.3. **Potassium Hydrogen Phthalate–** Purchased Commercially.
6.3.4. **Purified Water –** In-House or Purchased Commercially.
6.4. Supplies
6.4.1. Graduated Cylinders
6.4.2. Assorted Beakers
6.4.3. Weight paper/funnel
6.4.4. Stir bar
6.5. Reference Standards
6.5.1. Traceable Potassium Hydrogen Phthalate (KHP) Standard (dried; stored in desiccator)

7. PROCEDURE:

7.1. Note: Loss on Drying result is required prior to performing analysis.
7.2. Standardize 0.1N Sodium Hydroxide as per the Standardization of Titrants SOP.
7.3. Accurately weigh sample into a suitable beaker (See Table Below).
7.4. Dissolve in 50 mL of Purified Water.
7.5. Titrate to a potentiometric endpoint with 0.1N Sodium Hydroxide.
7.6. Calculate % L-Histidine Monochloride Monohydrate (Dried Substance) using the following equation in the Metrohm® Tiamo™ software:

$$\%\ \text{L-Histidine Monochloride Monohydrate} = \frac{(\text{Sample Titrant Volume (mL)})(Titrant\ Normality)(19.16)}{Sample\ Weight\ (g)} \times \left(\frac{100}{(100 - LOD\ (\%))}\right)$$

7.7. Record Result.
7.8. Sample Preparations

ANALYST I SAMPLE PREPARATION TABLE					
Sample ID/Purpose		L-Histidine Monochloride Monohydrate Weight (mg)	Approximate 0.1N Sodium Hydroxide Volume Consumed	% Burette Volume Used	Analysis Level (%)
Blank / Specificity	1	0			0%
	2	0			
	3	0			
Linearity 1	1	125	7 mL	14%	25%
Accuracy Precision Linearity 2	1	250	13 mL	26%	50%
	2	250			
	3	250			
Accuracy Precision Linearity 3	1	400	21 mL	42%	80%
	2	400			
	3	400			
Accuracy Precision Linearity 4	1	500	26 mL	52%	100%
	2	500			
	3	500			
	4	500			
	5	500			
	6	500			
Accuracy Precision Linearity 5	1	600	31 mL	62%	120%
	2	600			
	3	600			

ANALYST II SAMPLE PREPARATION TABLE					
Sample ID/Purpose		L-Histidine Monochloride Monohydrate Weight (g)	Approximate 0.1N Sodium Hydroxide Volume Consumed	% Burette Volume Used	Analysis Level (%)
Int. Precision	1	500	26 mL	52%	100%
	2	500			
	3	500			
	4	500			
	5	500			
	6	500			

8. PERFORMANCE PARAMETERS:

8.1. System Suitability / Standardization:

8.1.1. System Suitability was assessed by standardizing 0.1N Sodium Hydroxide in triplicate and reporting the average as the Titer Value.

8.1.2. Acceptance Criteria:

8.1.2.1. Ensure each replicate of the triplicate standardization is within ± 0.0005 units of the others.

8.2. Accuracy and Precision:

8.2.1. Accuracy and Precision was assessed across four (4) concentration levels.

8.2.2. Accuracy was assessed by comparing the measured L-Histidine Monochloride Monohydrate Assay value (%) to the L-Histidine Monochloride Monohydrate Assay data on the Certificate of Analysis (CoA) and calculating the Percent Recovery (%).

$$Percent\ Recovery\ (\%) = \frac{Measured\ Assay\ Value\ (\%)}{\text{L-Histidine Monochloride Monohydrate Assay Value (\%)}} \times 100$$

8.2.3. Precision was assessed by determining the Standard Deviation and Relative Standard Deviation (%RSD) at each concentration level.

8.2.4. Acceptance Criteria:

8.2.4.1. Percent Recovery (%): All replicates are between 99.0% and 101.0%.

8.2.4.2. Standard Deviation: Report.

8.2.4.3. Relative Standard Deviation (%RSD): NMT 1.0%.

8.3. Linearity:

8.3.1. Linearity was assessed across five (5) concentration levels.

8.3.2. Plot and report the Calibration Coefficient (r^2), Slope, and Y-Intercept of the endpoint (mL) to weight of L-Histidine Monochloride Monohydrate (g) analyzed.

8.3.3. Acceptance Criteria:

8.3.3.1. Calibration Coefficient (r^2): NLT 0.99.

8.3.3.2. Slope: Report

8.3.3.3. Y-Intercept: Report

8.4. Specificity:

8.4.1. Specificity was assessed by demonstrating acceptable Accuracy and Precision data and a lack of meaningful response to triplicate analysis of the blank.

8.5. Range:

8.5.1. Range was assessed by showing an acceptable degree of Accuracy, Precision, and Linearity.

8.5.2. Acceptance Criteria:

8.5.2.1. A range of 80% to 120% of the 100% test weight minimum.

8.6. Intermediate Precision:

8.6.1. Intermediate precision was assessed by having a second analyst on a separate day perform a standardization and an additional six (6) determinations at 100% of the test concentration. The Standard Deviation and Relative Standard Deviation (%RSD) was calculated for individual analyst results and combined (Analyst I and II) results.

8.6.2. Acceptance Criteria:

8.6.2.1. Standard Deviation of Individual and Combined Results: Report

8.6.2.2. Relative Standard Deviation (%RSD) of Individual and Combined Results: NMT 1%.

9. DOCUMENTATION PROCEDURES:

9.1. All data sheets, including notebooks, were signed and dated by the employee who executed the Protocol. Pages were copied and uploaded as supporting material into Master Control.
9.2. All testing equipment was calibrated and ensured that there is a certificate on file or appropriate standards are used if calibration is required.
9.3. Any critical changes that were made to the analytical procedure are noted in the Validation Report with supporting evidence for the change.

10. VALIDATION SUMMARY:

Validation Summary		
Performance Parameters	**Acceptance Criteria**	**Results**
System Suitability	• Report the average of the triplicate standardizations as the titer value • Each replicate of the triplicate standardization is within ±0.0005 units of the others	Analyst I • Titer Value: 0.1005N • Deviation: 0.0001N Analyst II • Titer Value: 0.1003N • Deviation: 0.0002N
Accuracy	• All samples must have a Percent Recovery of 99.0% to 101.0%.	50% Level • Replicate 1: 100.1% • Replicate 2: 100.0% • Replicate 3: 100.0% 80% Level • Replicate 1: 100.2% • Replicate 2: 100.4% • Replicate 3: 100.4% 100% Level • Replicate 1: 100.4% • Replicate 2: 100.4% • Replicate 3: 100.5% • Replicate 4: 100.5% • Replicate 5: 100.4% • Replicate 6: 100.5% 120% Level • Replicate 1: 100.6% • Replicate 2: 100.6% • Replicate 3: 100.5%

Validation Summary		
Performance Parameters	**Acceptance Criteria**	**Results**
Precision	• Standard Deviation: Report • Each level must have a %RSD of NMT 1.0%	50% Level • Standard Deviation: 0.0450% • %RSD: 0.05 80% Level • Standard Deviation: 0.0785% • %RSD: 0.08 100% Level • Standard Deviation: 0.0616% • %RSD: 0.06 120% • Standard Deviation: 0.0125% • % RSD: 0.01
Linearity	• Report the slope and Y-Intercept • The Calibration coefficient (r^2) must be NLT 0.99	• Calibration Coefficient (r^2): 1 • Slope: 47.745 • Y-Intercept: -0.0575
Specificity	• Requirements for Accuracy and Precision are met. • Triplicate analysis of the blank shows no meaningful response.	• Requirements for accuracy and precision were met. • No meaningful response was shown during triplicate analysis of the blank.
Range	• Range was established by showing an acceptable degree of Accuracy, Precision, and Linearity. • A minimum range of 80% to 120% of the 100% test weight is required.	• Range was established from 50% to 120% of the 100% test weight.
Intermediate Precision	• Report the individual and combined (Analyst I and II) Standard Deviation and %RSD • The individual and combined (Analyst I and II) %RSD is NMT 1%	Individual (Analyst II) • Standard Deviation: 0.1783% • %RSD: 0.18 Combined (Analyst I and II): • Standard Deviation: 0.1510% • %RSD: 0.15

11. VALIDATION RESULTS:

11.1. Materials and Equipment:

Equipment and Instrumentation				
Equipment	**Model/Part Number**	**Manufacturer**	**Serial Number**	**Calibration Due**
Metrohm 907 Auto titrator	907 Titrando	Metrohm	12155	7/24
Analytical Balance	Secura 124-1S	Sartorius	29212172	4/30/24
pH Probe	6.0278.300	Metrohm	20672630	Not Applicable

Reagents and Standards						
Reagent/Standard	**Lot ID**	**Manufacturer**	**Part Number**	**CAS**	**Expiry Date**	**Date of Opening**
L-Histidine Monochloride Monohydrate	BCCH6305	Sigma Aldrich	102494534	5934-29-2	9/30/28	9/16/23
Traceable Potassium Hydrogen Phthalate (KHP) Reference Standard	BSP41P63	In-House	Not Applicable	877-24-7	6/10/24	3/10/24
Purified Water	F9SA1428 4H	Millipore Sigma	Not Applicable	7732-18-5	6/30/24	Not Applicable
0.1N Sodium Hydroxide	43090398	LabChem	LC242704	1310-73-2 7732-18-5	9/27/25	1/2/24
Traceable Potassium Hydrogen Phthalate (KHP) Reference Standard	BSP41P09	In-House	Not Applicable	877-24-7	5/1/24	2/1/24

Supplies		
Supply	**Manufacturer**	**Part Number**
Weigh Boat / Weigh Paper	Not Applicable	Not Applicable
Beakers	Pyrex	Not Applicable
Stir Bars	Metrohm	Not Applicable

11.2. System Suitability / Standardization:

11.2.1. System Suitability was assessed by standardizing 0.1N Sodium Hydroxide in triplicate and reporting the average as the Titer Value.

11.2.2. Acceptance Criteria:

11.2.2.1. Ensure each replicate of the triplicate standardization is within ±0.0005 units of the others.

0.1N Sodium Hydroxide Standardization (Analyst I)			
Replicate	**KHP Standard Weight (g)**	**Titrant Volume (mL)**	**Result (N)**
1	0.6023	29.3406	0.1005
2	0.6037	29.4088	0.1005
3	0.6020	29.3481	0.1004
		Average:	0.1005
		Deviation:	0.0001

11.3. **Accuracy and Precision:**

11.3.1. Accuracy and Precision was assessed across four (4) concentration levels.

11.3.2. Accuracy was assessed by comparing the measured L-Histidine Monochloride Monohydrate Assay value (%) to the L-Histidine Monochloride Monohydrate Assay data on the Certificate of Analysis (CoA) and calculating the Percent Recovery (%).

$$Percent\ Recovery\ (\%) = \frac{Measured\ Assay\ Value\ (\%)}{\text{L-Histidine Monochloride Monohydrate CoA Assay Value (\%)}} \times 100$$

11.3.3. Precision was assessed by determining the Standard Deviation and Relative Standard Deviation (%RSD) at each concentration level.

11.3.4. Acceptance Criteria:

11.3.4.1. Percent Recovery (%): All replicates are between 99.0% and 101.0%.

11.3.4.2. Standard Deviation: Report.

11.3.4.3. Relative Standard Deviation (%RSD): NMT 1.0%.

Accuracy and Precision							
L-Histidine Monochloride Monohydrate CoA Assay Value (%): 99.9%							
Loss on Drying Value (%): 8.6159%							
Concentration Level (%)	Replicate	L-Histidine Monochloride Monohydrate (g)	Endpoint (mL)	Assay Result (%)	Percent Recovery (%)	Standard Deviation (%)	%RSD
0	1	0.0000	Not Applicable, No Response				
	2	0.0000					
	3	0.0000					
25	1	0.1254	5.9524	100.02	100.1	Not Applicable	
50	1	0.2503	11.8769	99.98	100.1	0.0450	0.05
	2	0.2500	11.8566	99.93	100.0		
	3	0.2503	11.8630	99.87	100.0		
80	1	0.4005	19.0324	100.13	100.2	0.0785	0.08
	2	0.4008	19.0818	100.32	100.4		
	3	0.4002	19.0406	100.25	100.4		
100	1	0.5003	23.8040	100.26	100.4	0.0616	0.06
	2	0.5003	23.8153	100.30	100.4		
	3	0.5004	23.8367	100.37	100.5		
	4	0.5004	23.8514	100.44	100.5		
	5	0.5002	23.8202	100.34	100.4		
	6	0.5007	23.8585	100.41	100.5		
120	1	0.6006	28.6381	100.47	100.6	0.0125	0.01
	2	0.6000	28.6048	100.46	100.6		
	3	0.6000	28.6002	100.44	100.5		

11.4. **Linearity:**

11.4.1. Linearity was assessed across five (5) concentration levels.

11.4.2. Plot and report the Calibration Coefficient (r^2), Slope, and Y-Intercept of the endpoint (mL) to weight of L-Histidine Monochloride Monohydrate (g) analyzed.

11.4.3. Acceptance Criteria:

11.4.3.1. Calibration Coefficient (r^2): NLT 0.99.

11.4.3.2. Slope: Report

11.4.3.3. Y-Intercept: Report

Linearity					
Concentration Level (%)	**Average L-Histidine Monochloride Monohydrate Weight (g)**	**Average Endpoint Volume (mL)**	**Calibration Coefficient (r^2)**	**Slope**	**Y-Intercept**
25	0.1254	5.9524	1	47.745	-0.0575
50	0.2502	11.8655			
80	0.4005	19.0516			
100	0.5004	23.8310			
120	0.6002	28.6144			

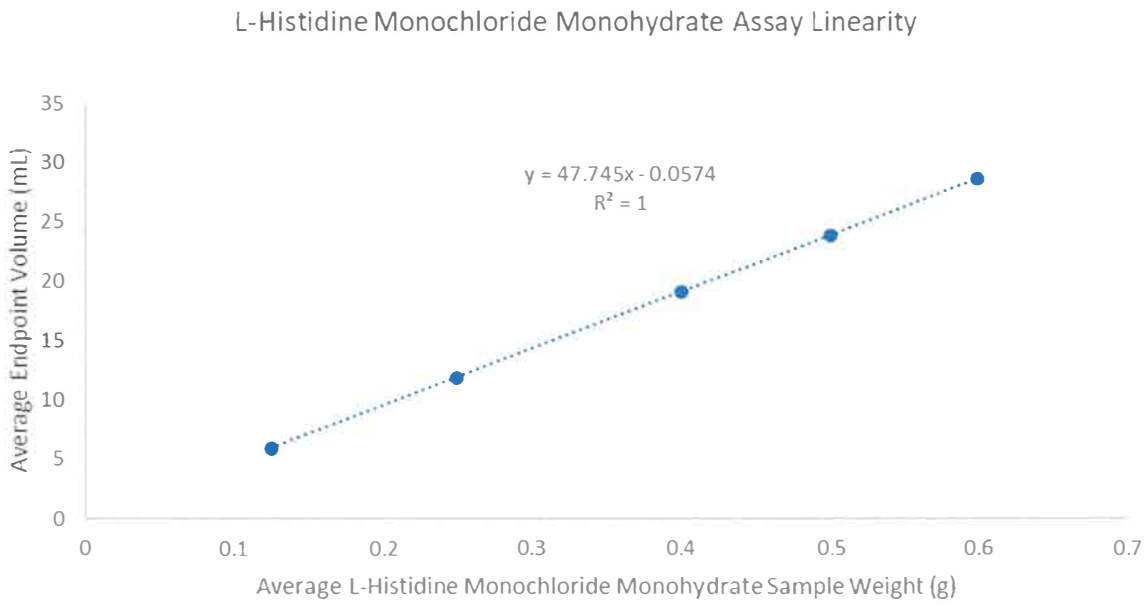

Figure 1: L-Histidine Monochloride Monohydrate Assay Linearity

11.5. **Specificity:**

11.5.1. Specificity was demonstrated by meeting requirements for accuracy and precision. If both accuracy and precision met requirements and there was lack of meaningful response to triplicate analysis of the blank then specificity was demonstrated.

Specificity	
Acceptance Criteria	**Result**
Meets requirements for Accuracy.	Pass
Meets requirements for Precision.	Pass
Triplicate analysis of the blank shows no meaningful response.	Pass

11.6. **Range:**

11.6.1. Range was assessed by showing an acceptable degree of Accuracy, Precision, and Linearity.

11.6.2. Acceptance Criteria:

11.6.2.1. A range of 80% to 120% of the 100% test weight minimum.

Range of Analysis: 50% - 120%

11.7. **Intermediate Precision:**

11.7.1. Intermediate precision was assessed by having a second analyst on a separate day perform a standardization and an additional six (6) determinations at 100% of the test concentration. The Standard Deviation and Relative Standard Deviation (%RSD) were calculated for individual analyst results and combined (Analyst I and II) results.

11.7.2. Acceptance Criteria:

11.7.2.1. Standard Deviation of Individual and Combined Results: Report

11.7.2.2. Relative Standard Deviation (%RSD) of Individual and Combined Results: NMT 1%.

11.7.3. Standardization

0.1N Sodium Hydroxide Standardization (Analyst II)			
Replicate	**KHP Standard Weight (g)**	**Titrant Volume (mL)**	**Result (N)**
1	0.6015	29.3423	0.1004
2	0.6021	29.3905	0.1003
3	0.6012	29.3815	0.1002
		Average:	0.1003
		Deviation:	0.0002

11.7.4. Intermediate Precision Data

Intermediate Precision						
Concentration Level (%)	**Analyst**	**L-Histidine Monochloride Monohydrate (g)**	**Endpoint (mL)**	**Assay Result (%)**	**Standard Deviation (%)**	**%RSD (1% Max)**
100	Analyst I	0.5003	23.8040	100.26	0.0616	0.06
		0.5003	23.8153	100.30		
		0.5004	23.8367	100.37		
		0.5004	23.8514	100.44		
		0.5002	23.8202	100.34		
		0.5007	23.8585	100.41		
	Analyst II	0.5001	23.8795	100.41	0.1738	0.18
		0.5009	23.8630	100.18		
		0.5007	23.8560	100.19		
		0.5008	23.8245	100.04		
		0.5004	23.9083	100.47		
		0.5001	23.7765	99.98		
Combined (Analyst I and II):					0.1510	0.15

12. CONCLUSION:

12.1. **Performance Summary:**

Performance Summary	
Method Performance Indicator	**Result**
System Suitability	Pass
Accuracy	Pass
Precision	Pass
Linearity	Pass
Range	50 – 120%
Specificity	Pass
Intermediate Precision	Pass

12.2. **Statement of Validation:**

12.2.1. The method of analysis of L-Histidine Monochloride Monohydrate determination by Potentiometric Titration with 0.1N Sodium Hydroxide is considered a validated method of analysis at the Bangor BioSpectra facility and is approved for use.

12.3. **Critical Changes, Deviations, or Failures:**

12.3.1. There were no critical changes, deviations, or failures during the execution of the protocol.

13. ADDENDUM:

13.1. Purpose:

13.1.1. The L-Histidine Monochloride Monohydrate Assay was carried out on the dried basis, using the Loss on Drying value, for this Analytical Method Validation. The L-Histidine Monochloride Monohydrate Assay can also be calculated on the anhydrous basis, using the Karl Fischer Water Content value. The purpose of this addendum is to demonstrate and validate the calculation of the L-Histidine Monochloride Monohydrate Assay on the anhydrous basis using the Karl Fischer Water Content value.

13.2. Scope:

13.2.1. This addendum applies to the L-Histidine Monochloride Monohydrate Assay calculated on the Anhydrous Basis using the Karl Fischer Water Content value.

13.3. Procedure:

13.3.1. Note: Karl Fischer Water Content result (%) is required prior to performing analysis.

13.3.2. Standardize 0.1N Sodium Hydroxide as per the Standardization of Titrants SOP.

13.3.3. Accurately weigh sample into a suitable beaker (See Sample Preparation Table in the Procedure Section).

13.3.4. Dissolve in 50mL of Purified Water.

13.3.5. Titrate to a potentiometric endpoint with 0.1N Sodium Hydroxide.

13.3.6. Calculate %L-Histidine Monochloride Monohydrate (Anhydrous Basis) using the following equation in the Metrohm® Tiamo™ software:

$$\%\ \text{L-Histidine Monochloride Monohydrate} = \frac{(\text{Sample Titrant Volume (mL)})(Titrant\ Normality)(19.16)}{Sample\ Weight\ (g)} \times \left(\frac{100}{(100 - KF\ (\%))}\right)$$

13.3.7. Record Result.

13.4. Validation Results:

13.4.1. System Suitability / Standardization, Precision, Linearity, Specificity, Range, and Intermediate Precision all remain unchanged when calculating assay results on the anhydrous basis.

13.4.2. **Accuracy:**

13.4.2.1. Accuracy was assessed by comparing the measured L-Histidine Monochloride Monohydrate Assay value (%) to the L-Histidine Monochloride Monohydrate Assay data on the Certificate of Analysis (CoA) and calculating the Percent Recovery (%).

$$Percent\ Recovery\ (\%) = \frac{Measured\ Assay\ Value\ (\%)}{\text{L-Histidine Monochloride Monohydrate CoA Assay Value (\%)}} \times 100$$

13.4.2.2. Acceptance Criteria:

13.4.2.2.1. Percent Recovery (%): All replicates are between 99.0% and 101.0%.

Assay (Anhydrous Basis) – Accuracy					
				CoA Assay Value (%):	99.9
				Karl Fischer Water Content Value (%):	8.69
Concentration Level (%)	**Replicate**	**L-Histidine Monochloride Monohydrate (g)**	**Endpoint (mL)**	**Assay (Anhydrous Basis) (%)**	**Percent Recovery (%)**
0	1	0.0000	Not Applicable	Not Applicable	Not Applicable
	2	0.0000	Not Applicable	Not Applicable	Not Applicable
	3	0.0000	Not Applicable	Not Applicable	Not Applicable
25	1	0.1254	5.9524	100.10	100.2
50	1	0.2503	11.8769	100.07	100.2
	2	0.2500	11.8566	100.01	100.1
	3	0.2503	11.8630	99.95	100.0
80	1	0.4005	19.0324	100.22	100.3
	2	0.4008	19.0818	100.40	100.5
	3	0.4002	19.0406	100.33	100.4
100	1	0.5003	23.8040	100.34	100.4
	2	0.5003	23.8153	100.38	100.5
	3	0.5004	23.8367	100.46	100.6
	4	0.5004	23.8514	100.52	100.6
	5	0.5002	23.8202	100.43	100.5
	6	0.5007	23.8585	100.49	100.6
120	1	0.6006	28.6381	100.55	100.7
	2	0.6000	28.6048	100.54	100.6
	3	0.6000	28.6002	100.52	100.6

13.5. **Critical Changes, Deviations, or Failures**

13.5.1. **Critical Changes – Water Content Reference Value –** The Water Content (%) value listed on the Certificate of Analysis for L-Histidine Monochloride Monohydrate is 6.6%. This value is inconsistent with the Loss on Drying (%) and Karl Fischer Water Content (%) values determined in-house which were 8.6159% and 8.69%, respectively. As the in-house values agree with one another, the in-house determined water content value was used to calculate the %L-Histidine Monochloride Monohydrate (Anhydrous Basis).

13.6. **Conclusion**

13.6.1. The L-Histidine Monochloride Monohydrate Assay on the anhydrous basis, using the Karl Fischer Water Content value met all acceptance criteria for System Suitability / Standardization, Accuracy, Precision, Linearity, Specificity, Range, and Intermediate Precision as is considered a validated method of analysis at the Bangor, PA BioSpectra Inc. facility and is approved for use.