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ANALYTICAL METHOD VERIFICATION REPORT: L-HISTIDINE MONOCHLORIDE MONOHYDRATE WATER DETERMINATION VIA KARL FISCHER UTILIZING METROHM 907 AUTO TITRATOR

TABLE OF CONTENTS

1. **PURPOSE:**

   1.1. The purpose of this Report is to:

       1.1.1. Provide performance data demonstrating that the L-Histidine Monochloride Monohydrate Water Determination procedure via Karl Fischer Utilizing the Metrohm 907 Auto-Titrator is adequately evaluated and validated.

       1.1.2. Provide verification that the procedure for determining the Water Content of L-Histidine Monochloride Monohydrate at the set specification met all requirements for System Suitability, Accuracy, Precision, Specificity, Limit of Detection (LoD), Limit of Quantitation (LoQ), Linearity, Range, and Intermediate Precision.

2. **SCOPE:**

   2.1. This Analytical Method Verification Report applies to the Water Determination method used on the Metrohm 907 Auto-Titrator to determine the water content of L-Histidine Monochloride Monohydrate.

   2.2. The targeted range of the analysis was 7.2% - 10.0% w/w water. Sample size was varied to work within the limits of 10 mL maximum titration volume of the Metrohm 10 mL auto-burette. Karl Fischer reagent Composite 5 generally titrates 5 mg of water per 1 mL of titrant, limiting the total titrated amount of water to at most 50 mg. For the higher levels of water spike analysis, sample size was reduced in order to not over-run the burette capacity. Sample size, therefore, may be varied depending on specification(s) and was investigated at varying levels in the verification protocol to thoroughly cover the range of moisture measurement required.

3. **RESPONSIBILITIES:**

   3.1. The Laboratory Manager, or designee, was responsible for the control, training, 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 Verification.

   3.3. The Analysts performing the test, with help from the Laboratory Manager if necessary, were responsible for completing the Method Verification Report using conclusions made from the results obtained from testing.

4. **REFERENCES:**

   4.1. BSI-PRL-0767, Analytical Method Verification Protocol: L-Histidine Monochloride Monohydrate Water Determination Via Karl Fischer Utilizing Metrohm 907 Auto Titrator

   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

   4.8. USP <1225>

   4.9. USP <1226>

5. **PRE-VERIFICATION REQUIREMENTS:**

   5.1. Equipment:

       5.1.1. All equipment used in this Verification was in proper working order and with current calibrations. This was documented in the Materials and Equipment portion of this document.

5.2. Personnel:

    5.2.1. All personnel performing this Verification were properly trained in accordance with the Analytical Methods Validation Master Plan. A copy of the documentation to support training is available in the Analysts' training binders or a Mark as Read training was assigned in the document management system.

5.3. Supplies:

    5.3.1. Any supplies to be used in the Verification were clean and appropriate for the intended use. A list of supplies used is included in the Materials and Equipment section of this 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 this document. This includes: reagent name, lot number, manufacturer, date of opening, part number, and expiration date (if applicable).

5.5. Reference Standards:

    5.5.1. Any standards used in this Verification are listed in the Materials and Equipment section of this document. Details regarding the storage conditions and usage of the reference standard (expiration date) is detailed in this list. The name of the reference standard, lot number, date of manufacture, date of opening, date of expiration and part number is provided in this document and was recorded during Verification testing.

6. MATERIALS AND EQUIPMENT:

6.1. All materials and equipment utilized in this Verification are outlined in this section. This is a list of the anticipated materials and equipment required. As part of the Analytical Method Verification Protocol, all materials and equipment used is documented in the respective section of this document, including the Instrument Name, Model Number, Manufacturer, Serial Number, Calibration Information, and Verification Information. Any specifications on materials or equipment is listed in the Method Verification Report.

6.2. Equipment:

    6.2.1. Analytical Balance

    6.2.2. Desiccator

    6.2.3. Double Pt-wire electrode

    6.2.4. Metrohm 907 Auto-Titrator

    6.2.5. Oven

6.3. Reagents:

    6.3.1. Composite 5 (Titrant)

    6.3.2. Formamide Dry

    6.3.3. Methanol Dry

    6.3.4. Purified Water

    6.3.5. L-Histidine Monochloride Monohydrate

6.4. Supplies:

    6.4.1. Syringe

    6.4.2. Micropipettes

    6.4.3. Micro Stir bars

    6.4.4. Micropipette Tips (LoRetention)

    6.4.5. Mortar and Pestle

    6.4.6. Glass Weighing Spoon

    6.4.7. Scoops

7. PROCEDURE:

7.1. Standardize Karl Fischer Titrant (Composite 5):
7.1.1. Standardize Karl Fischer Titrant (Composite 5) as per the Standardization of Titrants SOP.

7.2. L-Histidine Monochloride Monohydrate Drying Procedure:
7.2.1. Grind ~20 grams of L-Histidine Monochloride Monohydrate using a mortar and pestle to homogenize.

7.2.2. Dry the sample in the oven at 150 °C to a constant weight (NMT 0.5 mg difference between consecutive weights) and store in a desiccator until use.

7.2.3. Mix dried sample before use and store in a desiccator between analyses.

7.3. L-Histidine Monochloride Monohydrate Sample Analysis:
7.3.1. Weigh approximately 0.3 grams of sample into a glass weighing spoon and tare the balance.

7.3.2. Transfer the sample to the Karl Fischer vessel by removing the rubber septum and adding the sample into the titration vessel.

    7.3.2.1. Note: Do not leave the rubber septum open for longer than 20 seconds as this will allow moisture to enter the titration vessel.

7.3.3. Return the weighing spoon to the balance, making sure not to lose any sample that was left behind. Once the weight stabilizes, record the weight in the Tiamo Software.

7.3.4. The moisture content will then be determined by the KF titration using the Metrohm Titrando 907.

$$\%Moisture = \frac{(mL \text{ of Composite } 5)\left(\frac{mg}{mL} \text{ of Composite } 5\right)(0.1)}{Sample \text{ } weight \text{ } (g)}$$

7.4. Verification Sample Preparations:
7.4.1. 0% Limit:

    7.4.1.1. Weigh 0.3 grams of dried sample.

    7.4.1.2. Transfer to the Karl Fischer vessel.

    7.4.1.3. Record the weight by difference

    7.4.1.4. Analyze the sample.

    7.4.1.5. Record the result.

7.4.2. 3.5% Limit (~50% Limit Spike):

    7.4.2.1. Weigh 0.3 grams of dried sample.

    7.4.2.2. Transfer to the Karl Fischer vessel.

    7.4.2.3. Record the weight by difference.

    7.4.2.4. Pipette 10.5 µL of Purified Water into the vessel as quickly as possible to ensure environmental moisture does not enter the vessel.

    7.4.2.5. Titrate with Composite 5.

    7.4.2.6. Record the result.

    7.4.2.7. Repeat in triplicate.

7.4.3. 5.6% Limit (80% of the Lower Limit Specification):

    7.4.3.1. Weigh 0.3 grams of dried sample.

    7.4.3.2. Transfer to the Karl Fischer vessel.

    7.4.3.3. Record the weight by difference.

    7.4.3.4. Pipette 16.8 µL of Purified Water into the vessel as quickly as possible to ensure environmental moisture does not enter the vessel.

    7.4.3.5. Titrate with Composite 5.

    7.4.3.6. Record the result.

    7.4.3.7. Repeat in triplicate.

7.4.4. <u>7.2% Limit (100% of the Lower Limit Specification):</u>
    7.4.4.1. Weigh 0.3 grams of dried sample.
    7.4.4.2. Transfer to the Karl Fischer vessel.
    7.4.4.3. Record the weight by difference.
    7.4.4.4. Pipette 21 µL of Purified Water into the vessel as quickly as possible to ensure environmental moisture does not enter the vessel.
    7.4.4.5. Titrate with Composite 5.
    7.4.4.6. Record the result.
    7.4.4.7. Repeat in triplicate.

7.4.5. <u>10.0% Limit (100% of the Upper Limit Specification):</u>
    7.4.5.1. Weigh 0.3 grams of dried sample.
    7.4.5.2. Transfer to the Karl Fischer vessel.
    7.4.5.3. Record the weight by difference.
    7.4.5.4. Pipette 30 µL of Purified Water into the vessel as quickly as possible to ensure environmental moisture does not enter the vessel.
    7.4.5.5. Titrate with Composite 5.
    7.4.5.6. Record the result.
    7.4.5.7. Repeat in triplicate.

7.4.6. <u>12.0% Limit (120% of the Upper Limit Specification):</u>
    7.4.6.1. Weigh 0.3 grams of dried sample.
    7.4.6.2. Transfer to the Karl Fischer vessel.
    7.4.6.3. Record the weight by difference.
    7.4.6.4. Pipette 36 µL of Purified Water into the vessel as quickly as possible to ensure environmental moisture does not enter the vessel.
    7.4.6.5. Titrate with Composite 5.
    7.4.6.6. Record the result.
    7.4.6.7. Repeat in triplicate.

8. PERFORMANCE PARAMETERS:

8.1. System Suitability:
    8.1.1. System Suitability was assessed by standardizing Composite 5 in triplicate and reporting the average as the titer value.
    8.1.2. <u>Acceptance Criteria:</u>
        8.1.2.1. The Relative Standard Deviation (%RSD) between triplicate runs was NMT 2%.

8.2. Accuracy:
    8.2.1. Accuracy was assessed across five (5) concentration levels.
    8.2.2. Accuracy was assessed by comparing the water content result (%) with the water content spike (%) and calculating the Percent Recovery (%).
        8.2.2.1. Correct for intrinsic water content if required, L-Histidine Monohydrochloride Monohydrate should be dried and stored over desiccant to minimize correction factor if applicable.

$$Percent\ Recovery\ (\%) = \frac{Water\ Content\ Result\ (\%)}{Water\ Content\ Spike\ (\%) + 0\%\ Level\ Water\ Content\ (\%)} \times 100$$

    8.2.3. <u>Acceptance Criteria:</u>
        8.2.3.1. Percent Recovery (%): All replicates are between 80% and 120%.

8.3. Precision:

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

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

    8.3.3. Acceptance Criteria:

        8.3.3.1. Standard Deviation: Report.

        8.3.3.2. Relative Standard Deviation (%RSD): NMT 20%.

8.4. Specificity:

    8.4.1. Karl Fischer analysis is inherently selective to water.

    8.4.2. Specificity was demonstrated by meeting requirements for Accuracy and Precision.

    8.4.3. Acceptance Criteria:

        8.4.3.1. Requirements met for Accuracy and Precision.

8.5. Limit of Detection (LoD) / Limit of Quantitation (LoQ):

    8.5.1. The Limit of Detection was expressed as:

$$\text{Limit of Detection (LoD)} = \frac{3.3\sigma}{S}$$

        8.5.1.1. σ = Average Standard Deviation of the Water Content Results (%).

        8.5.1.2. S = Slope of the Water Content Results (%) vs. Water Content Spike (%).

    8.5.2. The Limit of Quantitation (LoQ) was expressed as:

$$\text{Limit of Quantitation (LoQ)} = \frac{10\sigma}{S}$$

        8.5.2.1. σ = Average Standard Deviation of the Water Content Results (%).

        8.5.2.2. S = Slope of the Water Content Results (%) vs. Water Content Spike (%).

    8.5.3. Acceptance Criteria:

        8.5.3.1. Limit of Detection (LoD): Report.

        8.5.3.2. Limit of Quantitation (LoQ): Report.

8.6. Linearity:

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

    8.6.2. Plot and report the Calibration Coefficient (r^2), Slope, and Y-Intercept of the endpoint (mL of Composite 5) to Water Content Spike (μL Water).

    8.6.3. Acceptance Criteria:

        8.6.3.1. Calibration Coefficient (r^2): NLT 0.95.

        8.6.3.2. Slope: Report.

        8.6.3.3. Y-Intercept: Report.

8.7. Range:

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

    8.7.2. Acceptance Criteria:

        8.7.2.1. A minimum range of 7.2% to 10.0% or the range of the Water Content specification is required.

8.8. Intermediate Precision:

    8.8.1. Intermediate Precision was assessed by having a second analyst on a separate day perform a standardization and an additional three (3) determinations at the 100% limits (7.2% and 10.0% Spikes). The Standard Deviation and Relative Standard Deviation (%RSD) will be calculated for individual (Analyst II) and combined (Analyst I and II) results.

8.8.2. Acceptance Criteria:
      8.8.2.1. Standard Deviation of Individual Combined Results: Report.
      8.8.2.2. Relative Standard Deviation (%RSD) of Individual and Combined Results: NMT 25%.

9. DOCUMENTATION PROCEDURES:

9.1. All data sheets, including notebooks, were signed and dated by the employee executing the Protocol. Pages were copied and uploaded as supporting material in Master Control.

9.2. All testing equipment were 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 this document 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 - The %RSD between the triplicate runs is NMT 2%	Analyst I - Titer Value: 5.1785 mg/mL %RSD: 0.50% Analyst II - Titer Value: 5.0690 mg/mL %RSD: 0.30%
Accuracy	All samples must have a Percent Recovery between 80% and 120%	50% Level - Replicate 1: 96% - Replicate 2: 103% - Replicate 3: 102% 80% Level - Replicate 1: 104% - Replicate 2: 93% - Replicate 3: 93% 100% Lower Limit Level - Replicate 1: 100% - Replicate 2: 102% - Replicate 3: 107% 100% Upper Limit Level - Replicate 1: 102% - Replicate 2: 84% - Replicate 3: 91% 120% Level - Replicate 1: 103% - Replicate 2: 99% - Replicate 3: 105%

Validation Summary		
Performance Parameters	**Acceptance Criteria**	**Results**
Precision	• Standard Deviation: Report • Each level must have a %RSD of NMT 20%	50% Level 　• Standard Deviation: 0.127% 　• %RSD: 3% 80% Level 　• Standard Deviation: 0.367% 　• %RSD: 7% 100% Lower Limit Level 　• Standard Deviation: 0.259% 　• %RSD: 4% 100% Upper Limit Level 　• Standard Deviation: 0.931% 　• %RSD: 10% 120% Level 　• Standard Deviation: 0.376% 　• %RSD: 3%
Specificity	• Requirements for Accuracy and Precision are met.	• Requirements for accuracy and precision were met.
Limit of Detection (LOD)	• Report	• LOD: 1.38%
Limit of Quantification (LOQ)	• Report	• LOQ: 4.17%
Linearity	• Report slope and y-intercept • Calibration coefficient (r^2) is NLT 0.95	• Calibration Coefficient (r^2): 0.9994 • Slope: 0.1979 • Y-Intercept: 0.0921
Range	• A minimum range of 7.2% to 10.0% or the range of the water content specification is required. • Range was assessed by showing an acceptable degree of Accuracy, Precision and Linearity	• Range: 3.5% – 12.0%

Validation Summary		
Performance Parameters	**Acceptance Criteria**	**Results**
Intermediate Precision	• Standard Deviation of Individual Combined Results: Report • Relative Standard Deviation (%RSD) of Individual and Combined Results: NMT 25%.	Individual 100% Lower Limit (Analyst II) • Standard Deviation: 0.006% • %RSD: 0% Individual 100% Upper Limit (Analyst II) • Standard Deviation: 0.366% • %RSD: 4% Combined 100% Lower Limit (Analyst I and II) • Standard Deviation: 0.313% • %RSD: 4% Combined 100% Upper Limit (Analyst I and II) • Standard Deviation: 0.737% • %RSD: 8%

11. EXECUTION DOCUMENTATION:

11.1. Materials and Equipment:

Equipment and Instrumentation				
Equipment	**Model/Part Number**	**Manufacturer**	**Serial Number**	**Calibration Due**
Auto-Titrator	907 Titrando	Metrohm	12155	7/24
Analytical Balance	MSE 224S	Sartorius	24801744	4/30/24
Analytical Balance	Secura 124-1S	Sartorius	29212172	4/30/24
Micropipette	Research Plus	Eppendorf	P53563H	7/31/24
Micropipette	Research Plus	Eppendorf	I05023M	8/31/24
Oven	Gravity Convection	VWR	1100001176D009	5/24

Reagents and Standards						
Reagent / Standard	**Lot ID**	**Manufacturer**	**Part Number**	**CAS Number**	**Expiry Date**	**Date of Opening**
L-Histidine Monochloride Monohydrate	BCCH6305	Sigma Aldrich	53370-500G	5934-29-2	9/30/28	9/6/23
Purified Water	F9SA14284H	Millipore Sigma	Not Applicable	7732-18-5	6/30/24	Not Applicable
Composite 5	N1020	Honeywell	34805-1L-US	111-90-0	3/27/26	3/13/24

Reagents and Standards						
Reagent / Standard	Lot ID	Manufacturer	Part Number	CAS Number	Expiry Date	Date of Opening
Methanol Dry	N018F	Honeywell	34741-1L-US	67-56-1	12/23/27	3/13/24
Formamide Dry	N1070	Honeywell	34724	75-12-7	4/1/26	3/13/24
Formamide Dry	M3400	Honeywell	34724-1L	75-12-7	11/20/25	3/15/24
Methanol Dry	N093A	Honeywell	34741-1L-US	67-56-1	3/7/28	3/15/24

Supplies		
Supply	Manufacturer	Part Number
Weight Boat / Weigh Paper	Metrohm	6.2412.000
Stir Bars	Metrohm	6.1903.030

11.2. **L-Histidine Monochloride Monohydrate Drying Procedure**:

    11.2.1. Grind ~20 grams of L-Histidine Monochloride Monohydrate using a mortar and pestle to homogenize.

    11.2.2. Dry the sample in the oven at 150 °C to a constant weight (NMT 0.5 mg difference between consecutive weights) and store in a desiccator until use.

    11.2.3. Mix dried sample before use and store in a desiccator between analyses.

L-Histidine Monochloride Monohydrate Dried Sample Preparation			
L-Histidine Monochloride Monohydrate Lot	Initial Sample Weight (g)	Final Sample Weight (g)	Dry Time (hours)
BCCH63305 exp 9/30/28	20.1491	18.4088	50

11.3. **System Suitability / Standardization**:

    11.3.1. System Suitability was assessed by standardizing Composite 5 in triplicate and reporting the average as the titer value.

    11.3.2. Acceptance Criteria:

        11.3.2.1. The Relative Standard Deviation (%RSD) between triplicate runs is NMT 2%.

System Suitability / Composite 5 Standardization			
Replicate	Purified Water (g) or (µL)	Titrant Volume (mL)	Result (mg/mL)
1	30 µL	5.7721	5.1975
2	30 µL	5.7816	5.1889
3	30 µL	5.8263	5.1491
Average Titer Value (mg/mL):			5.1785
Relative Standard Deviation (%RSD):			0.50

11.4. **Accuracy:**

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

    11.4.2. Accuracy was assessed by comparing the water content result (%) with the water content spike (%) and calculating the Percent Recovery (%).

        11.4.2.1. Correct for intrinsic water content if required, L-Histidine Monohydrochloride Monohydrate should be dried and stored over desiccant to minimize correction factor if applicable.

$$Percent\ Recovery\ (\%) = \frac{Water\ Content\ Result\ (\%)}{Water\ Content\ Spike\ (\%) + 0\%\ Level\ Water\ Content\ (\%)} \times 100$$

    11.4.3. Acceptance Criteria:

        11.4.3.1. Percent Recovery (%): All replicates are between 80% and 120%.

Accuracy						
Concentration Level (%)	Spike Level (%)	Replicate	Sample Weight (g)	Endpoint (mL)	Water Content Result (%)	Percent Recovery (%)
0%	Not Applicable	1	0.3095	0.0747	0.13	███
~50%	3.5%	1	0.3116	2.1070	3.50	96
		2	0.3076	2.2157	3.73	103
		3	0.3028	2.1722	3.71	102
80%	5.6%	1	0.3023	3.4821	5.96	104
		2	0.3259	3.3609	5.34	93
		3	0.3287	3.3708	5.31	93
100% Lower Limit	7.2%	1	0.3041	4.2019	7.16	100
		2	0.3016	4.2449	7.29	102
		3	0.3009	4.4520	7.66	107
100% Upper Limit	10.0%	1	0.3036	6.0662	10.35	102
		2	0.3625	5.9510	8.50	84
		3	0.3276	5.8509	9.25	91
120%	12.0%	1	0.3021	7.2714	12.46	103
		2	0.3055	7.0622	11.97	99
		3	0.3024	7.4230	12.71	105

11.5. **Precision:**
    11.5.1. Precision was assessed across five (5) concentration levels.
    11.5.2. Precision was assessed by determining the Standard Deviation and Relative Standard Deviation (%RSD) at each concentration level.
    11.5.3. Acceptance Criteria:
        11.5.3.1. Standard Deviation: Report.
        11.5.3.2. Relative Standard Deviation (%RSD): NMT 20%.

Precision				
Concentration Level (%)	Replicate	Water Content Result (%)	Standard Deviation (%)	%RSD
~50%	1	3.50	0.127	3
	2	3.73		
	3	3.71		
80%	1	5.96	0.367	7
	2	5.34		
	3	5.31		
100% Lower Limit	1	7.16	0.259	4
	2	7.29		
	3	7.66		
100% Upper Limit	1	10.35	0.931	10
	2	8.50		
	3	9.25		
120%	1	12.46	0.376	3
	2	11.97		
	3	12.71		

11.6. **Specificity:**
    11.6.1. Karl Fischer analysis is inherently selective to water.
    11.6.2. Specificity was demonstrated by meeting requirements for Accuracy and Precision.
    11.6.3. Acceptance Criteria:
        11.6.3.1. Requirements met for Accuracy and Precision.

Specificity	
Acceptance Criteria	**Result**
Meets Requirements for Accuracy.	Pass
Meets Requirements for Precision.	Pass

11.7. **Limit of Detection (LoD) / Limit of Quantitation (LoQ):**
    11.7.1. The Limit of Detection was expressed as:

$$\text{Limit of Detection (LoD)} = \frac{3.3\sigma}{S}$$

        11.7.1.1. σ = Average Standard Deviation of the Water Content Results (%).
        11.7.1.2. S = Slope of the Water Content Results (%) vs. Water Content Spike (%).
    11.7.2. The Limit of Quantitation (LoQ) was expressed as:

$$\text{Limit of Quantitation (LoQ)} = \frac{10\sigma}{S}$$

        11.7.2.1. σ = Average Standard Deviation of the Water Content Results (%).
        11.7.2.2. S = Slope of the Water Content Results (%) vs. Water Content Spike (%).
    11.7.3. Acceptance Criteria:
        11.7.3.1. Limit of Detection (LoD): Report.
        11.7.3.2. Limit of Quantitation (LoQ): Report.

Limit of Detection (LoD) / Limit of Quantitation (LoQ)			
Average Standard Deviation of Water Content Results, σ (%)	**Slope of Water Content Results vs. Water Content Spike, S**	**Limit of Detection (%)**	**Limit of Quantitation (%)**
0.412	0.9881	1.38	4.17

11.8. **Linearity:**

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

  11.8.2. Plot and report the Calibration Coefficient (r^2), Slope, and Y-Intercept of the endpoint (mL of Composite 5) to Water Content Spike (μL Water).

  11.8.3. <u>Acceptance Criteria:</u>

    11.8.3.1. Calibration Coefficient (r^2): NLT 0.95.

    11.8.3.2. Slope: Report.

    11.8.3.3. Y-Intercept: Report.

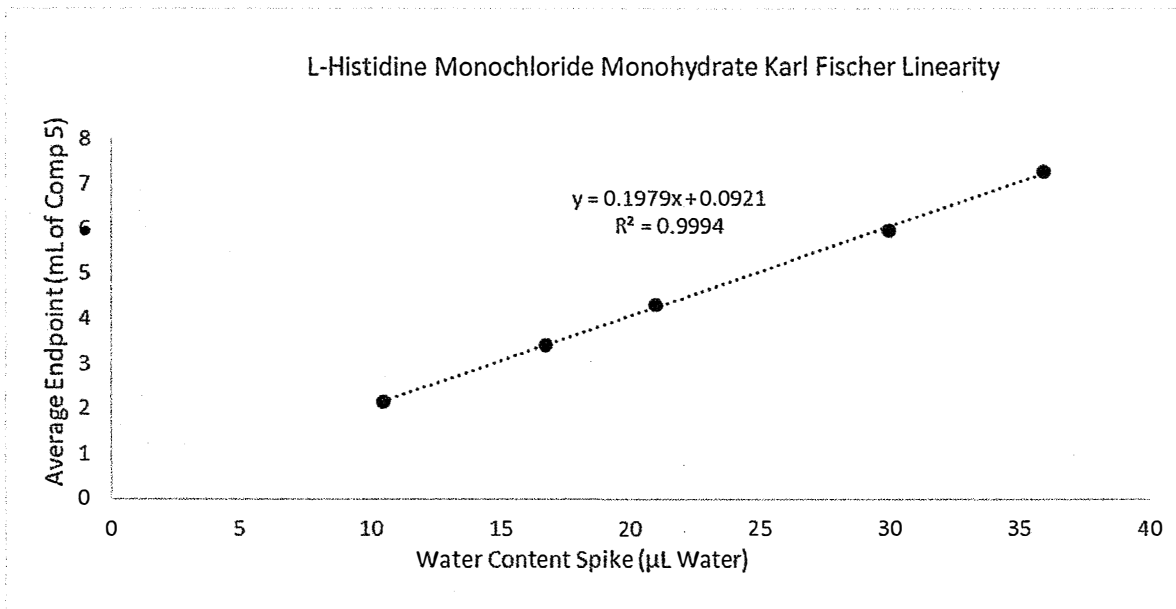| Linearity						
Water Content Spike (μL Water)	Replicate	Endpoint (mL)	Average Endpoint (mL)	Slope	Y-Intercept	Calibration Coefficient (r^2)
10.5	1	2.1070	2.1650			
10.5	2	2.2157	2.1650			
10.5	3	2.1722	2.1650			
16.8	1	3.4821	3.4046			
16.8	2	3.3609	3.4046			
16.8	3	3.3708	3.4046			
21	1	4.2019	4.2996	0.1979	0.0921	0.9994
21	2	4.2449	4.2996			
21	3	4.4520	4.2996			
30	1	6.0662	5.9560			
30	2	5.9510	5.9560			
30	3	5.8509	5.9560			
36	1	7.2714	7.2522			
36	2	7.0622	7.2522			
36	3	7.4230	7.2522			

Figure 1: L-Histidine Monochloride Monohydrate Karl Fischer Linearity

11.9. **Range:**

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

    11.9.2. Acceptance Criteria:

        11.9.2.1. A minimum range of 7.2% to 10.0% or the range of the Water Content specification was required.

Range of Analysis: 3.5% - 12.0%

11.10. **Intermediate Precision:**

    11.10.1. **L-Histidine Monochloride Monohydrate Drying Procedure**

        11.10.1.1. Grind ~20 grams of L-Histidine Monochloride Monohydrate using a mortar and pestle to homogenize.

        11.10.1.2. Dry the sample in the oven at 150°C to a constant weight (NMT 0.5mg difference between consecutive weights) and store in a desiccator until use.

        11.10.1.3. Mix dried sample before use and store in a desiccator between analyses.

L-Histidine Monochloride Monohydrate Dried Sample Preparation			
L-Histidine Monochloride Monohydrate Lot	**Initial Sample Weight (g)**	**Final Sample Weight (g)**	**Dry Time (hours)**
BCCH6305	20.1491	18.4088	50

11.10.2. **Standardization/System Suitability:**

    11.10.2.1. System Suitability was assessed by standardizing Composite 5 in triplicate and reporting the average as the titer value.

    11.10.2.2. <u>Acceptance Criteria:</u>

        11.10.2.2.1. The Relative Standard Deviation (%RSD) between triplicate runs is NMT 2%.

System Suitability / Composite 5 Standardization			
Replicate	**Purified Water (g) or (µL)**	**Titrant Volume (mL)**	**Result (mg/mL)**
1	30 µL	5.9129	5.0737
2	30 µL	5.9382	5.0521
3	30 µL	5.9040	5.0813
Average Titer Value (mg/mL):			5.0690
Relative Standard Deviation (%RSD):			0.30

11.10.3. **Intermediate Precision:**

    11.10.3.1. Intermediate Precision was assessed by having a second analyst on a separate day perform a standardization and an additional three (3) determinations at the 100% limits (7.2% and 10.0% Spikes). The Standard Deviation and Relative Standard Deviation (%RSD) were calculated for individual (Analyst II) and combined (Analyst I and II) results.

    11.10.3.2. <u>Acceptance Criteria:</u>

        11.10.3.2.1. Standard Deviation of Individual Combined Results: Report.

        11.10.3.2.2. Relative Standard Deviation (%RSD) of Individual and Combined Results: NMT 25%.

Intermediate Precision							
Concentration Level (%)	Analyst	Replicate	Sample Weight (g)	Endpoint (mL)	Water Content Result (%)	Standard Deviation (%)	%RSD
100% Lower Limit	I	1	0.3041	4.2019	7.16	0.259	4
		2	0.3016	4.2449	7.29		
		3	0.3009	4.4520	7.66		
	II	1	0.3060	4.1620	6.89	0.006	0
		2	0.3035	4.1180	6.88		
		3	0.3069	4.1642	6.88		
		100% Lower Limit Combined:				0.313	4
100% Upper Limit	I	1	0.3036	6.0662	10.35	0.931	10
		2	0.3625	5.9510	8.50		
		3	03276	5.8509	9.25		
	II	1	0.2987	5.9683	10.13	0.366	4
		2	0.3077	5.8662	9.66		
		3	0.2878	5.8907	10.38		
		100% Upper Limit Combined:				0.737	8

12. CONCLUSION:

12.1. Performance Summary:

Performance Summary	
Method Performance Indicator	**Result**
System Suitability	Pass
Accuracy	Pass
Precision	Pass
Specificity	Pass
Limit of Detection (LoD)	1.38%
Limit of Quantitation (LoQ)	4.17%
Linearity	Pass
Range	3.5% - 12.0%
Intermediate Precision	Pass

12.2. Statement of Verification:
12.2.1. The method of analysis of Water in L-Histidine Monohydrochloride Monohydrate by means of Karl Fischer Titration in samples is considered a Verified method of analysis at the Bangor, PA facility and is approved for use.

12.3. Excursions or Critical Changes to Method Verification Protocol:
12.3.1. There were no excursions, critical changes, or failures found during the execution of the verification protocol.