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ANALYTICAL METHOD VERIFICATION: BIS TRIS HCL WATER DETERMINATION VIA KARL FISCHER UTILIZING METROHM 907 AUTO TITRATOR

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1. PURPOSE:

1.1. The purpose of this Verification Protocol Document is to:

1.1.1. Ensure that the Karl Fischer procedure used on the Metrohm 907 Auto-Titrator for Bis Tris HCl samples is adequately evaluated and verified.

1.1.2. Provide verification that the set specification of Bis Tris HCl meets all requirements for accuracy, precision, linearity, range, LOQ, and specificity.

1.1.3. The targeted range of the analysis is 1.0% w/w water maximum. Sample size will be varied to work within the limits of 10mL max. titration volume of the Metrohm 10mL auto-burette. Karl Fischer reagent Composite 5 generally titrates 5mg of water per 1mL of titrant, limiting the total titrated amount of water to at most 50 mg. For the higher levels of water spike analysis, sample size will be reduced in order to not over-run the burette capacity. Sample size therefore may be varied depending on specification(s) and will be investigated at varying levels in this verification protocol to thoroughly cover the range of moisture measurement required.

1.1.4. Ensure that the proper reagents and testing materials are used and the correct documentation is provided for evaluation.

2. SCOPE:

2.1. This Analytical Method Verification protocol applies to the Water Determination method used on the Metrohm 907 Auto-Titrator to determine the water content of Bis Tris HCl.

3. RESPONSIBILITIES:

3.1. The Associate Director of Product Life Cycle, or designee is responsible for the control, training, implementation and maintenance of this procedure.

3.2. Trained chemists, or qualified designees, are responsible for performing the testing stated in Section 7 and 8 of the Protocol and for performing the Verification.

4. REFERENCES:

4.1. BSI-SOP-0098, Balance SOP
4.2. BSI-SOP-0126, Laboratory Notebooks
4.3. BSI-SOP-0140, Standardization of Titrants
4.4. BSI-SOP-0143, Metrohm Titrando 907 Auto Titrator SOP
4.5. BSI-SOP-0436, Analytical Methods Validation Master Plan
4.6. USP<1225>
4.7. USP<1226>

5. PRE-VERIFICATION REQUIREMENTS:

5.1. Equipment:

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

5.2. Personnel:

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

5.3. Supplies:

5.3.1. Any supplies to be used in the Verification must be clean and appropriate for the intended use. A list of supplies used will be in the Materials and Equipment section of this and will be identified with the supplier and description.

5.4. Reagents:
5.4.1. All reagents must be current, meet required specifications, and be suitable for the intended use. A list of reagents used will be included in this document. This should include: 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 will be listed in the Materials and Equipment section of this document. Details regarding the storage conditions and usage of the reference standard (expiration date) will be detailed in this list. The name of the reference standard, lot number, date of manufacture, date of opening, date of expiration and part number must be provided in this document and recorded during Verification testing.

6. MATERIALS AND EQUIPMENT:

6.1. All materials and equipment utilized in this Verification will be 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 will be documented in the respective section of this document, including the Instrument Name, Model Number, Manufacturer, Serial Number, Calibration Information, and Verification Information. All information as required in Section 5 of this Protocol will be documented. Any other pertinent information regarding the materials and equipment will be documented.
6.2. Equipment:
6.2.1. Analytical Balance
6.2.2. Double Pt-wire electrode
6.2.3. Metrohm 907 Auto-Titrator
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. Bis Tris HCl (Dried, Minimum 20g, homogenized and stored over desiccant.)
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. Glass Weighing Spoon
6.4.6. Scoops

7. PROCEDURE:

7.1. Standardization Composite 5:

7.1.1. Standardize Karl Fischer titrant (Composite 5) using Standardization of Titrants, DCN: BSI-SOP-0140, as guidance as well as Metrohm instrument operation manual (electronic or print).

7.2. Bis Tris HCl Test Procedure:

7.2.1. Weigh approximately 1.0g of dried sample into a glass weighing spoon and tare the balance.

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

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

7.2.3. 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.

7.2.4. Bis Tris HCl may not fully dissolve in the 50/50 Methanol/Formamide mix. Ensure that all sample that was added to the KF vessel is suspended in the solution.

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

$$\%\ Moisture = \frac{(mL\ of\ Composite\ 5)\ (mg/mL\ of\ Composite\ 5)\ (0.1)}{Sample\ Weight\ (g)}$$

8. PERFORMANCE PARAMETERS:

8.1. This method is to be validated as a Category II Quantitative test.

8.1.1. System Suitability, Range, Accuracy, Precision, Specificity, Quantitation Limit, and Linearity will be assessed.

8.1.2. System suitability:

8.1.2.1. Standardize Composite 5 in triplicate as per Standardization of Titrants, BSI-SOP-0140.

8.1.2.1.1. % RSD of NMT 2% is required between triplicate runs.

8.1.3. Sample preparations:

8.1.3.1. 0% Limit (Dried Sample):

8.1.3.1.1. Weigh 1.0g of dried sample.

8.1.3.1.2. Transfer to Karl Fischer vessel.

8.1.3.1.3. Record weight by difference.

8.1.3.1.4. Analyze the sample.

8.1.3.1.5. Record result.

8.1.3.2. 0.10% Limit (10% Limit Spike, Low Level Analysis Method Verification):

8.1.3.2.1. Weigh 1.0 g of dried sample.

8.1.3.2.2. Transfer to Karl Fischer vessel.

8.1.3.2.3. Record weight by difference.

8.1.3.2.4. Pipette 1µL of Purified Water into vessel as quickly as possible to ensure environmental moisture does not enter vessel.

8.1.3.2.5. Titrate to analyze the sample.

8.1.3.2.6. Record result.

8.1.3.2.7. Repeat in triplicate for LOQ, Linearity, and Range data.

8.1.3.3. 0.50% Limit (50% Limit Spike):

8.1.3.3.1. Weigh 1.0 g of dried sample.

8.1.3.3.2. Transfer to Karl Fischer vessel.

8.1.3.3.3. Record weight by difference.

8.1.3.3.4. Pipette 5µL of Purified Water into vessel as quickly as possible to ensure environmental moisture does not enter vessel.
8.1.3.3.5. Titrate to analyze the sample.
8.1.3.3.6. Record result.
8.1.3.3.7. Analyze one time for Linearity.

8.1.3.4. 0.80% Limit (80% Limit Spike):
8.1.3.4.1. Weigh 1.0 g of dried sample.
8.1.3.4.2. Transfer to Karl Fischer vessel.
8.1.3.4.3. Record weight by difference.
8.1.3.4.4. Pipette 8µL of Purified Water into vessel as quickly as possible to ensure environmental moisture does not enter vessel.
8.1.3.4.5. Titrate to analyze the sample.
8.1.3.4.6. Record result.
8.1.3.4.7. Repeat in triplicate.

8.1.3.5. 1.00% Limit (100% Limit Spike):
8.1.3.5.1. Weigh 1.0g of dried sample.
8.1.3.5.2. Transfer to Karl Fischer vessel.
8.1.3.5.3. Record weight by difference.
8.1.3.5.4. Pipette 10µL of Purified Water into vessel as quickly as possible to ensure environmental moisture does not enter vessel.
8.1.3.5.5. Titrate to analyze the sample.
8.1.3.5.6. Record result.
8.1.3.5.7. Repeat in triplicate.

8.1.3.6. 1.20% Limit (120% Limit Spike):
8.1.3.6.1. Weigh 1.0 g of dried sample.
8.1.3.6.2. Transfer to Karl Fischer vessel.
8.1.3.6.3. Record weight by difference.
8.1.3.6.4. Pipette 12µL of Purified Water into vessel as quickly as possible to ensure environmental moisture does not enter vessel.
8.1.3.6.5. Titrate to analyze the sample.
8.1.3.6.6. Record result.
8.1.3.6.7. Repeat in triplicate.

8.2. Accuracy: (80-120% Recovery)
8.2.1. Calculate % Recovery for all samples analyzed as well as average % recovery.
8.2.2. Acceptance Criteria: 80-120% Recovery
8.2.3. Correct for intrinsic water content if required[1], Bis Tris HCl should be dried and stored over desiccant to minimize correction factor if applicable.

$$\%\ Recovery = \frac{Result\ (\%)}{(Spike\ Value\ (\%) + 0\%\ Level^{1})} \times 100$$

8.3. Precision: (20% RSD Max)
8.3.1. Calculate the % RSD at the 10%, 80%, 100% and 120% levels.
8.3.2. Acceptance Criteria: NMT 20% RSD at 100% level.

8.4. Ruggedness (Intermediate Precision): (25% RSD Max.)
8.4.1. A separate analyst will perform the 100% Limit (1% Spike) analyses in triplicate on a different day in which they perform a separate standardization.
8.4.2. The standard deviation and relative standard deviation (%RSD) of the individual and combined runs will be analyzed to determine. Ruggedness.

8.5. Specificity:
  8.5.1. Karl Fischer analysis is inherently selective to water, by meeting requirements for accuracy and precision, specificity will be demonstrated.
8.6. Quantitation Limit: (Report)
  8.6.1. The quantitation limit will be determined based on the lowest acceptable level that demonstrates acceptable accuracy and precision.
8.7. Linearity: (0.95 R^2 Min)
  8.7.1. Plot 5 levels of instrument response (mL Comp 5) vs. Spike level (µL Water).
    8.7.1.1. Level 1: 0%
    8.7.1.2. Level 2: 50%
    8.7.1.3. Level 3: 80%
    8.7.1.4. Level 4: 100%
    8.7.1.5. Level 5: 120%
8.8. Range: (Target: 50-120%)
  8.8.1. Report on accuracy and precision of samples analyzed for linearity at each concentration. Report suitable range, acceptance criteria at minimum is 50-120% range of the impurity (water).

9. DOCUMENTATION PROCEDURES:

9.1. All data sheets, including notebooks, are to be signed and dated by the employee executing the protocol. Pages may be copied as necessary to attach.
9.2. All testing equipment must be calibrated. Ensure that there is a certificate on file or appropriate standards are used if calibration is required.
9.3. Any critical changes that must be made to the analytical procedure should be noted in this document with supporting evidence for the change.

10. EXECUTION DOCUMENTATION:

10.1. Personnel:

Responsibility	Name	Title	Initial / Date
Analyst I			
Analyst II			
Reviewer I			
Reviewer II			
Approver I			
Approver II			

Training Verification Form(s) Attached: _____/_____

10.2. Materials and Equipment[1]:

Equipment and Instrumentation Information				
Equipment	**Model/Part Number**	**Manufacturer**	**S/N**	**Calibration Due**
Auto-Titrator				
Analytical Balance				
Analytical Balance				
Micropipette				
Micropipette				
Micropipette				
Micropipette				
Micropipette				
Weight Boat / Paper				
Oven				

Reagent and Standard Information				
Reagent/Standard	**Lot ID**	**Manufacturer**	**CAS**	**Expiry Date**
Bis Tris HCl				
Purified Water				
Composite 5				
Methanol				
Formamide				

[1]Lines Intentionally Left Blank for contingency equipment, if applicable.

Completed by: _____
Reviewed by: _____

10.3. Sample Preparation (Analyst 1): Homogenize ~20 g of Bis-Tris HCl sample with a mortar and pestle and dry by placing over desiccant for a minimum of 48 hours. Mix the powder before use and keep in the desiccator between trials to maintain low levels of intrinsic moisture.

Bis Tris HCl Dried Sample			
Bis Tris HCl Lot	Weight (g)	Desiccant Lot	Dry Time (Hours)

Performed by: _____
Reviewed by: _____

10.4. Standardization/System Suitability: (NMT 2% RSD)

System Suitability / Composite 5 Standardization				
Replicate	Purified Water (g) or (µL)	EP1 (mL)	Titer (mg/mL)	% RSD (NMT 2%)
1				
2				
3				

System Suitability: Pass / Fail

Performed by: _____
Reviewed by: _____

Example Calculation(s):

10.5. Accuracy: (80-120% Recovery) Accuracy was assessed over a minimum of 9 determinations over a minimum of 3 concentration levels. Accuracy was reported as the percent recovery by the assay of known amount of analyte in the sample. The data will be assessed by calculating the percent recovery for each concentration.

Percent Recovery = (Result %)/(Spike Level % + 0% Spike Result) x 100

	Accuracy				
Replicate	Sample Weight (g)	Concentration Level	Spike Level (%)	Result % Water	% Recovery (80-120%)
1		0% Spike	Not Applicable		
1		0.10% Spike	0.10%		
2					
3					
1		0.50% Spike	0.50%		
1		0.80% Spike	0.80%		
2					
3					
1		1.00% Spike	1.00%		
2					
3					
1		1.20% Spike	1.20%		
2					
3					

Accuracy : Pass / Fail

Performed by: _____
Reviewed by: _____

Example Calculation(s):

10.6. Precision: (NMT 20% RSD)

10.6.1. The precision of analytical procedure is determined by assaying a sufficient number of aliquots of a homogenous sample to be able to calculate statistically valid estimates of standard deviation or relative standard deviation (%RSD or %CV). Individual repeatability was be assessed at a minimum of 9 determinations covering the specified range of the analysis.

10.6.2. Acceptance Criteria: NMT 20% RSD

Individual Relative Standard Deviation = (standard deviation/average) x 100

Precision			
Determination	Concentration Level	Result % Water	% RSD (NMT 20%)
1	0.10%		
2			
3			
1	0.80%		
2			
3			
1	1.00%		
2			
3			
1	1.20%		
2			
3			

Precision : Pass / Fail

Performed by: _____

Reviewed by: _____

Example Calculation(s):

10.7. Specificity:

10.7.1. Specificity will be demonstrated by meeting requirements for accuracy and precision. Refer to section 10.5 and 10.6 for disposition. If both accuracy and precision met requirements then specificity is demonstrated.

Precision Result (10.6):

Pass / Fail

Accuracy Result (10.5):

Pass / Fail

Specificity Result:

Pass / Fail

Performed by: _____
Reviewed by: _____

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

10.8.1. LoD = The detection limit (DL or LoD) will be expressed as $3.3\sigma/S$

10.8.2. LoQ = The quantitation limit (QL or LoQ) will be expressed as $10\sigma/S$

10.8.2.1. Where:

10.8.2.1.1. S = Slope of the % Water Reported vs. Spike Level % Water

10.8.2.1.2. σ = Average Std. deviation of the % Water Reported.

LoD = 3.3 ( ) / ( ) = %
LoQ = 10 ( ) / ( ) = %

Performed by: _____
Reviewed by: _____

10.9. Linearity and Range:

10.9.1. The average area response of the injections at each level executed during the Accuracy and LoD/LoQ portion of this protocol will be analyzed to determine linearity and range over a range of 5 concentration levels.

10.9.1.1. Note: 10mg of spike is equivalent to 1.0% w/w water in test sample.

10.9.1.2. 10mg/1,000mg sample weight = 1.0%.

10.9.2. A correlation coefficient of 0.95 minimum is considered acceptable.

10.9.3. The range of the analytical method is determined by the highest concentration of analyte that produce suitable results for precision, accuracy and linearity and the LoQ value as determined in section 10.5 - 10.8.

Linearity Data					
Determination	Standard Spike (µL Water)	Response (mL)	Average Response (mL)	Slope	R^2 (NLT 0.95)
1	1				
2					
3					
1	5				
1	8				
2					
3					
1	10				
2					
3					
1	12				
2					
3					

R^2: _____

Linearity Result (NLT 0.99): Pass / Fail

Range of Analysis: _____% -_____%

Performed by: _____

Reviewed by: _____

10.10. Intermediate Precision (Analyst II): Homogenize ~20 g of Bis-Tris HCl sample with a mortar and dry by placing over desiccant for a minimum of 48 hours. Mix the powder before use and keep in the desiccator between trials to maintain low levels of intrinsic moisture.

Bis Tris HCl Dried Sample			
Bis Tris HCl Lot	Weight (g)	Desiccant Lot	Dry Time (Hours)

Performed by: _____
Reviewed by: _____

10.11. Standardization/System Suitability (Analyst II): (NMT 2% RSD)

System Suitability / Composite 5 Standardization				
Replicate	Purified Water (g) or (µL)	EP1 (mL)	Titer (mg/mL)	% RSD (NMT 2%)
1				
2				
3				

System Suitability: Pass / Fail

Performed by: _____
Reviewed by: _____

Intermediate Precision Data					
Determination	Concentration Level	Analyst	Sample Weight (g)	Result % Water	% RSD (NMT 25%)
1	1.00%	I			
2					
3					
1		II			
2					
3					

Intermediate Precision : Pass / Fail

Performed by: _____
Reviewed by: _____

11. CONCLUSION:

11.1. Performance Summary:

Method Performance Indicator	Result	Initial / Date
System Suitability	Pass / Fail	
Accuracy	Pass / Fail	
Precision	Pass / Fail	
Linearity	Pass / Fail	
Range	Pass / Fail	
Specificity	Pass / Fail	
Intermediate Precision	Pass / Fail	
LoD		
LoQ		

11.2. Statement of Verification: The method of analysis of Water in Bis Tris HCl by means of Karl Fischer Titration in samples is considered a Verified method of analysis at locations described below and is reviewed and approved by the review and approval team as described below 11.3.

11.3. Approval:

Method Verification Approval				
Locations Approved (Check All That Apply)				
☐ 100 Majestic Way, Bangor, PA 18013			Not Applicable ☐	
☐ 1474 Rockdale Lane, Stroudsburg, PA 18360			Not Applicable ☐	
☐ 11 University Place, Rensselaer, NY 12144			Not Applicable ☐	
Name	**Role**	**Title**	**Signature**	**Date**
	Analyst I			
	Analyst II			
	Quality Review			
	Management			

11.4. Excursions or Critical Changes to Method Verification Protocol:

Signature Manifest

Document Number: BSI-RPT-1448 **Revision:** 1.0

Title: Analytical Method Verification: Bis-Tris HCl Water Determination via Karl Fischer

Effective Date: 08 Aug 2023

All dates and times are in US/Eastern.

Analytical Method Verification: Bis-Tris HCl Water Determination via Karl Fischer

Change Request

Name/Signature	Title	Date	Meaning/Reason
Mark Uhlig (MARK.UHLIG)	Associate Director of Product Lifecycle	31 Jul 2023, 04:04:54 PM	Approved
Meghan Skeehan (MEGHAN.SKEEHAN)	Document Control Technician II	31 Jul 2023, 04:23:44 PM	Approved

Originator and Peer Review Collaboration Workspace

Name/Signature	Title	Date	Meaning/Reason
James Piper (JAMES.PIPER)	Director, Laboratory Testing	02 Aug 2023, 03:17:18 PM	Complete & Quit
Kyle Kearns (KYLE.KEARNS)	Senior Chemist	03 Aug 2023, 03:20:52 PM	Complete & Quit
Mark Uhlig (MARK.UHLIG)	Associate Director of Product Lifecycle	03 Aug 2023, 03:38:21 PM	Complete

Departmental Approval

Name/Signature	Title	Date	Meaning/Reason
Mark Uhlig (MARK.UHLIG)	Associate Director of Product Lifecycle	08 Aug 2023, 09:09:47 AM	Approved

Author Approval

Name/Signature	Title	Date	Meaning/Reason
Mark Uhlig (MARK.UHLIG)	Associate Director of Product Lifecycle	08 Aug 2023, 09:09:34 AM	Approved

Quality Approval

Name/Signature	Title	Date	Meaning/Reason
Carissa Albert (CARISSA.ALBERT)	Associate Director of Quality	08 Aug 2023, 12:48:35 PM	Approved

Set Date

Name/Signature	Title	Date	Meaning/Reason
Meghan Skeehan (MEGHAN.SKEEHAN)	Document Control Technician II	08 Aug 2023, 01:08:47 PM	Approved