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LIMIT OF AMMONIUM IN L-HISTIDINE MONOCHLORIDE MONOHYDRATE VIA ULTRA HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC) WITH UV DETECTION

TABLE OF CONTENTS

1. PURPOSE:

1.1. To provide Laboratory Analysts and/or qualified designees with a procedure for analyzing the Limit of Ammonium in L-Histidine Monochloride Monohydrate Via Ultra High-Performance Liquid Chromatography (UPLC) with UV Detection.

2. SCOPE:

2.1. This Analytical Test Method applies to the Limit of Ammonium in L-Histidine Monochloride Monohydrate Via Ultra High-Performance Liquid Chromatography (UPLC) with UV Detection.
2.2. Limit of Ammonium Specification: ≤0.02%.
2.3. **Reaction Chemistry:** 6-AminoQuinolyl-N-HydroxySuccinimidyl Carbamate (AQC) (AccQ · Tag Ultra Reagent) converts primary and secondary Amino Acids into stable derivatives adding both UV absorbance and fluorescent character. Any excess AQC hydrolyzes to produce 6-AminoQuinoline (AMQ), N-Hydroxy Succinimide (NHS), and Carbon Dioxide.

3. RESPONSIBILITIES:

3.1. The Laboratory Technology Manager is responsible for the control, training, implementation, and maintenance of this procedure.
3.2. The Laboratory Analysts and/or qualified designees are responsible for performing the testing stated in this procedure.
3.3. Safety: Standard laboratory safety regulations apply. Before working with any chemical, read and understand the Safety Data Sheet (SDS).

4. REFERENCE:

4.1. BSI-PRL-0820, Analytical Method Validation Protocol: Ammonium Analysis in Amino Acids Via Ultra High-Performance Liquid Chromatography (UPLC) with UV Detection
4.2. BSI-RPT-1906, Analytical Method Validation Report: Ammonium Analysis in L-Histidine Monochloride Monohydrate Via Ultra High-Performance Liquid Chromatography (UPLC) with UV Detection
4.3. BSI-SOP-0098, Balance SOP
4.4. BSI-SOP-0126, Laboratory Notebooks SOP
4.5. BSI-SOP-0134, Pipette SOP
4.6. Waters 2695 Separations Module Operator's Guide
4.7. Waters ACQUITY UPLC H-Class and H-Class Bio Amino Acid Analysis System Guide
4.8. Waters ACQUITY UPLC H-Class Quaternary Solvent Manager Operator's Overview and Maintenance Information
4.9. Waters ACQUITY UPLC H-Class Sample Manager – Flow Through Needle Operator's Overview and Maintenance Information
4.10. Waters *ACQUITY UPLC TUV Detector Operator's Overview and Maintenance Guide*

5. MATERIALS AND EQUIPMENT:

5.1. All materials and equipment utilized in this analysis are outlined in this section.
5.2. Equipment and Instrumentation
  5.2.1. Analytical / Micro Balance
  5.2.2. Calibrated Micropipettes
  5.2.3. Waters ACQUITY H-Class UPLC With TUV Detector
  5.2.4. LC Column
    5.2.4.1. Waters AccQ · Tag Ultra C18 Column
      5.2.4.1.1. Dimensions: 2.1mm x 100mm, 1.7µm ID
      5.2.4.1.2. Part Number: 186003837
5.3. Reagents
  5.3.1. **AccQ · Tag Ultra Borate Buffer:** Purchased Commercially.
  5.3.2. **AccQ · Tag Ultra Eluent A:** Purchased Commercially.
  5.3.3. **AccQ · Tag Ultra Eluent B:** Purchased Commercially.
  5.3.4. **AccQ · Tag Ultra Reagent Diluent:** Purchased Commercially.
  5.3.5. **AccQ · Tag Ultra Reagent Powder (6-AminoQuinolyl-N-HydroxySuccinimidyl Carbamate (AQC)):** Purchased Commercially.
  5.3.6. **Acetonitrile:** Purchased Commercially.
  5.3.7. **Ammonium Chloride:** Purchased Commercially.
  5.3.8. **Isopropanol:** Purchased Commercially.
  5.3.9. **L-Histidine Monochloride Monohydrate:** Purchased Commercially.
  5.3.10. **Methanol:** Purchased Commercially.
  5.3.11. **Phosphoric Acid:** Purchased Commercially.
  5.3.12. **Purified Water (HPLC Grade):** In-House or Purchased Commercially.
  5.3.13. **Water Amino Acid Hydrolysate Standard:** Purchased Commercially.
5.4. Supplies
  5.4.1. Class A Volumetric Flasks
  5.4.2. LCGC Certified Clear Glass, 12x32mm, Screw Neck Vial, Total Recovery with Cap and PTFE / Silicone Septum, 1mL volume
  5.4.3. Micropipette Tips
  5.4.4. Transfer pipettes
  5.4.5. Waters Low-Flow Tubing, 0.0025 ID, 10.5 inches
  5.4.6. Weigh Boats/ Papers/ Funnels or equivalent
5.5. Reference Standards
  5.5.1. Waters Amino Acid Hydrolysate Standard

6. UPLC PRE-ANALYSIS CLEANING PROCEDURE:

6.1. **Note:** UPLC Pre-Analysis Cleaning will be performed on an as needed basis.

6.2. **Cleaning Solution Preparation**

6.2.1. 50% Methanol: 50% Purified Water:

6.2.1.1. Combine 500 mL of Purified Water and 500 mL of HPLC Grade Methanol.

6.2.1.2. Mix thoroughly and allow to equilibrate to room temperature.

6.2.1.3. Transfer 1 mL to an autosampler vial and the rest to a 1 L Mobile Phase Bottle.

6.2.2. 30% Phosphoric Acid: 70% Purified Water:

6.2.2.1. Combine 700 mL of Purified Water and 300 mL of HPLC Grade Phosphoric Acid.

6.2.2.2. Mix thoroughly and allow to equilibrate to room temperature.

6.2.2.3. Transfer 1 mL to an autosampler vial and the rest to a 1 L Mobile Phase Bottle.

6.2.3. 100% Purified Water:

6.2.3.1. Place 1 mL of Purified Water in an autosampler vial and fill a 1 L Mobile Phase Bottle with Purified Water.

6.2.4. 100% Isopropanol:

6.2.4.1. Place 1 mL of HPLC Grade Isopropanol in an autosampler vial and fill a 1 L Mobile Phase Bottle with HPLC Grade Isopropanol.

6.3. **Cleaning Procedure**

6.3.1. Place all lines into the appropriate cleaning solution.

6.3.2. Prime each Solvent Line for 5 minutes.

6.3.3. Prime the Seal Wash for 1 minute.

6.3.4. Prime the Purge for 50 cycles.

6.3.5. Connect a flow restrictor to the outlet of the active preheater assembly in the column heater.

6.3.6. Connect a waste line from the outlet of the flow restrictor to a suitable waste container.

6.3.7. Transfer an autosampler vial containing the appropriate cleaning solution to the autosampler.

6.3.8. Create an instrument method incorporating the following parameters:

6.3.8.1. Flow Rate: 0.5 mL/min

6.3.8.2. Gradient Composition: 25%A, 25%B, 25%C, 25%D

6.3.9. Set the run time to 0.5 minutes and make 10 injections from the sample vial.

6.3.10. Repeat Section 6.3.1. to Section 6.3.9. with the following solvents in the order specified:

6.3.10.1. 50% Methanol: 50% Purified Water

6.3.10.2. 100% Isopropanol

6.3.10.3. 100% Purified Water

6.3.10.4. 30% Phosphoric Acid: 70% Purified Water

6.3.10.4.1. **Note:** Remove Solvent Reservoir Filters prior to placing lines in Phosphoric Acid to avoid damage.

6.3.10.5. 100% Purified Water

6.3.11. Reinsert the waste line into the original waste container, reattach the solvent line to the detector, replace the solvent reservoir filters on all lines, and place the seal wash into 100% Purified Water.

6.3.12. Repeat Section 6.3.1. to Section 6.3.4. and Section 6.3.7. to Section 6.3.9. using 50% Methanol: 50% Purified Water.

6.3.13. Remove the flow restrictor from the active preheater assembly on the column heater.

7. TESTING PROCEDURE:

7.1. Solution Preparation

7.1.1. **Note:** All solutions may be scaled as needed.

7.1.2. Solvent A: AccQ · Tag Ultra Eluent A.

7.1.2.1. After opening, solution stable for 3 days at room temperature or 30 days tightly capped in original bottle at 4°C.

7.1.3. Solvent B (90% Purified Water: 10% AccQ · Tag Ultra Eluent B):

7.1.3.1. Combine 100 mL of AccQ · Tag Ultra Eluent B and 900 mL of Purified Water.

7.1.3.2. Mix thoroughly and allow to equilibrate to room temperature.

7.1.3.3. Solution stable for 3 days at room temperature.

7.1.4. Solvent C: Purified Water.

7.1.4.1. Purified water stable for 3 days at room temperature.

7.1.5. Solvent D: AccQ · Tag Ultra Eluent B.

7.1.5.1. After opening, solution stable for 3 days at room temperature or 30 days tightly capped in original bottle at 4°C.

7.1.6. Needle / Seal / Purge Wash (50% Purified Water: 50% Acetonitrile):

7.1.6.1. Combine 500 mL of Acetonitrile and 500 mL of Purified Water.

7.1.6.2. Mix thoroughly and allow to equilibrate to room temperature.

7.1.7. Reconstituted AccQ · Tag Ultra Reagent Powder:

7.1.7.1. **Note:** Solution may be stored in a desiccator at room temperature for 1 week.

7.1.7.2. Tap the AccQ Tag Ultra Reagent Powder vial to ensure all reagent powder is at the bottom of the container.

7.1.7.3. Pipette 1.0 mL of AccQ · Tag Ultra Reagent Diluent into the AccQ · Tag Ultra Reagent Powder vial.

7.1.7.3.1. Rinse the pipette tip three (3) times with AccQ · Tag Ultra Reagent Diluent before use. Discard each rinse.

7.1.7.4. Cap the vial tightly and vortex until dissolved.

7.1.8. Derivatization Blank:

7.1.8.1. In a total recovery LC vial, add 80 µL of AccQ · Tag Ultra Borate Buffer and 20 µL of Reconstituted AccQ · Tag Ultra Reagent Powder.

7.1.8.2. Vortex immediately and allow to sit at room temperature for 1 minute.

7.1.9. Suitability Standard (250 pmol/µL Amino Acids; 125 pmol/µL Cysteine):

7.1.9.1. **Note:** Solution may be stored at room temperature for 1 week.

7.1.9.2. Allow the Waters Amino Acid Hydrolysate Standard to thaw completely before use.

7.1.9.3. In a total recovery LC vial, mix 100 µL of Waters Amino Acid Hydrolysate Standard with 900 µL of Purified Water, and mix well.

7.1.9.4. In another total recovery LC vial, add 70 µL of AccQ · Tag Ultra Borate Buffer, 10 µL of the diluted Waters Amino Acid Hydrolysate Standard, and 20 µL of Reconstituted AccQ · Tag Ultra Reagent Powder.

7.1.9.5. Vortex immediately and allow to sit at room temperature for 1 minute.

7.1.10. L-Histidine Monochloride Monohydrate Standard Solution (5000 ppm L-Histidine Monochloride Monohydrate Standard):

7.1.10.1. Weigh out 500 mg of L-Histidine Monochloride Monohydrate Standard, transfer to a 100 mL volumetric flask, dissolve in Purified Water, fill to volume with Purified Water, and mix well.

7.1.10.2. In a total recovery LC vial, add 70 µL of AccQ · Tag Ultra Borate Buffer, 10 µL of *L-Histidine Monochloride Monohydrate Standard Solution*, and 20 µL of Reconstituted AccQ · Tag Ultra Reagent Powder.

7.1.10.3. Vortex immediately and allow to sit at room temperature for 1 minute.

7.1.11. Ammonium Stock Solution (200 ppm Ammonium):

7.1.11.1. Weigh out the amount specified in the calculation below of Ammonium Chloride and transfer to a 100 mL volumetric flask, dissolve in Purified Water, fill to volume with Purified Water, and mix well.

$$Amount\ of\ Ammonium\ Chloride\ (g) = 0.0593g\ \times Ammonium\ Chloride\ CoA\ Purity\ \left(\frac{mg}{mg}\right)$$

7.1.12. Ammonium Standard Solution (5000 ppm L-Histidine Monochloride Monohydrate Standard; 1.0 ppm Ammonium):

7.1.12.1. Weigh out 500 mg of L-Histidine Monochloride Monohydrate Standard. Transfer to a 100 mL volumetric flask, pipette 0.50 mL of *Ammonium Stock Solution*, dissolve in Purified Water, fill to volume with Purified Water, and mix well.

7.1.12.2. In a total recovery LC vial, add 70 µL of AccQ · Tag Ultra Borate Buffer, 10 µL of *Ammonium Standard Solution*, and 20µL of Reconstituted AccQ · Tag Ultra Reagent Powder.

7.1.12.3. Vortex immediately and allow to sit at room temperature for 1 minute.

7.1.12.4. Calculation for Ammonia in Standard Amounts Table. Refer to Figure 7 in section 8.6.

$$\boldsymbol{Ammonium\ (\mu g) = ammonium\ chloride\ weight\ (mg) x \frac{18.04}{53.491}\ x\ 1000}$$

7.1.13. Sample Test Solution (5000 ppm L-Histidine Monochloride Monohydrate Sample):

7.1.13.1. Weigh out 500 mg of L-Histidine Monochloride Monohydrate Sample, transfer to a 100 mL volumetric flask, dissolve in Purified Water, fill to volume with Purified Water, and mix well.

7.1.13.2. In a total recovery LC vial, add 70 µL of AccQ · Tag Ultra Borate Buffer, 10 µL of *Sample Test Solution*, and 20 µL of Reconstituted AccQ · Tag Ultra Reagent Powder.

7.1.13.3. Vortex immediately and allow to sit at room temperature for 1 minute.

7.2. Instrument Setup

7.2.1. Waters ACQUITY H-Class UPLC Method Parameters:

TABLE 1: METHOD PARAMETERS

Parameter	Setting
Flow Type	Gradient
Solvent A	AccQ · Tag Ultra Eluent A
Solvent B	90% Purified Water: 10% AccQ · Tag Ultra Eluent B
Solvent C	Purified Water
Solvent D	AccQ · Tag Ultra Eluent B
Needle / Seal / Purge Wash	50% Purified Water: 50% Acetonitrile
Flow Rate	0.7 mL/min
Injection Volume	1.0 µL
Detector	TUV Detector – 260 nm
Detector Sampling Rate	10 Points/sec
Detector Sensitivity	2.00 AUFS
Column Temperature	43 °C
Sample Temperature	20 °C
Run Time	11 minutes

7.2.2. Gradient

TABLE 2: GRADIENT

Step	Time (min)	%A	%B	%C	%D	Curve
1	0.00	10.0	0.0	90.0	0.0	Not Applicable
2	0.29	9.9	0.0	90.1	0.0	11
3	5.49	9.0	80.0	11.0	0.0	7
4	7.10	8.0	15.6	57.9	18.5	6
5	7.30	8.0	15.6	57.9	18.5	6
6	7.69	7.8	0.0	70.9	21.3	6
7	7.99	4.0	0.0	36.3	59.7	6
8	8.59	4.0	0.0	36.3	59.7	6
9	8.68	10.0	0.0	90.0	0.0	6
10	10.20	10.0	0.0	90.0	0.0	6

7.2.3. Injection Sequence:

TABLE 3: INJECTION SEQUENCE	
System Suitability Injections	
Gradient Blank	1
Derivatization Blank	1
Suitability Standard	1
L-Histidine Monochloride Monohydrate Standard Solution	1
Ammonium Standard Solution	3
Sample Injections[1]	
Derivatization Blank	1
Sample Test Solution[2]	≤6
Ammonium Standard Solution	1
[1]Repeat the sample injection sequence if additional samples are to be analyzed. [2]Samples may be substituted with Gradient Blank injections.	

7.2.4. System Suitability:

TABLE 4: SYSTEM SUITABILITY	
System Suitability Parameter	**Acceptance Criteria**
Derivatization Blank: The first injection of the Derivatization Blank shows the AMQ peak and the Derivatization peak.	Appropriate Peaks Present
Suitability Standard: The Suitability Standard shows all appropriate peaks (Reference Suitability Standard Table).	Appropriate Peaks Present
Resolution: The resolution between the Ammonia and Histidine peaks in the Suitability Standard.	NLT 1.5
Instrument Precision: The %RSD of the Ammonia peak in the first three (3) *Ammonium Standard Solution* injections.	NMT 20%
Instrument Precision (QC Check): The %RSD of the Ammonia peak in all *Ammonium Standard Solution* injections.	NMT 20%

7.2.5. Suitability Standard:

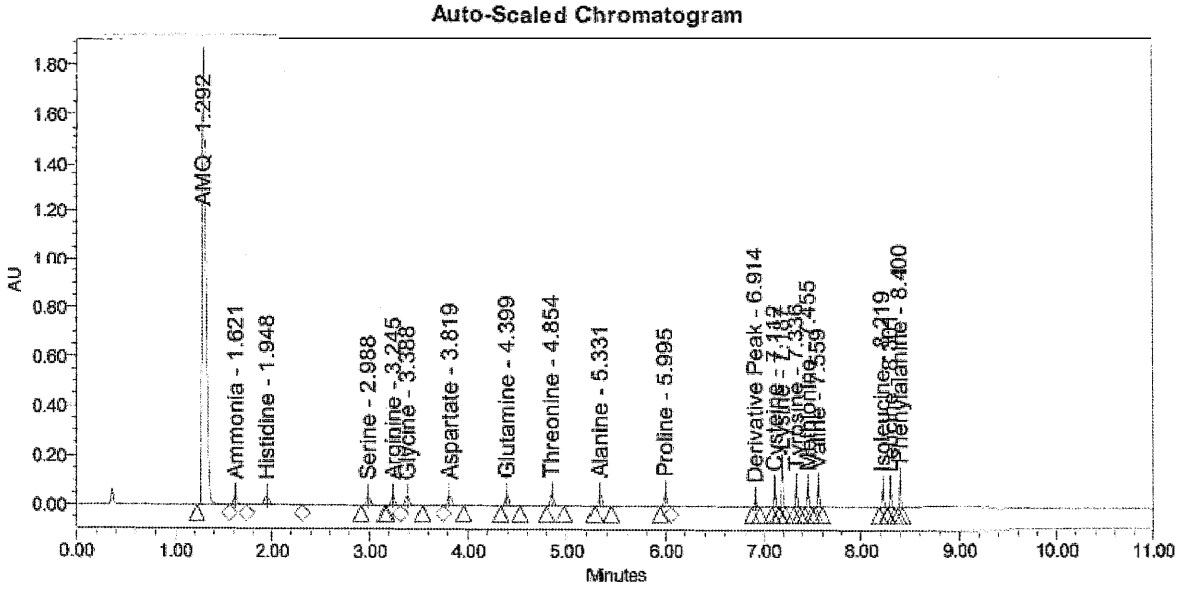


FIGURE 1: AUTO-SCALED CHROMATOGRAM

TABLE 5: SUITABILITY STANDARD

Analyte	Approximate Retention Time (min)
AMQ	1.3
Ammonia	1.6
Histidine	1.9
Serine	3.0
Arginine	3.2
Glycine	3.4
Aspartate	3.8
Glutamine	4.4
Threonine	4.9
Alanine	5.3
Proline	6.0
Derivative Peak	6.9
Cysteine	7.1
Lysine	7.2
Tyrosine	7.3
Methionine	7.5
Valine	7.6
Isoleucine	8.2
Leucine	8.3
Phenylalanine	8.4

7.3. **Acceptance Criteria:**

7.3.1. The area of the Ammonia peak in the *Sample Test Solution* is not greater than the area of the Ammonia peak in the *Ammonium Standard Solution* when corrected for the *L-Histidine Monochloride Monohydrate Standard Solution*.

7.3.2. If the area for Ammonia peak in the *Sample Test Solution* is greater than or equal to the Ammonia peak in the *Ammonium Standard Solution*, no results are to be reported until evaluated by Laboratory Management to determine if the result is valid/reportable or if any further action is required.

7.4. **Result Reporting**

TABLE 6: RESULT REPORTING

Result	Reporting
If Ammonia peak area in *Sample Test Solution* < Corrected Ammonia peak area in *Ammonium Standard Solution*	Report < 0.02%
If Ammonia peak area in *Sample Test Solution* = Corrected Ammonia peak area in *Ammonium Standard Solution*	Report as 0.02%
If Ammonia peak area in *Sample Test Solution* > Corrected Ammonia peak area in *Ammonium Standard Solution*	Report > 0.02%

8. CHROMATOGRAMS AND DATA PROCESSING:

8.1. Gradient Blank

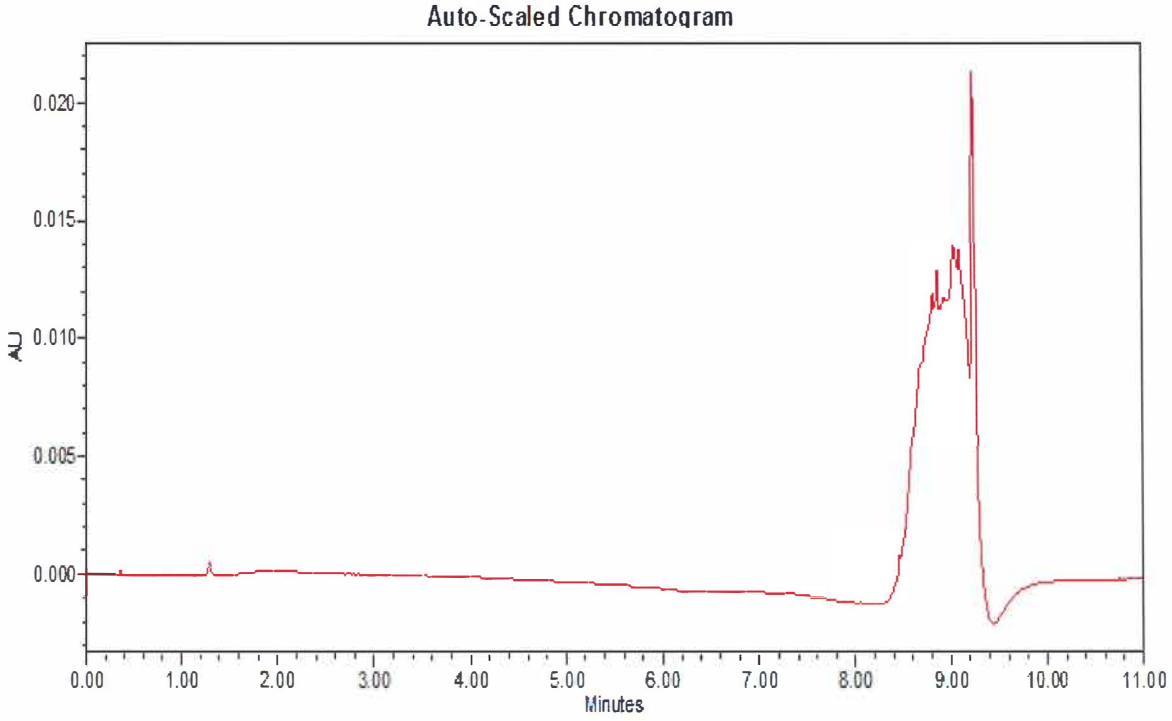


FIGURE 2: AUTO-SCALED CHROMATOGRAM

8.2. Derivatization Blank

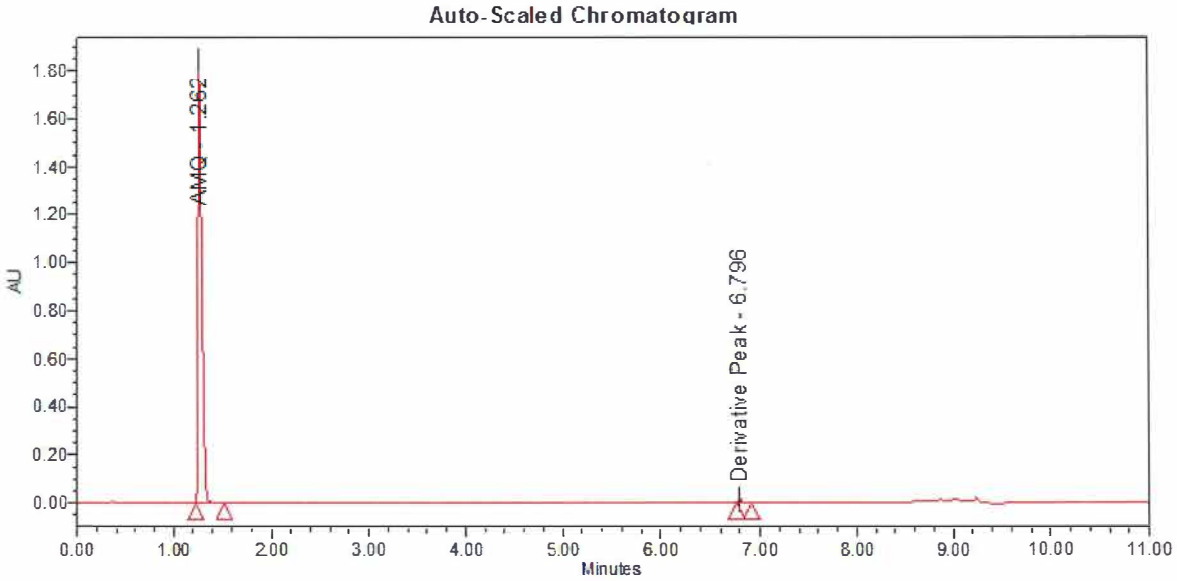


FIGURE 3: AUTO-SCALED CHROMATOGRAM

8.3. Suitability Standard

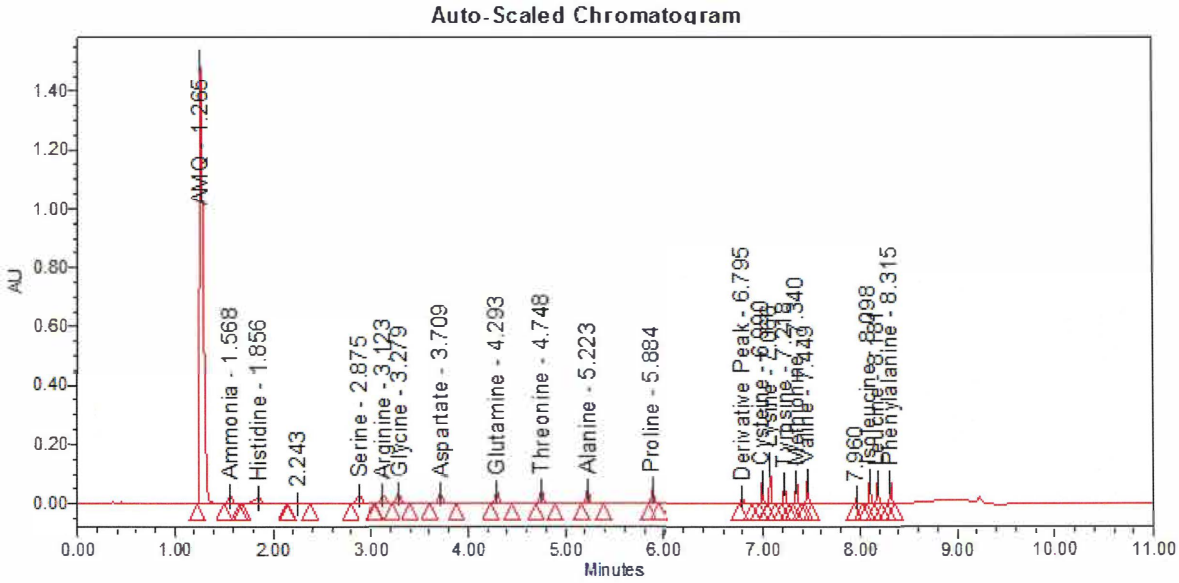


FIGURE 4: AUTO-SCALED CHROMATOGRAM

8.4. Ammonium Standard Solution

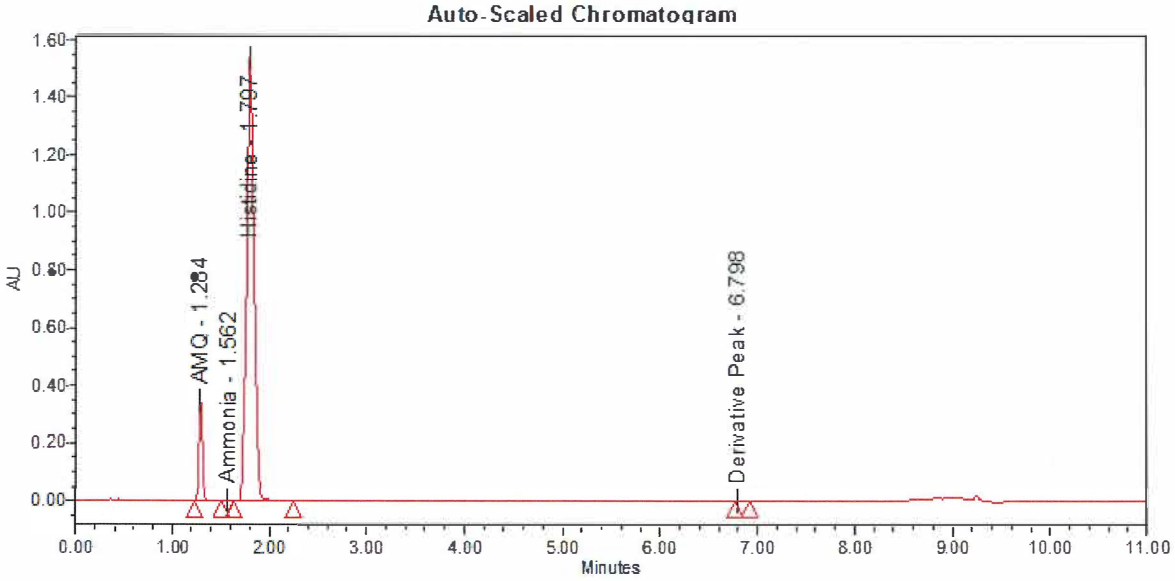


FIGURE 5: AUTO-SCALED CHROMATOGRAM

8.5. Example Sequence Table

122024 KJK Ammonium Meethod Va in 2024\Amino Acid Analysis\Method Validation as kkearns/RD_Chemist - Alter Sample Set

File Edit View Help

Apply Table Preferences | Alter Sample

	Plate/Well	Inj Vol (uL)	# of Inj	Label	SampleName	Sample Type	Level	Function	Method Set / Report or Export Method	Label Reference	Processing	RI Sensitivity	Solvent	SampleWeight	Dilution	Altered	Blank	Peak Ratio Reference	Sample Matrix	Column Name	Column Serial Number
1								Condition Column	StartUp_Hyd_TUV_HClass1011							☐					
2								Condition Column	Ammonia_Analysis_MS							☐					
3								Condition Column	Ammonia_Analysis_MS							☐					
4								Condition Column	Ammonia_Analysis_MS							☐					
5	1:A,1	0.0	1	G	Gradient Blank	Unknown		Inject Samples	Ammonia_Analysis_MS		Don't Process or Report			1.0000	1.0000	☑	☑	☐			
6	1:A,1	1.0	1	D	Derivitization Blank	Unknown		Inject Samples	Ammonia_Analysis_MS		Don't Process or Report			1.0000	1.0000	☑	☐	☐			
7	1:A,2	1.0	1	R	Suitability Standard	Unknown		Inject Samples	Ammonia_Analysis_MS		Don't Process or Report			1.0000	1.0000	☑	☐	☐			
8	1:A,3	1.0	3	S	Ammonium Standard Solution	Standard		Inject Standards	Ammonia_Analysis_MS		Don't Process or Report			1.0000	20000.0000	☑	☐	☐			
9	1:A,1	10	1	D	Derivitization Blank	Unknown		Inject Samples	Ammonia_Analysis_MS		Don't Process or Report			1.0000	1.0000	☑	☐	☐			
10	1:A,4	1.0	1	A1	L-Histidine HCl H2O 0% Level	Unknown		Inject Samples	Ammonia_Analysis_MS		Don't Process or Report			500600.0000	10000.0000	☑	☐	☐			
11	1A5	1.0	1	A2	L-Histidine HCl H2O 50% Rep #1	Unknown		Inject Samples	Ammonia_Analysis_MS		Don't Process or Report			500600.0000	10000.0000	☑	☐	☐			
12	1:A,6	1.0	1	A2	L-Histidine HCl H2O 50% Rep. #2	Unknown		Inject Samples	Ammonia_Analysis_MS		Don't Process or Report			500500.0000	10000.0000	☑	☐	☐			
13	1:A,7	1.0	1	A2	L-Histidine HCl H2O 50% Rep. #3	Unknown		Inject Samples	Ammonia_Analysis_MS		Don't Process or Report			500400.0000	10000.0000	☑	☐	☐			
14	1:A,8	1.0	1	A3	L-Histidine HCl H2O 150% Rep 1	Unknown		Inject Samples	Ammonia_Analysis_MS		Don't Process or Report			500900.0000	10000.0000	☑	☐	☐			
15	1:B,1	1.0	1	A3	L-Histidine HCl H2O 150% Rep 2	Unknown		Inject Samples	Ammonia_Analysis_MS		Don't Process or Report			500200.0000	10000.0000	☑	☐	☐			
16	1:A,3	1.0	1	Q1	Ammonium Standard Solution (QC1)	Standard		Inject Standards	Ammonia_Analysis_MS		Don't Process or Report			1.0000	20000.0000	☑	☐	☐			
17	1:A,1	1.0	1	D	Derivitization Blank	Unknown		Inject Samples	Ammonia_Analysis_MS		Don't Process or Report			1.0000	1.0000	☑	☐	☐			
18	1:B,2	1.0	1	A3	L-Histidine HCl H2O 150% Rep 3	Unknown		Inject Samples	Ammonia_Analysis_MS		Don't Process or Report			5007000000	10000000000	☑	☐	☐			
19	1:B,3	1.0	1	A4	L-Histidine HCl H2O 200% Rep 1	Unknown		Inject Samples	Ammonia_Analysis_MS		Don't Process or Report			500000.0000	10000.0000	☑	☐	☐			
20	1:B,4	1.0	1	A4	L-Histidine HCl H2O 200% Rep 2	Unknown		Inject Samples	Ammonia_Analysis_MS		Don't Process or Report			500400.0000	10000.0000	☑	☐	☐			
21	1:B,5	1.0	1	A4	L-Histidine HCl H2O 200% Rep 3	Unknown		Inject Samples	Ammonia_Analysis_MS		Don't Process or Report			500400.0000	10000.0000	☑	☐	☐			
22	1:A,3	1.0	1	Q2	Ammonium Standard Solution (QC2)	Standard		Inject Standards	Ammonia_Analysis_MS		Don't Process or Report			1.0000	20000.0000	☑	☐	☐			
23	1:A,1	1.0	1	D	Derivitization Blank	Unknown		Inject Samples	Ammonia_Analysis_MS		Don't Process or Report			1.0000	1.0000	☑	☐	☐			
24	1:A,5	1.0	6	L	L-Histidine HCl H2O 50% #1 LOB	Unknown		Inject Samples	Ammonia_Analysis_MS		Don't Process or Report			500600.0000	10000.0000	☑	☐	☐			
25	1:A,3	1.0	1	Q3	Ammonium Standard Solution (QC3)	Standard		Inject Standards	Ammonia_Analysis_MS		Don't Process or Report			1.0000	20000.0000	☑	☐	☐			
26								Clear Calibration	Ammonia_Analysis_MS		Normal					☐					
27								Calibrate	Ammonia_Analysis_MS	S	Normal					☐					
28								Clear Calibration	Ammonia_Analysis_MS		Normal					☐					
29								Calibrate	Ammonia_Analysis_MS	S Q*	Normal					☐					
30								Quantitate	Ammonia_Analysis_MS	G D R A* L	Normal					☐					

FIGURE 6: EXAMPLE SEQUENCE TABLE

8.6. Example Amount Window

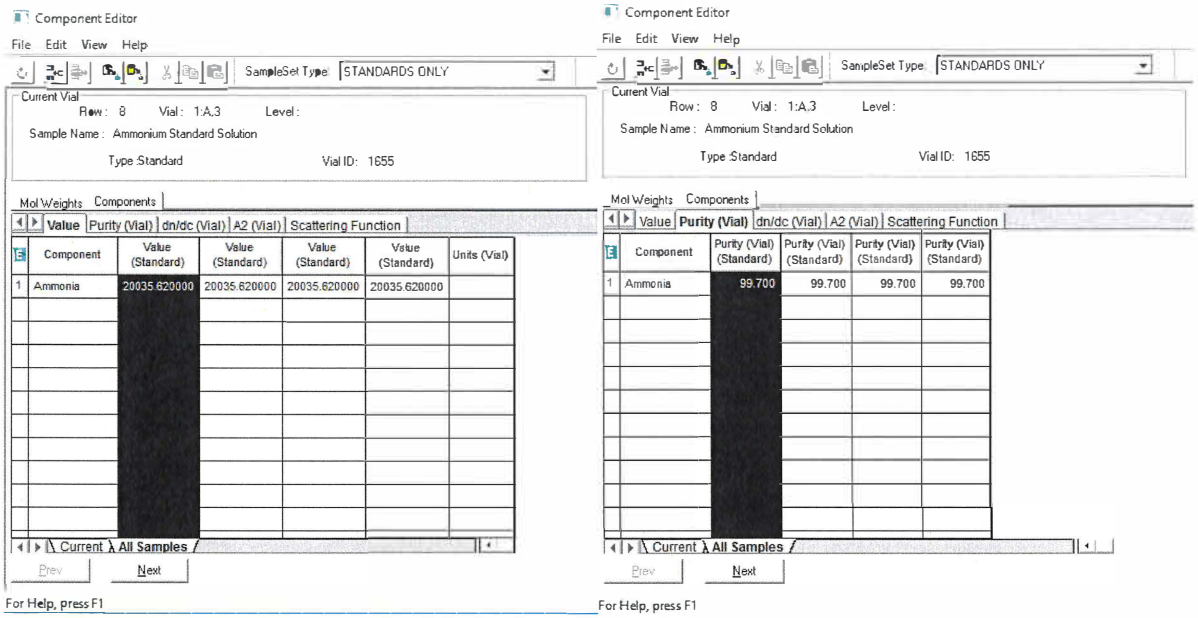
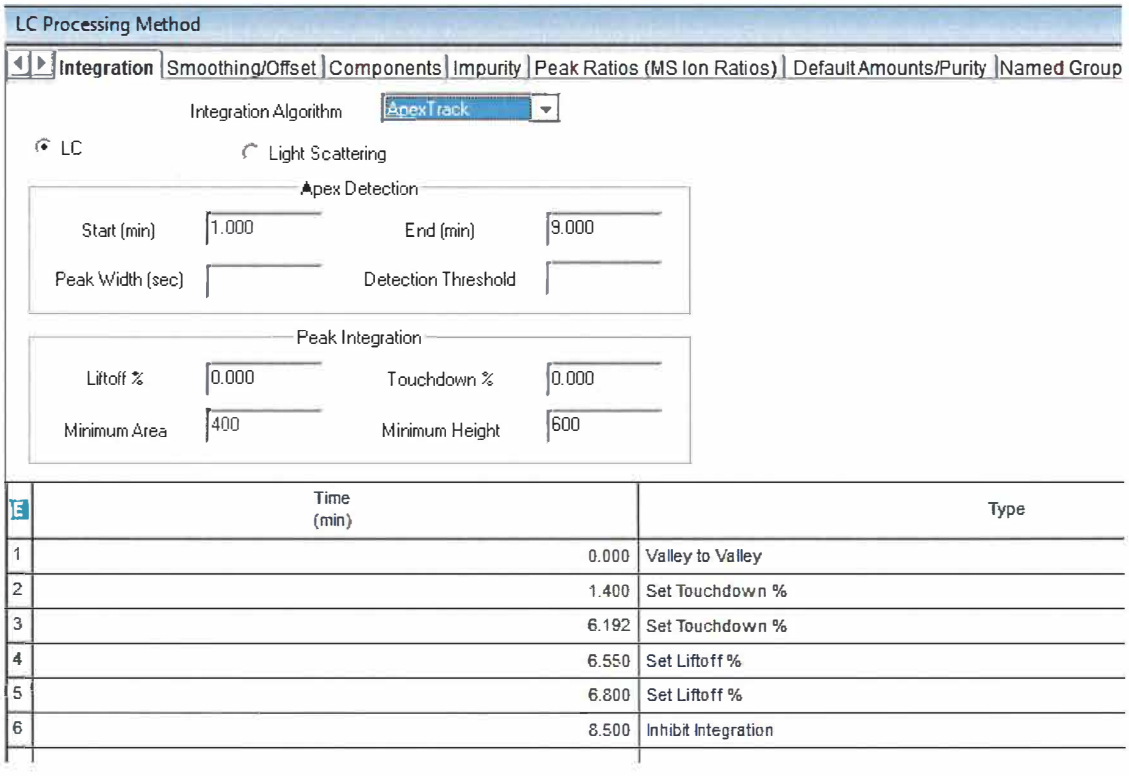


FIGURE 7: EXAMPLE AMOUNT WINDOW

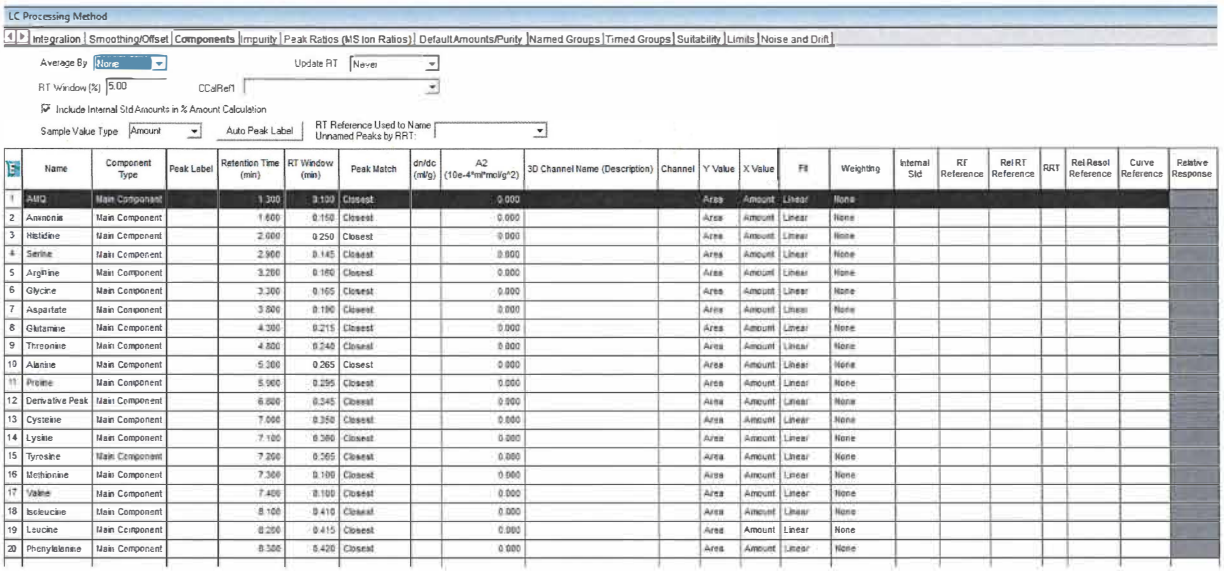
8.7. Example Processing Method Integration Tab



	Time (min)	Type
1	0.000	Valley to Valley
2	1.400	Set Touchdown %
3	6.192	Set Touchdown %
4	6.550	Set Liftoff %
5	6.800	Set Liftoff %
6	8.500	Inhibit Integration

FIGURE 8: EXAMPLE PROCESSING METHOD INTEGRATION TAB

8.8. Processing Method Component Tab



LC Processing Method

Integration | Smoothing/Offset | Components | Impurity | Peak Ratios (MS Ion Ratios) | DefaultAmounts/Purity | Named Groups | Timed Groups | Suitability | Limits | Noise and Drift

Average By: None — Update RT: Never

RT Window (%): 5.00 — CCalRef1:

Include Internal Std Amounts in % Amount Calculation

Sample Value Type: Amount — Auto Peak Label — RT Reference Used to Name Unnamed Peaks by RRT:

	Name	Component Type	Peak Label	Retention Time (min)	RT Window (min)	Peak Match	dn/dc (ml/g)	A2 (10e-4*ml*mol/g^2)	3D Channel Name (Description)	Channel	Y Value	X Value	Fit	Weighting	Internal Std	RF Reference	Rel RT Reference	RRT	Rel Resol Reference	Curve Reference	Relative Response
1	AAIQ	Main Component		1.300	0.130	Closest		0.000			Area	Amount	Linear	None							
2	Ammonia	Main Component		1.600	0.160	Closest		0.000			Area	Amount	Linear	None							
3	Histidine	Main Component		2.600	0.250	Closest		0.000			Area	Amount	Linear	None							
4	Serine	Main Component		2.900	0.145	Closest		0.000			Area	Amount	Linear	None							
5	Arginine	Main Component		3.200	0.160	Closest		0.000			Area	Amount	Linear	None							
6	Glycine	Main Component		3.300	0.165	Closest		0.000			Area	Amount	Linear	None							
7	Aspartate	Main Component		3.800	0.190	Closest		0.000			Area	Amount	Linear	None							
8	Glutamine	Main Component		4.300	0.215	Closest		0.000			Area	Amount	Linear	None							
9	Threonine	Main Component		4.800	0.240	Closest		0.000			Area	Amount	Linear	None							
10	Alanine	Main Component		5.300	0.265	Closest		0.000			Area	Amount	Linear	None							
11	Proline	Main Component		5.900	0.295	Closest		0.000			Area	Amount	Linear	None							
12	Derivative Peak	Main Component		6.800	0.340	Closest		0.000			Area	Amount	Linear	None							
13	Cysteine	Main Component		7.000	0.350	Closest		0.000			Area	Amount	Linear	None							
14	Lysine	Main Component		7.100	0.380	Closest		0.000			Area	Amount	Linear	None							
15	Tyrosine	Main Component		7.200	0.365	Closest		0.000			Area	Amount	Linear	None							
16	Methionine	Main Component		7.300	0.100	Closest		0.000			Area	Amount	Linear	None							
17	Valine	Main Component		7.400	0.100	Closest		0.000			Area	Amount	Linear	None							
18	Isoleucine	Main Component		8.100	0.410	Closest		0.000			Area	Amount	Linear	None							
19	Leucine	Main Component		8.200	0.415	Closest		0.000			Area	Amount	Linear	None							
20	Phenylalanine	Main Component		8.300	0.420	Closest		0.000			Area	Amount	Linear	None							

FIGURE 9: PROCESSING METHOD COMPONENT TAB