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UREA 6M TESTING METHOD VIA UPLC

TABLE OF CONTENTS

1. PURPOSE:

1.1. To provide the Analytical Chemistry Specialists and Quality Control (QC) Analysts with a procedure for determining the molarity of Urea solutions via UPLC.

2. SCOPE:

2.1. Applies to 6M Urea assay/normality testing on the Waters ACQUITY UPLC.

3. RESPONSIBILITIES:

3.1. The Laboratory Technology Manager is responsible for the control, training, implementation, and maintenance of this procedure.
3.2. The Analytical Chemistry Specialists, Quality Control Analysts, and/or qualified designee are responsible for performing the testing as stated in this procedure.
3.3. The Analytical Chemistry Specialists or Analysts performing this procedure, with help and training from the Laboratory Managers, are responsible for documenting the results obtained from testing.
3.4. 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-0466, Analytical Method Verification Protocol: Urea 6M Solution Molarity
4.2. BSI-RPT-0879, Analytical Method Verification Report: Urea 6M Solution Molarity
4.3. BSI-SOP-0098, Balance SOP
4.4. BSI-SOP-0348, Waters Acquity UPLC H-Class Plus SOP
4.5. *USP <621> Chromatography*
4.6. *USP <1225> Validation of Compendial Procedures*
4.7. *USP <1226> Verification of Compendial Procedures*
4.8. *USP Urea*

5. MATERIALS AND EQUIPMENT:

5.1. All materials and equipment utilized in this Verification are outlined in this section.
  5.1.1. Analytical Balance
  5.1.2. Microbalance
  5.1.3. Waters ACQUITY UPLC
5.2. Reagents
  5.2.1. UPLC Grade Acetonitrile
  5.2.2. UPLC Grade Water
  5.2.3. Formic Acid
5.3. Supplies
  5.3.1. Micropipettes
  5.3.2. Micropipette Tips
  5.3.3. Transfer pipettes
  5.3.4. Screw Top Vials, 2mL 10 mm x 32 mm
  5.3.5. 10 mm Screw Thread Autosampler Caps
5.4. Reference Standards
  5.4.1. USP Traceable Related Compound A Reference Standard
  5.4.2. USP Traceable Urea Reference Standard
  5.4.3. Certified In-House Urea Reference Standard
5.5. UPLC Column
  5.5.1. Ascentis Express 90Å OH5 15cm x 4.6 mm. 2.7 um
  Part number: 53778-U

6. PROCEDURE:

6.1. Method

6.1.1. The method on the UPLC follows USP parameters and is set as follows:

6.1.2. Method Parameters:

TABLE 1: LC PARAMETERS	
Parameter	**Setting**
Flow Type	Gradient Elution
Mobile Phase	See 6.2.2
Flow Rate	1.0mL/min
Injection Volume	2µL
Detector	UV 195nm
Column Temperature	30 °C
Sample Temperature	10 °C
Run Time	15 min

6.1.3. Gradient:

TABLE 2: LC GRADIENT		
Time (min)	**Solution A (%)**	**Solution B (%)**
0.0	2.5	97.5
7.0	10.0	90.0
7.01	2.5	97.5
15.0	2.5	97.5

6.2. Solution preparation:

6.2.1. Diluent

6.2.1.1. Prepare a 90:10 Acetonitrile: Water (UPLC Grade).

6.2.2. Mobile phase

6.2.2.1. Mobile Phase A (0.1% Formic acid in water)

6.2.2.1.1. Add 1 mL of formic acid to 1L UPLC grade water, mix thoroughly.

6.2.2.1.2. Note: Solution may be purchased commercially.

6.2.2.2. Mobile Phase B (Acetonitrile)

6.2.2.3. Wash Solvent

6.2.2.3.1. Use diluent listed above.

6.2.3. RCA Stock Solution (0.5 mg/mL Biuret)

6.2.3.1. Weigh ~5 mg biuret into a 10mL volumetric flask.

6.2.3.2. Dissolve and bring to volume with diluent, mix thoroughly.

6.2.4. System Suitability Solution (5 mg/mL Urea, 0.01 mg/mL RCA, prepare in duplicate)
- 6.2.4.1. Weigh 125 mg urea reference standard into a 25 mL volumetric flask.
- 6.2.4.2. Pipette 500 µL of RCA stock solution into the common flask.
- 6.2.4.3. Dissolve and bring to volume with diluent, mix thoroughly.
- 6.2.4.4. Label each solution SS1 and SS2 respectively.
- 6.2.4.5. The solutions are stable for 4 days at normal lighting and laboratory conditions.
- 6.2.4.6. Note: Record time and date of preparation if intended to be used more than once.

6.2.5. Standard Solution for Urea Assay
- 6.2.5.1. Refer to system suitability solution.

6.2.6. Sample Preparation (~5 mg/mL Urea, prepare in duplicate)
- 6.2.6.1. Thoroughly mix the 6M urea solution.
- 6.2.6.2. Pipette 0.700 mL of the 6M Urea solution into a tared 50 mL volumetric flask and record the weight.
- 6.2.6.3. Dilute to volume with diluent and mix.
- 6.2.6.4. The solutions are stable for 4 days at normal lighting and laboratory conditions.

6.3. Setting up the instrument:

6.3.1. Injection Sequence

TABLE 3: EXAMPLE INJECTION SEQUENCE

System Suitability Injections	
Diluent	≥ 2
System Suitability Solution 1 (SS1)	5
System Suitability Solution 2 (SS2)	1
Sample Injections	
Diluent	1
Samples[1]	≤ 6
QC Check (SS1)	1
Repeat the *Sample Injections* sequence if more than 6 samples are analyzed.	
[1]Additional blanks may be used as samples	

6.3.2. Refer to BSI-SOP-0348 ACQUITY UPLC H-Class Plus SOP
- 6.3.2.1. A System Suitability run will precede the standard/sample run to ensure the method conditions are suitable for analysis. QC check solutions will be injected throughout the injection sequence to ensure performance is maintained throughout the analysis.
  - 6.3.2.1.1. Pre-Run:
    - 6.3.2.1.1.1. % RSD: NMT 1.0% for Urea.
    - 6.3.2.1.1.2. Resolution: The resolution between Urea RCA and Urea should be NLT 1.5.
    - 6.3.2.1.1.3. Tailing: The USP tailing for Urea should be NMT 2.0.
    - 6.3.2.1.1.4. The standard agreement between the system suitability solutions is between 98.0% and 102.0%.
  - 6.3.2.1.2. Post Run:
    - 6.3.2.1.2.1. The percent agreement between the first 5 system suitability solutions and all QC checks is between 98.0% and 102.0%.

6.4. Calculations:

6.4.1. Percent Agreement = (R_{SS2}/R_{SS1}) x (C_{SS1}/C_{SS2}) x 100

6.4.1.1. R_{SS1} = average peak response from SS1

6.4.1.2. R_{SS2} = peak response from SS2

6.4.1.3. C_{SS1} = Concentration of SS1

6.4.1.4. C_{SS2} = Concentration of SS2

6.4.2. Weight Percent of Urea in 6M Urea = (R_u/R_{SS1}) x C_s x (50/W) x 100

6.4.2.1. R_u = peak response from Sample solution

6.4.2.2. R_{SS1} = average peak response from the Standard solution

6.4.2.3. C_s = concentration of Urea in the Standard solution(mg/mL)

6.4.2.4. 50 = Dilution Factor (50 mL volumetric flask)

6.4.2.5. W = Weight of 6M Urea sample.

6.4.2.5.1. Report to two decimal places

6.4.3. Urea Molarity (M): $(WP_S/100)$ x D_s x (1000/60.056)

6.4.3.1. WP_s = Calculated Weight Percent of the Sample.

6.4.3.2. D_s = Separate measured density of the sample

6.4.3.2.1. Report to two decimal places.

6.5. Example Chromatography:

6.5.1. Diluent Injection

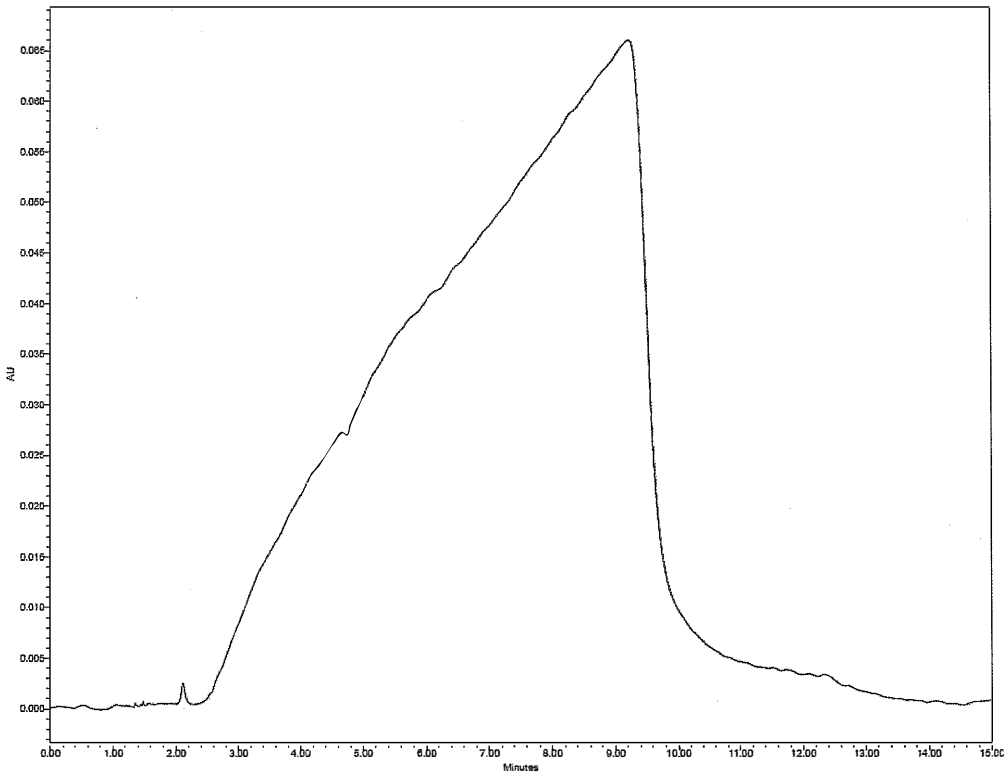

FIGURE 1: DILUENT INJECTION CHROMATOGRAPH

6.5.2. System Suitability Solution:

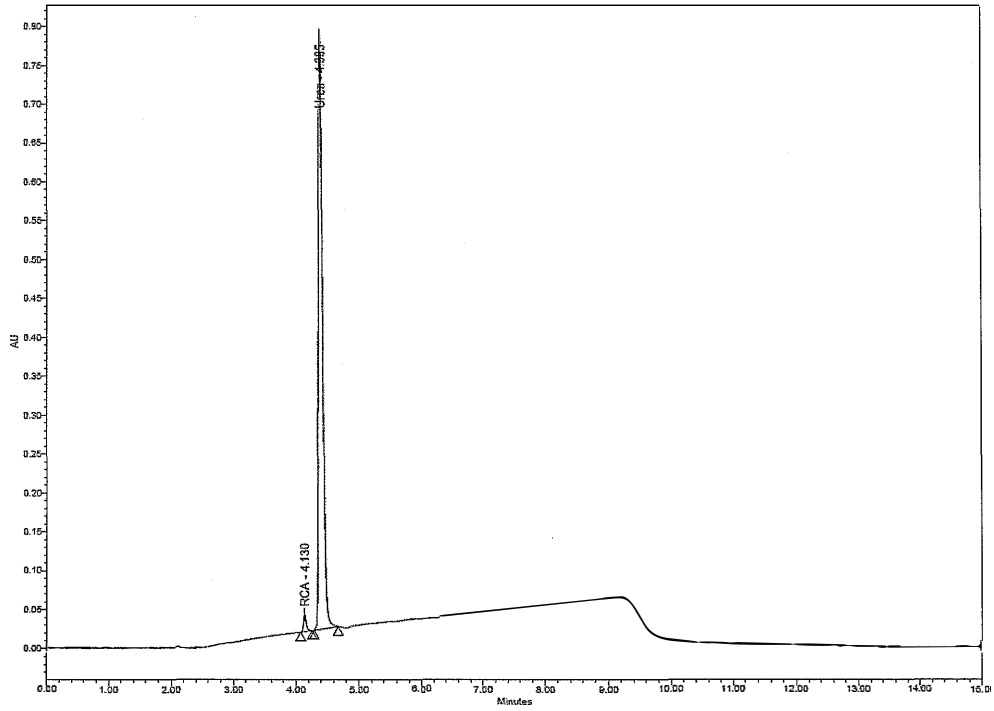

FIGURE 2: SYSTEM SUITABILITY SOLUTION CHROMATOGRAPH

6.5.3. System Suitability Solution (zoomed baseline):

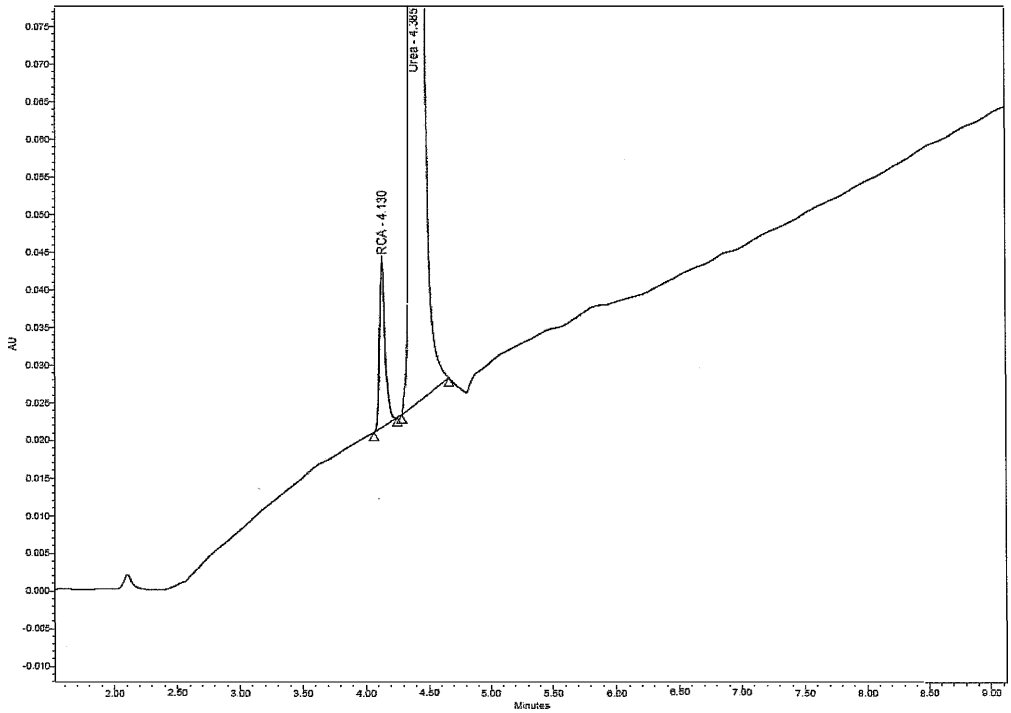

FIGURE 3: ZOOMED SYSTEM SUITABILITY CHROMATOGRAPH

6.5.4. Sample (zoomed baseline)

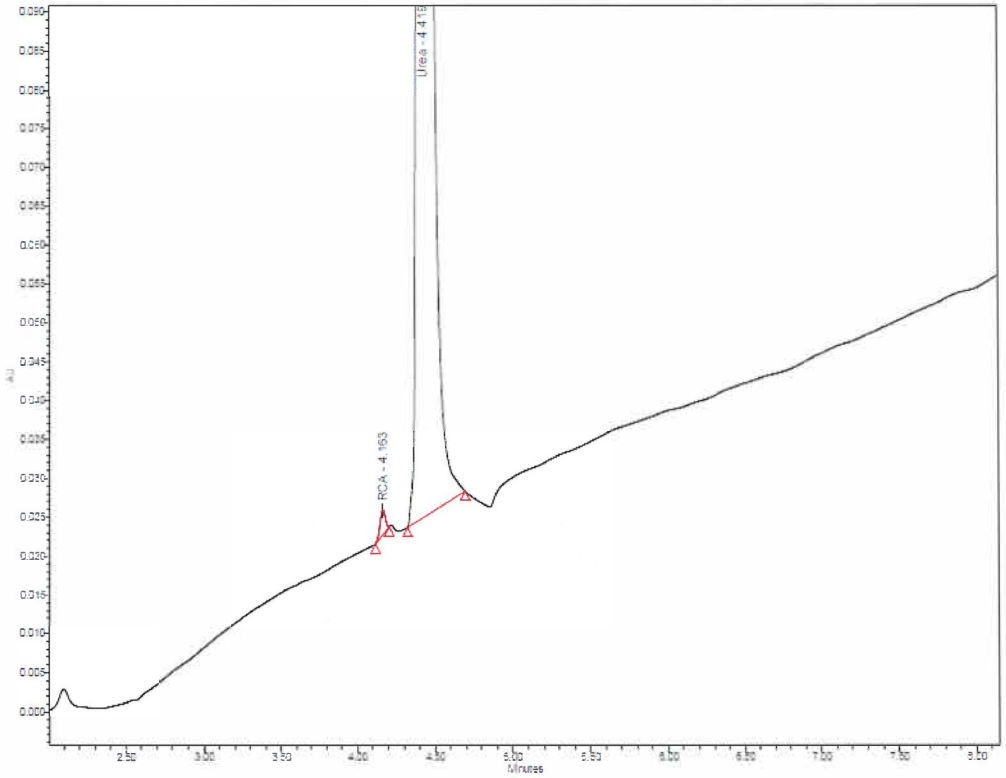

FIGURE 4: SAMPLE BASELINE CHROMATOGRAPH

6.6. Suggested Integration Parameters

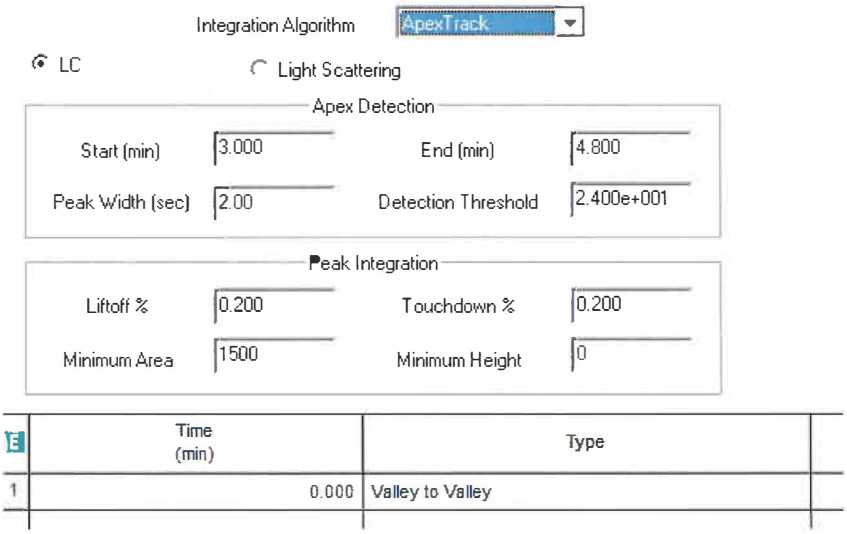

FIGURE 5: INTEGRATION PARAMETERS

6.6.1. Ensure Integrations for samples and standards are similar enough for accurate quantitation

6.6.2. Integration parameters may be adjusted in order to achieve similar integrations shown in section 6.5.