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TRIS IDENTITY AND RELATED SUBSTANCES VIA HPLC

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1. PURPOSE:

1.1. To provide the Quality Control (QC) Analysts with a procedure for Tris Identity and Related Substances determination and for operating the Waters Acquity UPLC.

2. SCOPE:

2.1. Applies to Tris Identity and Related Substances on the Waters Acquity UPLC.

3. RESPONSIBILITIES:

3.1. The Laboratory Technology Manager is responsible for the control, implementation, training, and maintenance of this procedure.
3.2. The Analytical Chemists and/or the qualified designee are responsible for complying with the requirements of this procedure.
3.3. If any abnormalities are determined during routine use of the HPLC or during calibration, the Laboratory Technology Manager shall be promptly notified. If necessary, the HPLC will be serviced and recalibrated by Waters before being approved for use.

4. REFERENCE:

4.1. BSI-PRL-0254, Analytical Method Validation Protocol: Limit of Tris(hydroxylmethyl) nitromethane
4.2. BSI-RPT-0440, Analytical Method Validation Report: Limit of Tris(hydroxylmethyl) nitromethane via HPLC
4.3. BSI-RPT-1756, Analytical Method Transfer Report: TRIS identity and Related Substances via HPLC with UV Detection
4.4. BSI-SOP-0098, Balance SOP
4.5. BSI-SOP-0422, Empower 3 General Procedure
4.6. *ACQUITY UPLC Quaternary Solvent Manager PLUS Series* USP *<621> Chromatography*
4.7. *ACQUITY UPLC TUV Detector Operator's Overview and Maintenance Guide*

5. MATERIALS AND EQUIPMENT:

5.1. Instrumentation
- 5.1.1. Analytical Balance
  - 5.1.1.1. Secura 124-1S or equivalent
- 5.1.2. HPLC
  - 5.1.2.1. Waters Acquity HPLC with a UV Detector Capability of 210 nm wavelength selectivity, or equivalent.
- 5.1.3. HPLC Column
  - 5.1.3.1. Agilent ZORBAX Carbohydrate 5µm, 4.6mm ID x 150mm, Part number: 843300-908, or equivalent.
- 5.1.4. Class A Volumetric Glassware
  - 5.1.4.1. Flasks, various sizes.
  - 5.1.4.2. Pipettes, various sizes.
- 5.1.5. Reagents
  - 5.1.5.1. HPLC Grade Acetonitrile
  - 5.1.5.2. HPLC Grade Purified Water or equivalent
- 5.1.6. Supplies
  - 5.1.6.1. Submicron Filters
  - 5.1.6.2. Syringes
- 5.1.7. Reference Standards
  - 5.1.7.1. TRIS Reference Standard
  - 5.1.7.2. Tris (hydroxymethyl) nitromethane, EP related Tris Compound CAS 126-11-4.

6. PROCEDURE:

6.1. Solution Preparations:

6.1.1. **Note - Possible sources of error in this analysis include but are not limited to: glassware/glove/weigh boat contamination, crystals on the analytical balance, improper transfer of sample and incorrect transfer of sample to vials. To avoid these errors; inspect weigh boats for contaminants prior to use, gloves should be checked for crystals after weighing samples, and clean the balance with an anti-static brush before each preparation.**

6.1.2. All solutions are to be thoroughly mixed after being prepared.

6.1.3. Mobile Phase: 75:25, (v:v), Acetonitrile: Water

6.1.3.1. Mix 750 mL of HPLC grade acetonitrile and 250 mL of HPLC grade purified water.

6.1.4. Tris (hydroxymethyl) nitromethane Stock Standard (600 mg/L)

6.1.4.1. Weigh 150 mg of Tris(hydroxymethyl)nitromethane standard to a 250 mL volumetric flask, dissolve and q.s. to volume with mobile phase.

6.1.5. Tris (hydroxymethyl) nitromethane Intermediate Standard (60 mg/L)

6.1.5.1. Dilute 10.0 mL of Tris (hydroxymethyl) nitromethane stock standard to a 100 mL volumetric flask, dissolve, and q.s. to a volume with mobile phase.

6.1.6. Tris (hydroxymethyl) nitromethane Working Standard (0.6 mg/L)

6.1.6.1. Dilute 1.0 mL of Tris (hydroxymethyl) nitromethane intermediate standard to a 100 mL volumetric flask, dissolve, and q.s. to volume with mobile phase.

6.1.7. Tris (tromethamine) Standard solution (2000 mg/L)

6.1.7.1. Weigh 200 mg of TRIS Reference Standard.

6.1.7.2. Transfer to a 100 mL volumetric flask and dissolve then q.s. to a volume with mobile phase.

6.1.8. Tris (tromethamine) sample solution (2000 mg/L)

6.1.8.1. Weigh 200 mg of Sample.

6.1.8.2. Transfer to a 100 mL volumetric flask and dissolve then q.s. to a volume with mobile phase.

6.1.8.3. **Prepare sample solution immediately before injection.**

6.2. Instrument Setup:

TABLE 1. INSTRUMENT SETUP

Parameter	Setting
Flow Type	Isocratic
Mobile Phase A	75:25, Acetonitrile: Water
Needle Wash	75:25, Acetonitrile: Water
Flow Rate	1.0 mL/min
Run Time	9 min
Injection Volume	20 µL
Column Temperature (°C)	30 °C
Sample Temperature (°C)	20 °C
Detector Settings	
Detector	UV-Vis
Wavelength	210 nm
Sampling Rate	5

6.3. Example Injection Sequence:

TABLE 2. EXAMPLE INJECTION SEQUENCE

Sample ID	Number of Injections
System Suitability	
Diluent (Mobile Phase)	≥1
Working Standard Solution	6
Diluent (Mobile Phase)	3
Standard Solution	1
Sample Solution	≤ 6
Working Standard Solution (QC Check)	1
Diluent (Mobile Phase)	1
• Tris Standard Solution may be omitted from sequence if ID D is not required. • Repeat the sample injection sequence if additional samples are to be analyzed • Samples may be substituted with diluent injections	

6.4. System Suitability Criteria:

TABLE 3. SYSTEM SUITABILITY CRITERIA

System Suitability Parameter	Acceptance Criteria
The relative standard deviation of the Tris (hydroxymethyl) nitromethane peak from the first six (6) injections of the Working Standard Solution.	NMT 15.0%
Tailing Factor	NMT 2.0
Bracket Standard Check	85-115% Recovery

6.4.1. Peak Identification:

6.4.1.1. Report Retention Time of Tris(hydroxymethyl)nitromethane related compound for system suitability replicate set.

6.4.1.2. Retention time of the primary peak of the standard TRIS should correspond the primary peak in the sample TRIS

6.5. Calculations:

6.5.1. %RSD = (standard/mean) x 100

6.5.2. 100 x (Peak Area of Standard Check/Average peak area of Initial 6 injections)

6.5.3. % w/w Tris (hydroxymethyl) nitromethane calculation:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

6.5.3.1. r_u= peak response of Tris(hydroxylmethyl)nitromethane from theSample Solution

6.5.3.2. r_s= peak response of Tris(hydroxylmethyl)nitromethane from the Standard Solution

6.5.3.3. C_s= concentration of Tris(hydroxylmethyl)nitromethane in the Standard Solution (mg/L)

6.5.3.4. C_u= concentration of Tris in the Sample Solution (mg/L)

6.5.4. % w/w Unspecified Impurity calculation:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

6.5.4.1. r_u= peak response of unspecified peak from the Sample Solution

6.5.4.2. rs= peak response of Tris(hydroxylmethyl)nitromethane from the Standard Solution

6.5.4.3. Cs= concentration of Tris(hydroxylmethyl)nitromethane in the Standard Solution (mg/L)

6.5.4.4. C_u= concentration of Tris in the Sample Solution (mg/L)