

BIOSPECTRA

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TRIS RELATED SUBSTANCES VIA HPLC

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Page 1 of 6

TABLE OF CONTENTS

1. PURPOSE:.....	3
2. SCOPE:.....	3
3. RESPONSIBILITIES:	3
4. REFERENCE:.....	3
5. MATERIALS AND EQUIPMENT:.....	3
6. PROCEDURE:	4

1. PURPOSE:

- 1.1. To provide the Quality Control (QC) Analysts with a procedure for Tris Related Substances determination and for operating the Perkin Elmer Flexar HPLC.

2. SCOPE:

- 2.1. Applies to Tris Related Substances on the Perkin Elmer Flexar HPLC.

3. RESPONSIBILITIES:

- 3.1. QC Management or other qualified designated individual, is responsible for the control, implementation, training and maintenance of this procedure.
- 3.2. The QC Analysts 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 QC Manager shall be promptly notified. If necessary, the HPLC will be serviced and recalibrated by Perkin Elmer before being approved for use.

4. REFERENCE:

- 4.1. BSI-PRL-0254, Analytical Method Validation Protocol: Limit of Tris (hydroxymethyl) nitromethane
- 4.2. BSI-RPT-0440, Analytical Method Validation Report: Limit of Tris (hydroxymethyl) nitromethane via HPLC,
- 4.3. BSI-SOP-0098, Balance SOP
- 4.4. BSI-SOP-0448, Perkin Elmer Flexar HPLC and TotalChrom Software
- 4.5. USP <621> *Chromatography*

5. MATERIALS AND EQUIPMENT:

- 5.1. Instrumentation
 - 5.1.1. Analytical Balance
 - 5.1.1.1. Secura 124-IS or equivalent
 - 5.1.2. HPLC
 - 5.1.2.1. Perkin Elmer Flexar Instrument with UV Detector Capability of 210nm 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 (hydroxymethyl) nitromethane, EP related Tris Compound CAS126-11-4.

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 750mL of HPLC grade acetonitrile and 250mL of HPLC grade purified water.
- 6.1.4. Tris (hydroxymethyl) nitromethane Stock Standard (600mg/L)
 - 6.1.4.1. Weigh 150mg of Tris(hydroxymethyl)nitromethane standard to a 250.0mL volumetric flask, dissolve and q.s. to volume with mobile phase.
- 6.1.5. Tris (hydroxymethyl) nitromethane Intermediate Standard (60mg/L)
 - 6.1.5.1. Dilute 10.0mL of Tris (hydroxymethyl) nitromethane stock standard to a 100.0mL volumetric flask, dissolve and q.s. to a volume with mobile phase.
- 6.1.6. Tris (hydroxymethyl) nitromethane Working Standard (0.6mg/L)
 - 6.1.6.1. Dilute 1.0mL of Tris (hydroxymethyl) nitromethane intermediate standard to a 100.0mL volumetric flask, dissolve, and q.s. to volume with mobile phase.
- 6.1.7. Tris (tromethamine) sample solution (2000mg/L)
 - 6.1.7.1. Weigh 200mg of Sample.
 - 6.1.7.2. Transfer to a 100.0mL volumetric flask and dissolve and q.s. to a volume with mobile phase.
 - 6.1.7.3. **Prepare sample solution immediately before injection.**

6.2. Instrument Setup:

Parameters	Setting
Column	Agilent ZORBAX Carbohydrate 5µm 4.6mm x150mm
Mobile Phase	75:25, Acetonitrile: Water
Needle Wash	75:25, Acetonitrile: Water
Flow Rate	1.0mL/min
Column Temperature	30°C
Column Pressure	Monitor
Autosampler Temperature	Controlled ambient
Injection Volume	20µL
Detector	210nm
Run Time	9 minutes

- 6.2.1. Ensure correct column is installed. If column is not installed, install the column by putting it in the HPLC oven and screwing both ends in to the respective flow lines. Do not over tighten
- 6.2.2. Injection Sequence:
 - 6.2.2.1. Inject ≥ 1 blank
 - 6.2.2.2. Inject working standard solution (section 6.1.6) in 6 replicates.
 - 6.2.2.3. Inject the blank in triplicate after system suitability to ensure no carryover is present in the system.
 - 6.2.2.4. Inject ≤ 6 samples
 - 6.2.2.5. Inject 1 QC check (working standard solution, section 6.1.6)
 - 6.2.2.6. Inject 1 blank
- 6.2.3. System Suitability Specifications:
 - 6.2.3.1. Tailing Factor: No more than 2.0
 - 6.2.3.2. %RSD: 15% maximum
 - 6.2.3.3. Bracket Standard Check: 85-115% Recovery
- 6.2.4. Peak Identification: Report Retention Time of Tris (hydroxymethyl) nitromethane related compound for system suitability replicate set.
- 6.2.5. Processing Figures:
 - 6.2.5.1. T=0 Disable Peak Detection
 - 6.2.5.2. T=2 Enable Peak Detection
 - 6.2.5.3. T=2 Set bunching factor 2
 - 6.2.5.4. Ensure related compound elutes after peak detection is enabled.

6.3. Calculations:

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

6.3.2. 100 x (Peak Area of Standard Check/Average peak area of Initial 6 injections)

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

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

6.3.3.1. r_u = peak response of Tris (hydroxymethyl) nitromethane from the Sample Solution

6.3.3.2. r_s = peak response of Tris (hydroxymethyl) nitromethane from the Standard Solution

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

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

6.3.4. % w/w Unspecified Impurity calculation:

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

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

6.3.4.2. r_s = peak response of Tris (hydroxymethyl) nitromethane from the Standard Solution

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

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