BIOSPECTRA
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HEPES IDENTITY BY HPLC WITH UV DETECTION

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1. PURPOSE:

1.1. To provide Laboratory analysts with a procedure for determining HEPES Identity by liquid chromatography with UV detection.

2. SCOPE:

2.1. This analytical method applies to the HEPES Identity by liquid chromatography with UV detection method using BioSpectra's Waters Acquity UPLC.
2.2. Specification: Retention time of the primary peak of the HEPES standard should correspond the primary peak in the HEPES sample.

3. RESPONSIBILITIES:

3.1. The Director of Laboratory Systems, and/or a qualified designee, is responsible for the control, training, implementation, and maintenance of this procedure.
3.2. The Analytical chemists, and/or the qualified designee, are responsible for performing the testing as stated in this procedure.
3.3. The Laboratory analysts performing this procedure with help from the Laboratory Technology Manager, if necessary, 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-ATM-0070, HEPES Testing Method
4.2. BSI-SOP-0098, Balance SOP
4.3. BSI-SOP-0134, Pipette SOP
4.4. BSI-SOP-0422, Empower 3 General Procedure
4.5. BSI-SOP-0436, Analytical Methods Validation Master Plan
4.6. *ACQUITY UPLC Quaternary Solvent Manager PLUS Series*
4.7. *ACQUITY UPLC TUV Detector Operator's Overview and Maintenance Guide*
4.8. *USP <621> Chromatography*
4.9. *USP <1225> Validation of Compendial Procedures*
4.10. *USP <1226> Verification of Compendial Procedures*
4.11. *USP-NF Current*

5. MATERIALS AND EQUIPMENT:

5.1. Equipment
  5.1.1. Analytical Balance
  5.1.2. Analytical Microbalance
  5.1.3. Liquid Chromatograph
    5.1.3.1. Waters Acquity HPLC with UV-Vis Detector.
5.2. Reagents
  5.2.1. HPLC Grade Water
  5.2.2. Ammonium Formate
  5.2.3. Ammonium Hydroxide
5.3. Supplies
  5.3.1. Class A Volumetric Flasks
  5.3.2. LC auto-sampler vials and caps
  5.3.3. Micropipettes
  5.3.4. Micropipette tips
  5.3.5. Polypropylene Transfer Funnels, Aluminum Weighing Boats, or equivalent
  5.3.6. Transfer pipettes

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5.4. Reference Standards
5.4.1. HEPES Reference Standard
5.5. LC Column
5.5.1. Description: Lua 5µm C18(2) 100Å, 150 x 3.9mm
5.5.2. Supplier: Phenomenex
5.5.3. Part Number: 00F-4252-C0

6. TESTING PROCEDURE:

6.1. Solution Preparation
6.1.1. All solutions are to be thoroughly mixed after being prepared. Ensure the amounts to be weighed are NLT than the minimum weight requirement of the balance. Solutions may be scaled as needed.

6.1.2. Mobile Phase 0.1% Ammonium Formate pH (9-10):
6.1.2.1. Weigh 1 g of Ammonium Formate and dissolve into 1000 mL with HPLC Grade water or equivalent, pH to 9-10 and mix thoroughly.

6.1.3. Diluent: Purified water

6.1.4. Identity Standard Solution (2 mg/mL HEPES):
6.1.4.1. Accurately weigh 20 mg of HEPES CRS (Reference Standard) and transfer aliquot quantitatively to a 10 mL volumetric flask. Dissolve in diluent.
6.1.4.2. Dilute to volume with diluent and mix thoroughly.

6.1.5. Identity Sample Solution (2 mg/mL HEPES):
6.1.5.1. Accurately weigh 20 mg of HEPES Sample and transfer aliquot quantitatively to a 10 mL volumetric flask. Dissolve in diluent.
6.1.5.2. Dilute to volume with diluent and mix thoroughly.

6.2. Instrument Setup

Parameter	Setting
Flow Type	Isocratic
Mobile Phase	Refer to Section 6.1.2.
Needle Wash	Water
Flow Rate	1.0 mL/min
Run Time	5 min
Injection Volume	1 µL
Column Temperature (°C)	Ambient
Sample Temperature (°C)	Ambient
Detector Settings	
Detector	UV-Vis
Wavelength	195 nm
Sampling Rate	5

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6.3. Injection Sequence:

Sample ID	Number of Injections
System Suitability	
Diluent (Mobile Phase)	≥1
Standard Solution	1
Sample Solution	1
Diluent	1
• Repeat the sample injection sequence if additional samples are to be analyzed • Samples may be substituted with diluent injections	

6.4. Result Reporting:

6.4.1. Identity:

6.4.1.1. Retention time of the primary peak of the HEPES standard should correspond the primary peak in the HEPES sample.

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