1.0 Purpose

The purpose of this procedure is to define a method for the quantitative analysis and/or identification of synephrine in complex matrices and raw materials using HPLC and UV/VIS spectrophotometry.

2.0 Scope

This procedure applies to the quantification and identification of synephrine. Some excipients and dietary ingredients used in finished products can interfere with the analysis of synephrine.

3.0 Responsibility

3.1 It is the responsibility of QC and analytical chemists to follow this procedure.

3.2 It is the responsibility of QC Laboratory Management to implement this procedure and to ensure that the procedure is being followed.

4.0 Definitions

4.1 **Synephrine** - 4-[1-hydroxy-2-(methylamino)ethyl]phenol

4.2 **UV/VIS** – Ultraviolet and Visible Electromagnetic Spectrums

4.3 **NaCl** – Sodium Chloride

4.4 **H_3PO_4** – Phosphoric Acid

4.5 **CofA** – Certificate of Analysis

4.6 **RT** – Room Temperature

4.7 **H_2O** – Millipore water

4.8 **STD** – Standard

4.9 **QC** – Quality Control

5.0 References

5.1 MV-LAB-13-072, Protocol, Synephrine Determination by HPLC

6.0 Reagents, Supplies, Glassware and Equipment

6.1 Reagents: all reagents are HPLC or better.

6.1.1 Millipore Water

6.1.2 NaCl

6.1.3 H_3PO_4

6.1.4 Synephrine

6.2 Supplies and Glassware

6.2.1 HPLC vials, 12mm X 32mm with screw cap enclosures w/ septa

6.2.2 1L mobile phase container

6.2.3 10mL, 50mL, 100mL, 500mL, and 1L volumetric flasks

6.2.4 200µL, 1mL, and 10mL pipette tips

6.2.5 10mL Plastic luer-lock syringes

6.2.6 0.2µM or 0.45µM 25mm Nylon syringe filters

6.2.7 22mL screw cap vials

6.2.8 1.5mL and 2.0mL micro centrifuge tubes

6.2.9 Weigh paper and weigh boats

6.3 Equipment

6.3.1 Suitable HPLC system consisting of gradient capable pump, autosampler, column compartment, and diode array detector.

6.3.2 Phenomonex Luna 5µM SCX 100Å, 4.6mm X 250mm or equivalent

6.3.3 Analytical Balance

6.3.4 Stir Plate

6.3.5 Wrist Action Shaker

6.3.6 Vortex

6.3.7 Sonicator Bath

6.3.8 200µL, 1mL, and 10mL Pipettes- adjustable

6.4 Mobile Phase Preparation

6.4.1 Mobile Phase A (0.1% H_3PO_4 in H_2O) - prepared by adding 1mL H_3PO_4 to a 1L volumetric flask then diluting to 1L with H_2O.

6.4.2 Mobile Phase B (0.1% H_3PO_4 1M NaCl) - prepared by adding 1mL H_3PO_4 and 58.44g NaCl to a 1L volumetric flask then diluting to 1L with H_2O.

6.4.3 Dissolution buffer- Mobile Phase A

7.0 Procedure

7.1 Standard Preparation

7.1.1 Use the actual purity from the CofA for synephrine in your calculations. The Standard Preparation reflects 100% of the label quantity for synephrine.

Synephrine standards need to be made fresh daily.

Example: Synephrine, 99.5% purity

Prepare 50mL of a 1mg/mL solution

1) 1mg/mL X 50mL = 50mg

2) 50mg/0.995 = 50.3mg

3) Dissolve 50.3mg up to 50mL = 1.0mg/mL

7.1.2 All standards are prepared by weighing no less than 50mg then bring up to two thirds their final volumes in an appropriate volumetric flask using Mobile Phase A. Mix on a wrist action shaker for 20 minutes then inspect to ensure complete dissolution. Sonication for 10 minutes can also be used to assist dissolution. Once the standard is fully dissolved, bring standard to final volume before beginning dilutions.

7.1.3 Standards and dilutions are prepared using Mobile Phase A. Dilutions can be made using volumetric flasks or using 1mL and 200uL variable pipettes. Working standard concentrations will approximate the concentration expected to be found in the product being tested based on the sample dilution and calculated from the label. Final dilutions may be prepared directly in HPLC vials.

7.2 Sample Preparation

7.2.1 The linear range of the method is 0.004 mg/mL – 0.4 mg/mL. All standards and samples to be injected must be within the linear range.

7.2.2 At least 20 dosage units are pooled and ground by mortar and pestle as necessary.

7.2.3 Based on the fill weight or tablet weight per dose weigh a portion of the pooled dosages to generate an analyte concentration that is within the validated linearity and solubility range for the analyte being tested. Raw materials samples can be

prepared using 100% as purity.

7.2.4 Samples can be dissolved in Mobile Phase A at any volume starting from 25mL. The volume chosen must be in the solubility range of synephrine. To manage large volumes the sample can be initially dissolved in a smaller volume that is within the solubility range and a portion further diluted to bring the analyte concentration into the linear range of measurement. See Attachment 1 for validated assay range.

7.2.5 No less than 50mg of sample can be weighed.

7.2.6 Dilute the sample to 2/3's the calculated volume with Mobile Phase A, cap and shake for 20 minutes to facilitate dissolution. Sonication for 10 minutes can also be used to assist dissolution. Once the synephrine is completely dissolved, bring sample up to volume with Mobile Phase A before beginning dilutions.

7.2.7 If sonication is used allow sample to cool to RT before continuing.

7.2.8 For filtration, using the final large scale diluted sample withdraw up to 10mL using a 10mL plastic syringe then filter and discard the first 0.5mL of sample before collecting. From the collected sample dilute as needed then add 1mL to an HPLC vial for analysis.

7.2.9 For centrifugation using the final large scale diluted sample, fill an even number of 1.5 or 2.0mL micro-centrifuge tubes and pellet insoluble matter for 5 minutes at 6000rpm.

7.2.10 For finished products or raw materials being analyzed for the first time using this method an in process validation is required to demonstrate spectral purity, baseline separation of peaks and extraction efficiency as a part of system suitability before data can be reported using this method.

7.3 Test Conditions

7.3.1 Standards, Pure Raw Materials, and Blanks

7.3.1.1 Isocratic 55% Mobile Phase A : 45% Mobile Phase B

7.3.2 Finished Products and Complex Raw Materials

7.3.2.1

Time (min)	%A	%B
0.0	55	45
8.0	55	45
8.01	0	100
16.0	0	100
16.1	55	45
20.0	55	45

7.3.3 Column - Luna 5µM SCX 100Å, 4.6mm X 250mm or equivalent

7.3.4 Flow Rate - 1.0 mL/min

7.3.5 UV Detection - 220nm

7.3.6 Injection Volume - 20µL

7.3.7 Temperature - 35°C

7.3.8 3-D Spectral Range- 190nm to 700nm

7.4 Recommended Sequence

7.4.1 Perform at least one injection of Diluent

7.4.2 Perform five injections of the Working Standard

7.4.3 Perform a single injection of each Sample Preparation

7.4.4 Perform a single injection of the Working Standard after every six samples and at the end of the run.

7.5 System Suitability

7.5.1 The %RSD of 5 injections of the Working Standard is NMT 3.0%

7.5.2 The %RSD of all injections of the Working Standard is NMT 3%

7.5.3 The spectral match is NLT 900.

7.5.4 The retention time of the sample is within 0.3 min of the standard.

7.6 Calculations

$$\%\ \text{assay} = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{V_{spl}}{SA} \times \frac{SS}{LA} \times 100$$

R_u Sample peak area

R_s Mean standard peak area

Wt_{std} Weight of reference standard in mg

V_{std} Volume of the standard preparation accounting for dilutions in mL

P Purity of the reference standard in decimal format

SA Sample amount in mg (solids) or mL (liquids)

V_{spl} Volume of the sample preparation accounting for dilutions in mL

SS Serving size: Weight of a single dosage unit in mg for tablets and capsules, volume of a single serving from the theoretical formula in mL for liquids, or 1 for raw materials.

LA Label amount in mg per dose or 1 for raw materials

7.7 Column Wash and Storage

7.7.1 Rinse and store the column with Water / MeOH (70/30)

8.0 Revision History

Revision	Date	Description of Changes	CCR #	By
1	01/10/14	New	14-0053	B. Johns
2	04/01/16	Biennial review: Updated SOP to current format	16-0204	J. Maignan
3	09/03/19	Scheduled review: update SOP with current laboratory practices.	19-0597	I. Garrett
4	03/23/22	Update with current practices. Add bracketing standard as system suitability requirement. Remove references to specific HPLC systems.	CC-22-0119	S. Sassman