1.0 Purpose

The purpose of this procedure is to describe a method for the quantitative analysis and identification of 2-phenylethylamine and hordenine in finished products and raw materials using HPLC and UV/VIS spectrophotometry.

2.0 Scope

This procedure applies to the quantification and identification of 2-phenylethylamine and hordenine. This procedure is valid for 2-phenylethylamine and hordenine. Some excipients and dietary ingredients used in the finished products can interfere with the analysis of 2-Phenylethylamine. Other wavelengths can be used if interferences are present.

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.

3.3 It is the responsibility of QC Laboratory Management to keep this procedure current with latest Ion Labs practices.

4.0 Definitions

4.1 **ACN** – Acetonitrile

4.2 **H_2O** – Deionized water

4.3 **HCl** – Hydrochloride

4.4 **2-PEA** – 2-Phenylethylamine

4.5 **QC** – Quality Control

4.6 **CofA** – Certificate of Analysis

5.0 References

5.1 MV-LAB-14-012, Protocol, Open Validation Protocol for Amino Acids and Amines

6.0 Reagents, Supplies, Glassware and Equipment

6.1 Reagents: all reagents are HPLC grade or better.

6.1.1 Deionized water

6.1.2 ACN

6.1.3 Ammonium Acetate

6.1.4 2-phenylethylamine and/or hordenine

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 50mL, 100mL, and 500mL volumetric flasks

6.2.4 50mL and 100mL beakers

6.2.5 200uL, 1mL, and 10mL pipette tips

6.2.6 1.5mL and 2.0mL micro centrifuge tubes

6.2.7 10mL plastic luer lock syringe

6.2.8 0.2 or 0.45µm 25mm Nylon syringe filters

6.2.9 22mL screw cap vials (scintillation)

6.2.10 Weigh boats

6.3 Equipment

6.3.1 Suitable gradient HPLC system consisting of a pump, autosampler, column oven and UV detector with a chromatographic data handling system

6.3.2 Analytical Balance

6.3.3 Vortex

6.3.4 Stir Plate

6.3.5 Wrist action shaker

6.3.6 Micro-centrifuge

6.3.7 200uL, 1mL and 10mL pipettes

7.0 Procedure

7.1 Mobile Phase A - 20mM Ammonium acetate (aq)

7.1.1 Dissolve 1.54 g of ammonium acetate in 1000 mL H_2O. Scale as necessary.

7.2 Mobile Phase B (ACN) - Acetonitrile

7.3 Diluent - Mobile Phase A

7.4 Standard Preparation

7.4.1 Use the actual purity from the CofA for 2-Phenylethylamine/hordenine in your calculations. All Standards are prepared by weighing no less than the minimum weight of the analytical balance.

7.4.2 Dissolve standard in two-thirds its final volume in an appropriately sized volumetric flask using Diluent. Mix on the wrist action shaker for 30 minutes then inspect to ensure complete dissolution. Once standard is fully dissolved, bring standard to final volume before using.

7.4.3 Dilutions are prepared using diluent. Dilutions can be made using volumetric flasks or using 10mL, 1mL and 200uL variable pipettes. Specific 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. Dilutions can be prepared directly in HPLC vials.

7.5 Sample Preparation

7.5.1 The Sample Preparation must be within the linear range of the method:

7.5.1.1 2-Phenethylamine – 0.006 mg/mL – 0.4 mg/mL

7.5.1.2 Hordenine – 0.01 mg/mL – 0.5 mg/mL

7.5.2 10 or more dosage units can be pooled and ground by mortar and pestle as necessary.

7.5.3 Based on the fill 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.

7.5.4 Samples can be dissolved in diluent at any volume starting from 10mL. 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.

7.5.5 The final diluted sample must be filtered or centrifuged before analyzing by HPLC. Filter through a 0.45 µm membrane, discarding at least 0.5 mL before collecting a portion for analysis. From the collected sample dilute as needed then add 1mL to an HPLC vial for analysis.

7.5.5.1 Alternatively centrifugation using the final diluted sample can be performed by filling an even number of 1.5mL or 2.0mL micro centrifuge tubes, then pellet out insoluble matter for 3 minutes at 6000rpm.

7.5.6 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 at the detection wavelength and extraction efficiency before quantitation can be completed.

7.6 Test Conditions

7.6.1 Gradient-Isocratic

Time	%A	%B	Gradient type
0.00	85	15	0
10.00	85	15	0

7.6.2 Column- Luna C18(2), 5µ, 120A, 4.6 X 250mm

7.6.3 Flow Rate- 1.0mL/min

7.6.4 UV detection- 210nm

7.6.5 Injection volume- 20uL

7.6.6 Column Temperature- 40°C

7.6.7 Recommended 3-D Spectral Range- 200nm to 700nm

7.7 Recommended Sequence

7.7.1 Make at least 2 injections of a Blank (Diluent).

7.7.2 Make five injections of the Working Standard.

7.7.3 Make a single injection of each Sample Preparation.

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

7.8 System Suitability

7.8.1 The %RSD of five consecutive injections of the Working Standard is NMT 5.0%.

7.8.2 The %RSD of all standard injections is NMT 5%.

7.8.3 Non-conforming results may trigger execution of a product specific method optimization per D-126/D-103

7.9 Column Wash and Storage

7.9.1 Rinse the column with H_2O / ACN (50/50) at 1 mL/min for at least 30 min.

7.9.2 Store the column with H_2O / ACN (50/50).

7.10 Example calculations for determining finished product % label or raw material % purity

7.10.1 $$\%\ \text{assay} = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{V_{spl}}{SA} \times \frac{SS}{LA} \times 100$$

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)

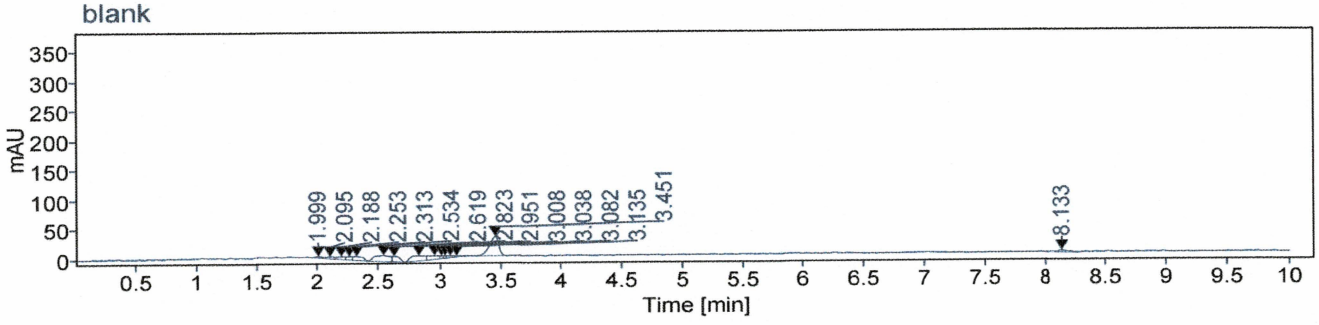
V_{spl} Volume of the sample preparation accounting for dilutions in mL

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.

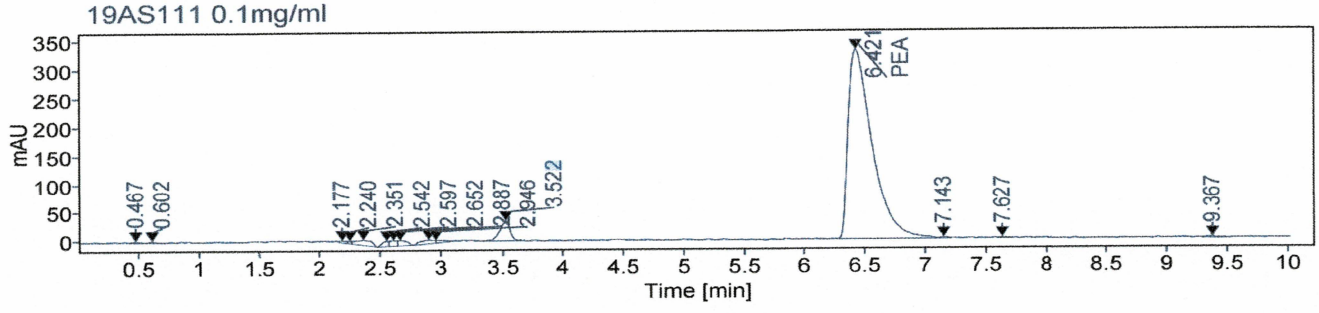
LA Label amount in mg per dose or 1 for raw materials

7.11 Example Chromatography

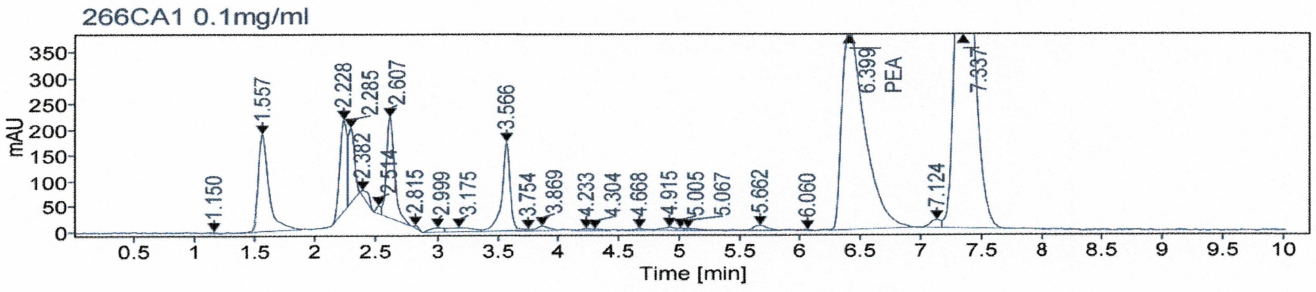
7.11.1 Blank



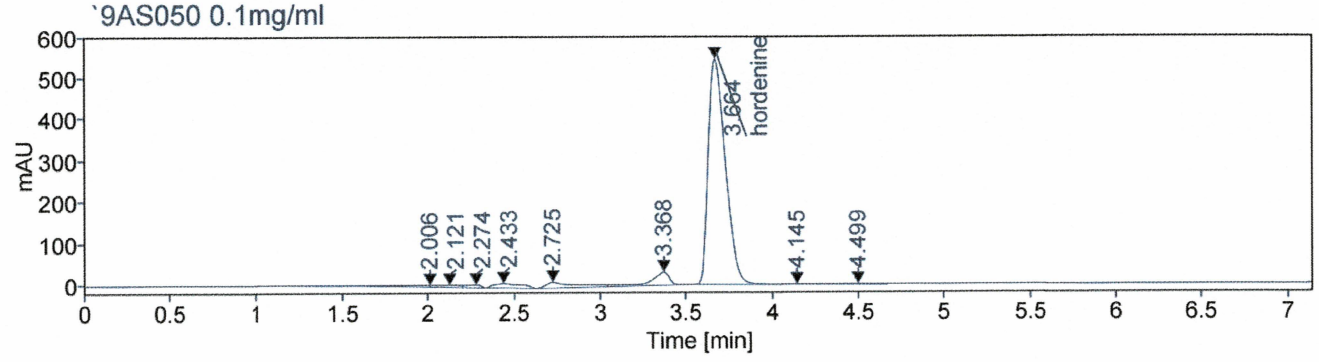
7.11.2 Working Standard (2-phenethylamine)



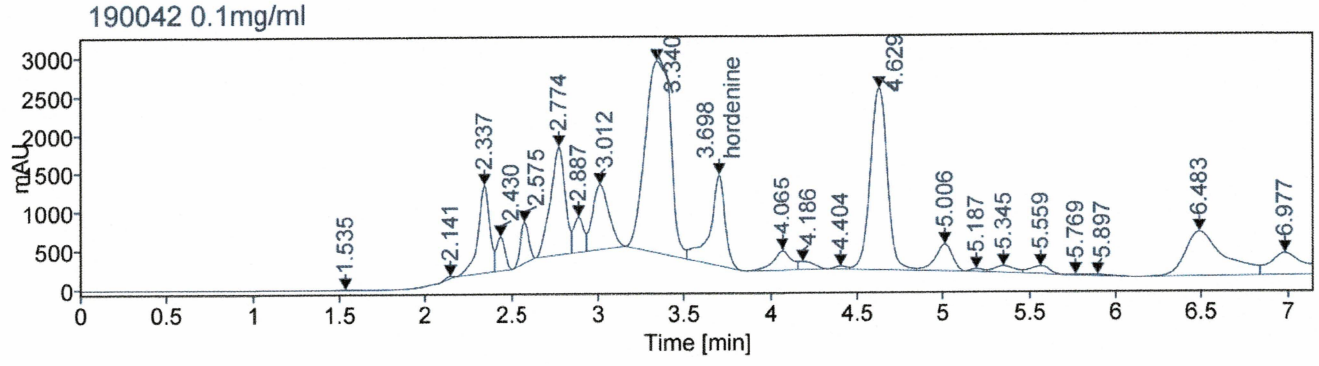
7.11.3 Sample (2-phenethylamine)



7.11.1 Working Standard (hordenine)



7.11.2 Sample (hordenine)



8.0 Revision History

Revision	Date	Description of Changes	CCR #	By
1	01/06/15	New	15-0008	X. Shao
2	01/14/19	Scheduled review: addition of hordenine to valid analytes, Updated format to match current Ion practices	19-0040	J. Maignan
3	04/15/22	Update to reflect current practices. Simplify mobile phase preparation. Add recommended sequence section. Replace requirements section with system suitability. Update example calculation for consistency with current methods. Add example chromatography.	CC-22-0180	S. Sassman
4	12/05/22	Minor edits. Added "recommended" to spectral range.	CC-22-0459	J. Sassman