1.0 Purpose

The purpose of this procedure is to define the method for the quantitation and/or identification of individual yohimbe alkaloids in raw materials and finished products using HPLC coupled with UV/VIS spectrophotometry.

2.0 Scope

This procedure applies to the identification and quantitation of yohimbe alkaloids in raw materials and finished products in the QC laboratory at Ion Labs. Yohimbe alkaloids are determined as Yohimbine equivalents by comparison against a Yohimbine HCl reference standard without regard to their individual relative response factors.

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 ensure that this procedure is being followed.

3.3 It is the responsibility of QC Laboratory Management and/or Analytical Development to keep this procedure aligned with current practices.

4.0 Definitions

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

4.2 **H_2O** – Water

4.3 **QC** – Quality Control

5.0 References

5.1 MV-LAB-15-043, Protocol, Total Yohimbine Alkaloids Determination using HPLC coupled with UV/VIS and Mass Spectroscopy.

5.2 D-793, SOP, Cryogenic Grinding of Chewable Gels

6.0 Procedure

6.1 Chemicals: All reagents are HPLC grade or better.

6.1.1 H_2O ($\geq$ 18.2 MΩ·cm)

6.1.2 Triethylamine

6.1.3 Methanol

6.1.4 Yohimbine HCl reference standard

6.2 Glassware

6.2.1 HPLC vials, 12mm x 32mm with screw cap enclosures with septa

6.2.2 Scintillation vials

6.2.3 Mobile phase containers

6.2.4 Volumetric glassware as required by standard and sample preparations

6.3 Disposables

6.3.1 Tips for adjustable pipettes

6.3.2 Micro-centrifuge tubes

6.3.3 16mL Test Tubes

6.3.4 Disposable plastic luer lock syringes

6.3.5 Nylon syringe filters, 0.45µm

6.3.6 Weigh paper

6.4 Equipment

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

6.4.2 Analytical balance

6.4.3 Shaker

6.4.4 Vortex

6.4.5 Stir Plate

6.4.6 Eppendorf centrifuge

6.4.7 Adjustable pipettes

7.0 Preparation of Mobile Phase, Diluent, Samples, and Standards

7.1 Mobile Phase A (0.5% triethylamine in H_2O)

7.1.1 Transfer 1000 mL H_2O to a mobile phase bottle.

7.1.2 Add 5.0 mL of triethylamine, and mix well.

7.2 Mobile Phase B (0.5% triethylamine in methanol)

7.2.1 Transfer 1000 mL methanol to a mobile phase bottle.

7.2.2 Add 5.0 mL of triethylamine, and mix well.

7.3 Diluent (Methanol/H_2O 50/50)

7.3.1 Transfer 500 mL methanol to a mobile phase bottle.

7.3.2 Add 500 mL H_2O, and mix well

7.3.3 **Equilibrate to room temperature before use.**

7.4 Stock Standard Preparation

7.4.1 Accurately weigh and transfer about 25 mg of Yohimbine HCl reference standard into a 100-mL volumetric flask.

7.4.2 Dissolve in and dilute to volume using Diluent.

7.5 Working Standard Preparation

7.5.1 Transfer 5.0 mL of Stock Standard to a 50-mL volumetric flask.

7.5.2 Dilute to volume using Diluent.

7.6 Sample Preparation

7.6.1 Specific sample testing details are provided in each products profile. If a specific testing details section is not available, then follow preparation procedure as described below, maintaining concentration within the linear range listed below.

7.6.2 The linear range of the method is 2.5 – 750 µg/mL. The concentration of the sample preparation must be within the linear range of the method. If the potency is given only for total alkaloids, assume Yohimbine is the only alkaloid present to calculate sample weight and volume.

7.6.3 For raw materials: Based on the expected Yohimbine content, weigh a portion into a suitably sized volumetric flask to generate an analyte concentration of about 25 µg/mL. Dilute the sample to 2/3 of the flask volume with Diluent, sonicate for 5 min with occasional shaking, shake mechanically for 30 minutes, and dilute to volume using Diluent.

7.6.4 For solid and liquid dose finished products: Combine and homogenize at least 10 dosage units. Based on the weight per dose and the label amount, weigh a portion of the homogenized sample into a suitably sized volumetric flask to generate a Yohimbine concentration of about 25 µg/mL. Dilute the sample to 2/3 of the flask volume with Diluent, sonicate for 5 min with occasional shaking, shake mechanically for 30 minutes, and dilute to volume using Diluent.

7.6.5 For chewable gels (gummies): homogenize at least 10 dosage units according to the procedure outlined in D-793 Cryogenic Grinding of Chewable Gels. Quickly weigh a portion of the homogenized sample into a suitably sized beaker. Use several small portions of diluent to completely transfer the sample into a suitably

sized volumetric flask to generate a Yohimbine concentration of about 25 µg/mL. Dilute the sample to 2/3 of the flask volume with Diluent, sonicate for 5 min with occasional shaking, shake mechanically for 30 minutes, and dilute to volume using Diluent.

7.6.6 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 using Diluent to bring the analyte concentration into the linear range of measurement.

7.6.7 The final sample must be filtered or centrifuged before analyzing by HPLC.

7.6.7.1 For filtration: filter a portion through a 0.45µm nylon membrane discarding the first 2-3 mL of filtrate before collecting an aliquot for analysis.

7.6.7.2 For centrifugation: centrifuge for 5 min at 10,000 rpm.

8.0 Test Conditions

8.1 Gradient (Blanks and Standards)

Time	%A	%B	Gradient Type
0.00	69%	31%	0
35.00	69%	31%	0

8.2 Gradient (Samples)

Time	%A	%B	Gradient Type
0.00	69%	31%	0
80.00	69%	31%	0
80.10	0%	100%	step
85.00	0%	100%	0
85.10	69%	31%	step
90.0	69%	31%	0

8.3 Column – Kinetex EVO C18 100 Å 5µ, 150mm x 3.0mm

8.4 Flow Rate – 0.7mL/min

8.5 UV Detection – 282nm

8.6 Recommended 3-D Spectral Range – 220nm – 400nm

8.7 Injection Volume - 20µL

8.8 Column Temperature – 30°C

8.9 Relative Retention Times

8.9.1 Ajmaline – 0.31

8.9.2 Unknown 1 – 0.69

8.9.3 Yohimbine – 1.00

8.9.4 Coryanthine – 1.13

8.9.5 Unknown 2 – 1.38

8.9.6 Rauwolscin – 2.19

8.9.7 Ajmalicine – 3.94

8.9.8 Reserpine – 5.13

8.10 Recommended Sequence

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

8.10.2 Make five injections of the Working Standard.

8.10.3 Make a single injection of each Sample Preparation.

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

8.11 System Suitability

8.11.1 The %RSD of five consecutive standard injections is NLT 5.0%.

8.11.2 The %RSD of all standard injections is NLT 5%.

8.12 Column Wash and Storage

8.12.1 Rinse and store the column with H_2O / methanol (50/50).

9.0 Calculations

9.1 $$\% \text{ assay} = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{V_{spl}}{SA} \times \frac{SS}{LA} \times 100$$

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)

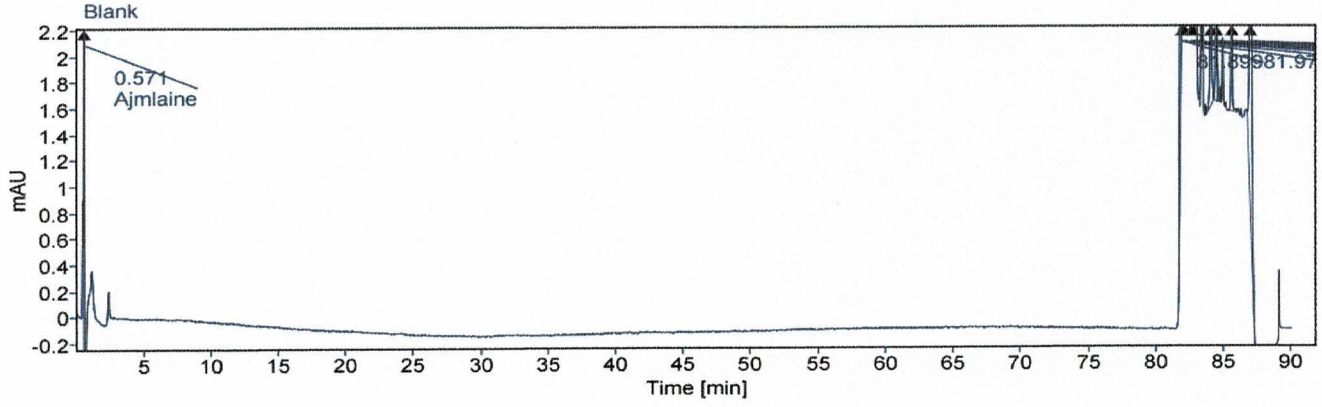
V_{spl} Volume of the sample preparation accounting for dilutions in mL

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.

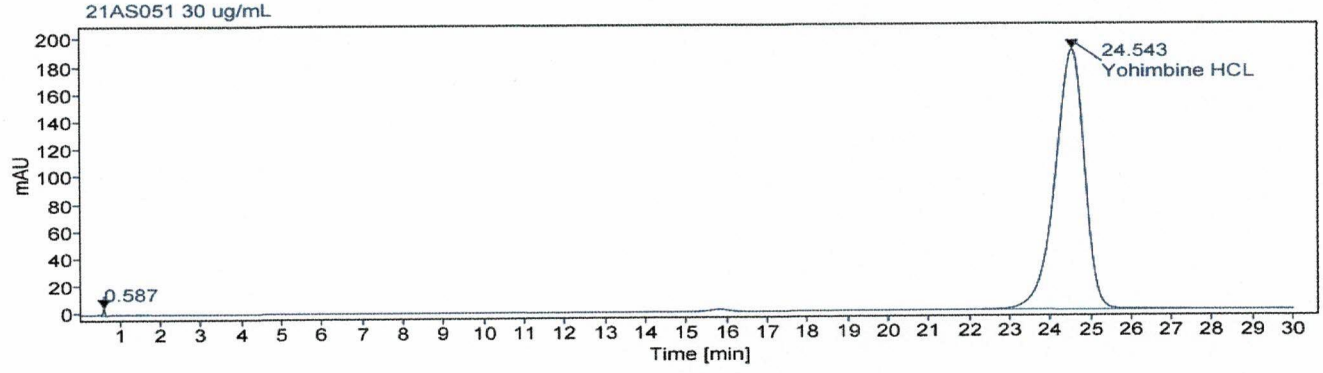
LA Label amount in mg per dose or 1 for raw materials

10.0 Example Chromatography

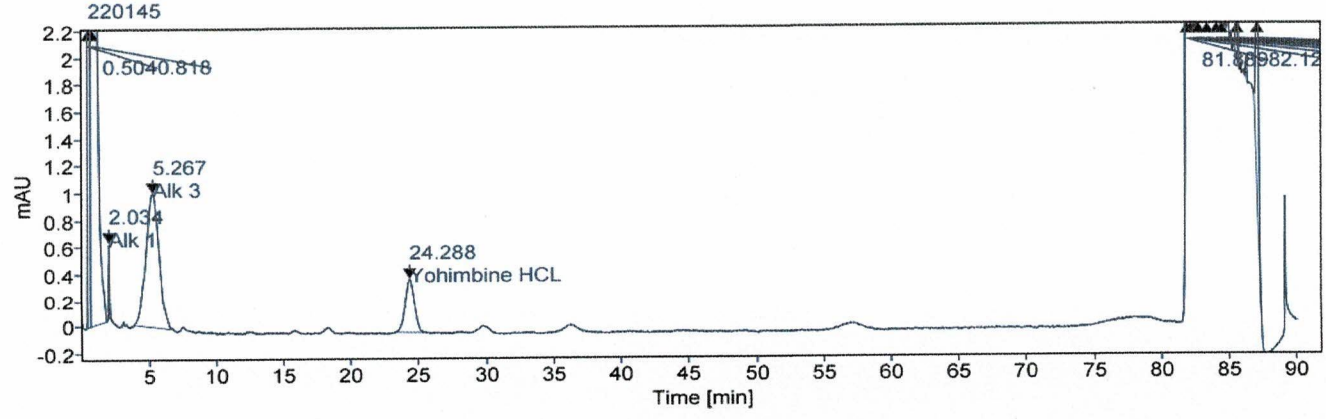
10.1 Diluent



10.2 Standard



10.3 Sample



11.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	09/28/15	New procedure	15-0961	J. Maignan
1	01/14/19	Scheduled review: updated format to match current Ion practices.	19-0039	J. Maignan
2	06/01/22	Update for consistency with current methods, simplify mobile phase preparation, simplify standard and sample preparation, add short gradient for standards and blanks, add column wash and storage, add example chromatography.	CC-22-0253	S. Sassman
3	05/10/23	Update for consistency with current methods, add instruction to follow test details, add specific sample preparation for different dosage forms.	CC-23-0248	S. Sassman