

IDENTIFICATION AND DETERMINATION OF YOHIMBINE FROM HORSE URINE SAMPLES – LC-MS/MS METHOD

I. INTRODUCTION

Yohimbine is a selectively competitive alpha-2 adrenergic receptor antagonist. It has been successfully used to reverse the CNS depressant and other potentially adverse side-effects of xylazine in horses (Kollias-Baker, *et al.*, 1993). Yohimbine has been classified by the Association of Racing Commissioners International (ARCI) as a class 2 violation and is not a Drug Enforcement Administration controlled substance.

In the horse, Yohimbine is excreted in its parent form and also as hydroxylated metabolites in urine.

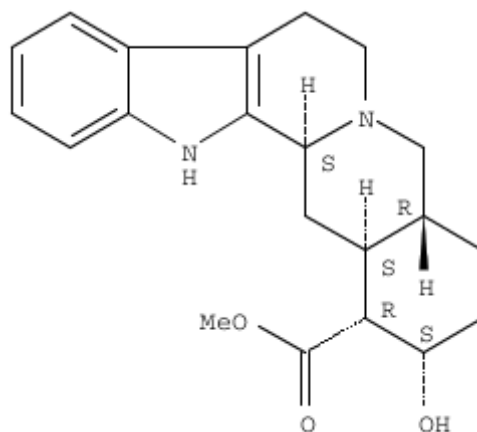


Figure 1: Yohimbine

II. SCOPE

This standard operating procedure is to be used for the identification and quantitation of Yohimbine. The lower limit of quantitation for this method is approximately 5.0 ng/mL of urine.

III. PRINCIPLE OF METHOD

This standard operating procedure is to be used for the identification and quantification of Yohimbine from hydrolysates of horse urine. In this procedure, the analytes are purified by mixed-mode SPE and identified and determined by liquid chromatography / mass spectrometry operated under electrospray ionization in positive ion mode. The concentration of Yohimbine is determined by the internal standard method using the peak area ratio and linear regression analysis. The Yohimbine calibration curve can be used to estimate levels of OH-Yohimbine, if present, as an authentic standard is not available for this metabolite. Reserpine is used as the internal standard.

IV. REAGENTS

- A. Water for reagents, use double distilled water in any reagent or procedure requiring the use of water except for HPLC.

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B. HPLC Solvent A (Acetonitrile + 0.2% Formic Acid)

1. Reagents
 - a) Acetonitrile, HPLC Grade (cat no. 015-4, Burdick & Jackson)
 - b) Formic Acid, Certified ACS Grade (98%, Code: 147932500, Acros Organics)
2. Procedure
 - a) Add 1000 mL acetonitrile to a HPLC solvent reservoir bottle. Remove and discard 2 mL using a glass syringe.
 - b) Add 2 mL of Formic acid using a glass syringe. Volumes may be adjusted as long as the correct ratio is maintained.
 - c) Store at room temperature in a glass container. Prepare the reagent fresh every two weeks.

C. HPLC Solvent B (Water + 0.2% Formic Acid)

1. Reagents
 - a) Water for HPLC, HPLC Grade (cat no. AH365, Burdick & Jackson).
 - b) Formic Acid, Certified ACS Grade (98%, Code: 147932500, Acros Organics)
2. Procedure
 - a) Add 1000 mL Water to a HPLC solvent reservoir bottle. Remove and discard 2 mL using a glass syringe.
 - b) Add 2 mL of Formic acid using a glass syringe. Cap and mix thoroughly before placing on HPLC. Volumes may be adjusted as long as the correct ratio is maintained.
 - c) Store at room temperature in a glass container. Prepare the reagent fresh every two weeks.

D. Sample Redissolving Solvent (5% ACN + 0.2% Formic Acid)

1. Reagents
 - a) HPLC Solvent A (HPLC Grade Acetonitrile + 0.2% Formic Acid)
 - b) HPLC Solvent B (HPLC Grade Water + 0.2% Formic Acid)
2. Procedure
 - a) Mix 500µL HPLC Solvent A with 9.5 mL of HPLC Solvent B, vortex.
3. Storage Requirements
 - a) Prepare the reagent fresh weekly. Store at room temperature

E. 1.6 M Acetate Buffer (pH 5.0)

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1. Reagent
 - a) sodium acetate TRIHYDRATE, anhydrous, reagent grade
 - b) concentrated glacial acetic acid, reagent grade
 - c) water
2. Procedure
 - a) Dissolve 136 g of sodium acetate in approximately 200 mL of water.
 - b) Add 33 mL of concentrated glacial acetic acid.
 - c) Dilute to 1000 mL with water and mix. Adjust the pH to 5.0 ± 0.1 with 1 *N* sodium hydroxide solution or concentrated glacial acetic acid, if necessary
3. Storage Requirements
 - a) Store at approximately 4°C in a glass container.
 - b) Discard 3 months after preparation. Inspect for contamination before use.

F. 1.0 M Acetic Acid

1. Reagents
 - a) glacial acetic acid, reagent grade
 - b) water
2. Procedure
 - a) **Prepare under a fume hood.**
 - b) To ~400 mL DI water pipette 28.6 mL of glacial acetic acid. Dilute to 500 mL and mix.
3. Storage Requirements
 - a) Store at room temperature in a glass container.
 - b) Discard 6 months after preparation.

G. β -Glucuronidase enzyme (10,000 units/mL in sodium acetate buffer)

1. Reagents
 - a) β -Glucuronidase from *Patella vulgata* (cat. no. G8132-2MU, Sigma Chemical Co. or equivalent)
 - b) 1.6 M acetate buffer (pH 5.0)
2. Procedure

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- a) Dilute the contents of one vial (2,000,000 units per vial) of β -glucuronidase enzyme to 200 mL with 1.6 M acetate buffer. Mix.

3. Storage Requirements

- a) Store at approximately 4°C in a glass container.
- b) Discard 1 month after preparation.

H. Methanol (Fisher Scientific Co. or equivalent)

I. 2 N Hydrochloric acid solution (certified grade, Fisher Scientific or equivalent)

J. 2 N Sodium Hydroxide solution (certified grade, Fisher Scientific or equivalent)

K. 0.6 M Phosphate Buffer, pH 6.5 (1 L)

1. Reagents

- a) Na_2HPO_4 and $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$
- b) water

2. Procedure

- a) Weigh 45.9 g of Na_2HPO_4 and 38.2 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$. Dilute to 1 L in a mixing cylinder or volumetric flask and mix. Adjust pH to 6.5 ± 0.1 with 1 N sodium hydroxide or 1 N Hydrochloric acid, if necessary.

3. Storage Requirements

- a) Store at room temperature in a glass container. Inspect for contamination before use.
- b) Discard 2 months after preparation.

L. Elution solvent: methylene chloride:isopropanol:ammonium hydroxide (78:20:2; v/v/v)

1. Reagents

- a) methylene chloride (Fisher, Optima grade or equivalent)
- b) concentrated ammonium hydroxide, reagent grade
- c) isopropanol (Fisher, Optima grade or equivalent)

2. Procedure

- a) **Prepare under a fume hood**
- b) Combine 20 mL isopropanol and 2 mL of ammonium hydroxide. Mix.

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- c) Add 78 mL methylene chloride, mix
- 3. Storage Requirements
 - a) Store at room temperature in a glass container Prepare the reagent fresh daily.
- M. 0.1 M sodium phosphate buffer (pH 6.0)
 - 1. Reagents
 - a) monobasic sodium phosphate, reagent grade
 - b) dibasic sodium phosphate, reagent grade
 - c) water
 - 2. Procedure
 - a) Dissolve 1.7g of Na_2HPO_4 and 12.1g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 800 mL water. Mix.
 - b) Dilute to 1000 mL with water. Mix.
 - c) Adjust the solution to $\text{pH } 6.0 \pm 0.1$ with 2 N sodium hydroxide or 2 N Hydrochloric acid, if necessary.
 - 3. Storage Requirements
 - a) Store at approximately 4 °C in a glass container. Inspect for contamination before use.
 - b) Discard 2 months after preparation.

V. MATERIALS

- A. HPLC autosampler vials with inserts (Agilent Technologies or equivalent).
- B. 12 x 75 mm glass tubes with caps.
- C. Pipettes and tips.

Note: Use the following positive displacement pipettes to pipette the standard solutions and working standard solutions.

- 1. 0.1-10 μL adjustable volume pipette (Eppendorf 2100, Brinkmann).
- 2. 2.0-20 μL adjustable volume pipette (Eppendorf 2000, Brinkmann).
- 3. 10-100 μL adjustable volume pipette (Eppendorf 2000, Brinkmann).

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4. 20-200 μ L adjustable volume pipette (Eppendorf 2000, Brinkmann).
 5. 100-1000 μ L adjustable volume pipette (Eppendorf 2000, Brinkmann).
 6. 500-5000 μ L adjustable volume pipette (Eppendorf 2100, Brinkmann).
- D. Vortex mixer (Glas-Col® Apparatus Co. or equivalent).
- E. pH meter (Corning 445, Fisher Scientific Co. or equivalent).
- F. Bransonic Ultrasonic Water Bath, 5510 (Fisher Scientific Co. or equivalent).
- G. Centrifuge (Sorvall Super T21, Kendro Laboratory Products or equivalent).
- H. Rotorack (Glas-Col® Apparatus Co. or equivalent).
- I. Cerex 24 or 48-place solid phase extraction apparatus (Cera Inc.).
- J. Solid Phase Extraction Columns, 35mg/3cc Cerex™ PolyCrom Clin II (cat. no. 691-0353, Cera Inc.).
- K. Tissue paper wipers (e.g. Kimwipes®).
- L. Evaporator (TurboVap, Zymark or equivalent).
- M. Nitrogen gas.
- N. Glass pasteur pipettes, disposable.
- O. Bottle Top Dispensers (0.4-2 ml, 1-5 ml, 1-10 ml, 2-10ml) Brinkmann Dispensette Digital (Brinkmann).

VI. TEST SUBSTANCE

Horse urine

VII. VOLUME REQUIRED

1 mL or appropriate dilution. Use duplicate 1-mL aliquots of undiluted test sample if the estimated concentration of Yohimbine in the test sample is between 5 and 200 ng/mL. If the estimated concentration of Yohimbine is greater than 200 ng/mL, prepare an appropriate dilution of an aliquot of the test sample with water. Repeat this process for each test sample.

VIII. WORKING STANDARD SOLUTIONS

- A. Yohimbine working solution in methanol – 1.0 ng/ μ L

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1. Yohimbine 1 mg/mL Reference Standard.
 2. Dilute 10.0 μ L of the 1.0 mg/mL Yohimbine reference standard solution to 10.0 mL with methanol in a volumetric flask.
 3. Prepare two separate working standard solutions; use one solution for the preparation of the calibrators (ws1) and the standard mixture and the other solution for the preparation of the control samples (ws2).
 4. Store the standard and working standard solutions at $< 0^{\circ}\text{C}$.
- B. Reserpine-*IS* working standard solution - 5.0 ng/ μ L
1. Reserpine standard solution in methanol - 1.0 mg/mL.
 2. Dilute 10 μ L of the 1.0 mg/mL Reserpine standard solution to 2 mL with methanol.
 3. Store the standard and working standard solutions at $< 0^{\circ}\text{C}$.

IX. CONTROL SAMPLES

- A. Negative Control Sample - Horse urine demonstrated by analysis to contain no detectable Yohimbine and/or OH-Yohimbine.
1. Dilute the negative control sample with water to the same extent as the test sample (see Section VII. **VOLUME REQUIRED**).
 2. Store control urine at $< 0^{\circ}\text{C}$.
- B. Positive Control Sample - Horse urine supplemented with Yohimbine.
1. See Table 1 for description of positive control sample preparation.
- C. Administration Positive Control Sample – Duplicate aliquots of administration positive control horse urine collected after administration of Yohimbine may be included.

X. SAMPLE REQUIREMENTS FOR ANALYSIS

Prepare the following samples and standards for each analysis:

1. Calibrators designated **C₁**, **C₂**, **C₃**, **C₄**, **C₅**, **C₆**, and **C₇**; prepare calibrators from negative control urine or diluted negative control urine and Yohimbine working standard solution as described in Table 1 below.

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2. System washes designated **SYS₁** and **SYS₂**; prepare system washes from sample redissolving solvent (5% ACN + 0.2% formic acid).
3. Negative control sample designated **NC**; prepare negative control sample from negative control urine or diluted negative control urine.
4. Test sample(s) designated **TS_{1a...TS_{nb}}** where n is the total number of test samples; a and b are designations for sample replicates.
5. Solvent blank(s) designated **SB_{1a...SB_{nb}}** where n is the total number of test samples; a and b are designations for sample replicates.
6. Positive control samples designated **PC_a**, **PC_b**, and **PC_c** where a, b, and c are designations for sample replicates.
7. Standard mixture designated **S₁**.

XI. CALIBRATOR AND SAMPLE PREPARATION

- A. Pipette 10 µL of Reserpine-*IS* working standard solution into each labeled 12 × 75 mm test tube except those labeled **SYS₁**, **SYS₂**, **SB_{1a...SB_{nb}}** and **S₁**.
- B. Pipette Yohimbine working standard solution (ws1) into the calibrator tubes labeled **C₁**, **C₂**, **C₃**, **C₄**, **C₅**, **C₆**, and **C₇**. See Table 1. Evaporate solvent to dryness under nitrogen.
- C. Pipette 100 µL of Yohimbine working standard solution (ws2) into the tubes labeled **PC_a**, **PC_b**, and **PC_c**. See Table 1. Evaporate solvent to dryness under nitrogen.

Table 1. Volumes of working standard solutions required to prepare calibrators, control samples and test samples.

TUBE NO.	Volume of Yohimbine Working Standard Solution, µL	Volume of Reserpine-IS Working Standard Solution, µL	Equivalent to Yohimbine in the Urine, ng/mL	Equivalent to Reserpine-IS in the Urine, ng/mL
C ₁	5	10	5	50
C ₂	10	10	10	50
C ₃	20	10	20	50
C ₄	50	10	50	50
C ₅	100	10	100	50
C ₆	150	10	150	50
C ₇	200	10	200	50

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SYS ₁₋₂	0	0	na	na
NC	0	10	0	50
TS _{1a-1b}	0	10	unknown	50
SB _{1a-1b}	0	0	na	na
PC _{a-c}	100	10	100	50
S ₁	100	10	na	na

na = not applicable

- D. Pipette 1 mL of negative control urine or diluted negative control urine into the tubes labeled **NC**, labeled **PC_a**, **PC_b**, **PC_c**, **C₁**, **C₂**, **C₃**, **C₄**, **C₅**, **C₆**, and **C₇**.
- E. Pipette duplicate 1-mL aliquots of the test sample or diluted test sample into the tubes labeled **TS_{1a}** and **TS_{1b}**.
- F. Pipette 1 mL of water into each tube labeled **SB_{1a}...SB_{nb}**.
- G. Vortex the contents of each tube for 5-10 seconds.

XII. ENZYME HYDROLYSIS OF CONJUGATES

- A. Pipette 0.4 mL of β -glucuronidase enzyme into each tube.
- B. Vortex mix the contents of each tube for 5 -10 seconds.
- C. Adjust the contents of each tube to pH 4.5 - 5.5 with 2 N hydrochloric acid or 2 N sodium hydroxide, if necessary.
- D. Place the tubes in a Branson Ultrasonic Cleaner and incubate at approximately 65°C for 2 hours with sonication for 99 minutes.
- E. Remove the tubes from the incubator and allow them to cool to room temperature.

XIII. SAMPLE PREPARATION FOR SOLID PHASE EXTRACTION

- A. Dilute and adjust the pH of hydrolyzed urine specimens with 1.6 mL 0.6 M phosphate buffer, pH 6.5. Check and adjust pH to 6 - 7 using either 2 N HCl or 2 N NaOH.
- B. Centrifuge the tubes for 5-10 minutes at ~ 4000 rpm to remove sediment, if necessary.

XIV. SOLID PHASE EXTRACTION PROCEDURE

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- A. Wipe off the gasket with methanol soaked Kimwipe, then a dry Kimwipe.
- B. Place the Cerex™ PolyCrom Clin II solid phase columns on the manifold rack.
- C. Decant each solution into the corresponding column reservoir and adjust the flows so that the solutions flow through the columns in not less than 2 minutes.
- D. Rinse each column with 3 mL of water.
- E. Rinse each column with 2 mL of 1.0 M acetic acid.
- F. Rinse each column with 3 mL of methanol.
- G. Dry the columns under full vacuum for 2 minutes at ~20 psi.
- H. Place labeled 12 × 75 mm test tube into position under the corresponding cartridges. Verify that the cartridge tips are positioned into the tubes. Elute to collect with 3.0 mL of the elution solvent (methylene chloride:isopropanol-ammonium hydroxide: 78:20:2; v/v/v) after allowing to soak for ~ 1 minute.
- I. Prepare the standard mixture by adding 100 µL of Yohimbine working standard solution (ws1) and 10 µL of Reserpine I.S. solution to a 12 x 75 mm glass tube labeled S₁.
- J. Evaporate the contents of each tube to dryness at 50 ± 5 °C under nitrogen.

XV. REDISSOLVING PROCEDURE

- A. Prepare the system wash tubes by labeling two autosampler vials: SYS₁ & SYS₂
- B. Add 160 µL of redissolving solvent (5% Acetonitrile with 0.2% Formic acid) to each tube.
- C. Cap and vortex. Mix the contents of each tube for ~ 60 seconds. Transfer to an HPLC autosampler vial with insert.
- D. Submit the for LC/MS analysis.

XVI. LIQUID CHROMATOGRAPHIC/MASS SPECTRAL IDENTIFICATION OF YOHIMBINE AND/OR OH-YOHIMBINE

- A. Liquid Chromatographic and Mass Spectrometer Operating Parameters
 1. Instrumentation:
Thermo Scientific LTQ Linear Ion-Trap Mass Spectrometer and Agilent Technologies Model 1100 HPLC pump, autosampler, column compartment, and

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degasser. Xcalibur™ software (Thermo Scientific Inc., Santa Clara, CA) used for system control and data processing

2. LC column:
- a) type: ACE C18 (cat no. ACE-111-1002, Mac-Mod or equivalent)
 - b) length: 100 mm
 - c) i.d.: 2.1 mm
 - d) particle size: 3 µm
 - e) temperature: 25 °C

3. Mobile Phase

Time (min)	% ACN +0.2% Formic Acid	% Water +0.2% Formic Acid
0.0	10	90
0.5	10	90
7.0	90	10
7.5	90	10
7.51	10	90
13.0	10	90

- a) Solvents: HPLC Grade, Burdick & Jackson
- b) flow rate: 0.4 mL/min

4. Injection volume: 40 µL

5. Ionization and Detection:
Positive ion electrospray with LC/MS/MS detection of Yohimbine, OH-Yohimbine and Reserpine-IS.

- a) Yohimbine MS/MS Transitions followed: 355.2 amu (isolation width 1.5, collision energy 30%) → Full Scan MS (95-360 amu)
- b) OH-Yohimbine MS/MS Transitions followed: 371.2 amu (isolation width 1.5, collision energy 30%) → Full Scan MS (100-380 amu)
- c) Reserpine-IS MS/MS Transitions followed: 609.3 amu (isolation width 1.5, collision energy 25%) → Full Scan MS (165-615 amu)

Note: Collision energy is instrument dependent and may be modified.

6. Program:
Name: Yohimbine_confirm
Initial divert time: ca. 2.5 minutes or as appropriate for the retention times of the compounds of interest

B. Procedure

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1. Perform analyses in the order and with the acquisition methods specified in Table 2:

Table 2. Run number, vial number, acquisition method, and sample designation for LC-MS/MS analysis for identification and determination of Yohimbine and/or OH-Yohimbine from urine.

Run #	Vial	Method	Sample
1-7	1-7	Yohimbine_confirm	C₁, C₂, C₃, C₄, C₅, C₆, C₇
8-9	8-9	Yohimbine_confirm	SYS₁, SYS₂
10	10	Yohimbine_confirm	NC
11	11	Yohimbine_confirm	TS_{1a}
12	12	Yohimbine_confirm	SB_{1a}
13	13	Yohimbine_confirm	TS_{1b}
14*	14*	Yohimbine_confirm	SB_{1b}
15-17	15-17	Yohimbine_confirm	PC_a, PC_b, and PC_c
18	18	Yohimbine_confirm	S₁

Analyze additional test sample extracts and solvent blanks by duplicating the runs 11-14.

C. Evaluation of Mass Spectral Data for Yohimbine and OH-Yohimbine

Table 3. Quantifying and qualifying ions for analysis of Yohimbine and/or OH-Yohimbine in extracts of horse urine. The most abundant qualifying ions are indicated in **bold** type the least abundant qualifying ions are underlined.

Analyte	Qualifying ions (amu)	Quantifying Ions (amu)
Yohimbine MS/MS product ions	212.1 + 144 + <u>224.1</u>	212.1
OH-Yohimbine* MS/MS product ions	212.1 + 224.1 + 160.1	212.1
Reserpine-IS MS/MS product ions	58, 86 (ions are ~ equivalent in abundance)	448.2 + 397.2 + 577.3

* More than one hydroxy metabolite may be present.

1. Obtain the ion chromatogram for Yohimbine and OH-Yohimbine (if present) and the integrated MS/MS product ion areas for the quantifying ions (See Table 3) at the retention time of Yohimbine, OH-Yohimbine and Reserpine-IS for each calibrator, test sample, positive control sample, and the standard.
2. Obtain the integrated MS/MS product ion areas for the qualifying ions (See Table 3) at the retention time of Yohimbine for each test sample and the standard.

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3. Calculate the peak area ratio for Yohimbine for each calibrator, test sample, and positive control sample by dividing the quantifying ion areas of each analyte peak by the quantifying ion areas of the Reserpine-IS peak (see Table 3).
 4. Calculate the peak area ratio for the OH-Yohimbine metabolite(s) for each test sample by dividing the quantifying ion areas of each analyte peak by the quantifying ion areas of the Reserpine-IS peak (see Table 3)
 5. Measure the signal-to-noise of the least abundant MS/MS product ion at the retention time of Yohimbine for each replicate of each test sample.
 6. At the retention time of the Yohimbine and/or OH-Yohimbine, select and print the full scan spectrum from the negative control sample extract, test sample extracts, and solvent blanks from the corresponding data files.
- D. Criteria for Identification of Yohimbine from Urine Extracts
1. The retention times of the Yohimbine qualifying ions in the test sample must be within ± 0.1 min of the RT of the same ions from the corresponding standard.
 2. The relative ion area ratios of the Yohimbine qualifying ions must be within $\pm 20\%$ of the values of the same ions from the corresponding standard. The most abundant ion must be at m/z that shown in Table 3. The qualifying ions must maximize at the same retention time ± 0.1 min of each other.
 3. The MS/MS spectra for Yohimbine from each test sample extract and from the standard or Admin sample must have essentially the same fragmentation patterns.
 4. The chromatographic peak shape of Yohimbine qualifying ions must be approximately Gaussian, with a narrow base, with baseline separation from neighboring peaks, and with little evidence of tailing. The following criteria will define an acceptable peak:
 - a) The width of the peak at its base should be less than 1 minute.
 - b) The peak should appear to be Gaussian, *i.e.*, symmetrical about the vertical mid-line.
 - c) There should be no interfering peaks. A neighboring peak is considered to be interfering if the height from the baseline to the lowest part of the valley between the peaks is greater than 20% of the height of the peak of interest.
 - d) There is no significant peak tailing. Unacceptable peak tailing is defined

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as the condition in which the ratio of b to a is greater than 1.5 at 20% of the peak height where a is the time from the leading edge of the peak to the mid-line and b is the time from the mid-line to the trailing edge.

- E. Determination of the Concentration of Yohimbine and/or OH-Yohimbine in Urine
1. Plot the peak area ratios of the quantifying ions for each calibrator versus the concentration of the analyte in the calibrator. Perform linear regression analysis on these data to obtain the slope, intercept, and correlation coefficient of the standard curve.
 2. Calculate the concentration of Yohimbine in each test sample and positive control sample from the peak area ratios of the quantifying ions and the slope and intercept of the corresponding standard curve.
 3. Estimate the concentration of the OH-Yohimbine metabolite(s), if present, from the peak area ratios of the quantifying ions and the slope and intercept of the Yohimbine standard curve.
 4. Determine the average concentration for each test sample and positive control sample.

XVII. CRITERIA FOR REPEATING THE ANALYSIS

Repeat the analysis of the test sample if any of the following conditions apply:

- A. The peak area ratio of any test sample replicate is greater than the peak area ratio of the highest calibrator. Repeat the analysis after diluting the urine sample with water as described in Section VII of this Standard Operating Procedure.
- B. The negative control sample or the solvent blanks contain Yohimbine and/or OH-Yohimbine as evidenced by the presence of the characteristic ions and ion ratios within the expected retention time window.
- C. The standard curve for Yohimbine has a correlation coefficient less than 0.98.
- D. The Reserpine-IS ions are not detectable within the expected retention time window for any of the sample replicates.

XVIII. CRITERIA FOR REPORTING A SAMPLE POSITIVE FOR YOHIMBINE

Report a test sample as positive per this standard operating procedure for Yohimbine if all of the following criteria are met:

- A. The test sample contains Yohimbine according to the criteria described in XVI.D

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- B. The average concentration of Yohimbine in the test sample is greater than the lowest calibrator.
- C. The signal-to-noise ratio of the least abundant qualifying ion for Yohimbine in each replicate of the test sample is greater than 3.

XIX. INTERFERING SUBSTANCES

No known substances have been found to interfere with the determination of Yohimbine and/or OH-Yohimbine by this procedure.

XX. FITNESS FOR USE

This Standard Operating Procedure for identification and determination of Yohimbine and/or OH-Yohimbine from horse urine samples by LC-MS/MS was validated using in-house criteria for method evaluation and is fit for the intended use.

XXI. RESPONSIBLE PERSONS

- A. Analysts assigned to the Confirmation Section
- B. Supervisor of the Confirmation Section

XXII. REFERENCES

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