

Rapid Method for Identification and Quantitation of Acepromazine and its Major Metabolite in Horse Serum using an Ion Trap Mass Spectrometer

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Overview

Purpose: 1) Develop a method for identifying and quantitating acepromazine (ACE) and its major metabolite 2-(1-hydroxyethyl) promazine sulfide (HEPS) in horse serum.
2) Determine limits of detection (LOD) and quantitation (LOQ) of these two compounds in serum.
3) Demonstrate low level detection of acepromazine in a complex biological matrix.

Methods: Reversed phase HPLC with full scan MS/MS analysis using a Finnigan™ Deca™ ion trap mass spectrometer.

Results: 1) Full scan MS/MS analysis using a Finnigan Deca XP Plus ion trap mass spectrometer provides the selectivity and sensitivity necessary to support ADME studies of acepromazine in horse serum.
2) Acepromazine was detected in horse serum up to 2 hrs after dosing whereas its metabolite was detected for up to 6 hrs.
3) The limit of detection (LOD) for acepromazine in horse serum was determined to be 0.5 ng/mL, while the LOQ for its metabolite was 0.5 ng/mL.

Introduction

Acepromazine is widely used as a sedative in horses as it serves as a tranquilizer which facilitates handling of these large mammals before transportation or surgical procedures. Acepromazine has been classified by the Association of Racing Commissioners International, Inc. as a Class 3 drug in horses. Abuse of the drug may result in 60 days-6 months of suspension and up to \$1500 fine and loss of purse. Acepromazine has a slow elimination rate and stays in the body for an extended time. This can be problematic if the drug is administered before events since its calming effect can lead to enhanced performance by the horse.

Methods

HPLC system: Surveyor™ MS pump with surveyor autosampler
Column: 50 x 2.1 mm i.d. packed with 5 µm BDS Hypersil C18 stationary phase (Thermo Hypersil-Keystone)
Injection volume: 20 µL. Flow Rate: 300 µl/min
Mobile phase A: Water containing 10 mM ammonium acetate and 0.1% formic acid
B: Acetonitrile containing 0.1% formic acid
Gradient: 5-40% B in 4 min, 40-65% B in 1 min, 65-90% B in 0.5 min, 90-5% B in 1 min, at 5% B for 4 min.

Mass Spectrometer: Ion Trap Mass Spectrometer, Thermo Finnigan LCQ Deca XP Plus
Ionization mode: Positive electrospray ionization (ESI). Capillary temperature: 300 °C. Spray voltage: 4.5 kV. Sheath gas: 45 units. Sweep gas: 6 units, isolation width: 1.5

Table 1. MS/MS parameters for 1-(1-hydroxymethyl) promazine sulfide (HEPS), Acepromazine (ACE) and Chlorpromazine.

Analyte	MHP	Collision energy %	Scan Range
2-(1-hydroxyethyl)promazine sulfide (HEPS)	345.1	40	230-347
Acepromazine (ACE)	327.1	38	238-330
Chlorpromazine (internal standard)	319.1	40	230-322

Samples and internal standard

Acepromazine was administered to the horse and a serum sample drawn after 30, 45 min and 1, 2, 4, 6 and 24 hrs post dose. Chlorpromazine at a concentration of 100 ng/mL (0.1 ACN:1M acetic acid) was used as an internal standard.

Sample preparation

0.5 mL of the calibration standard/horse serum sample was mixed with 0.6 mL of chlorpromazine internal standard. The standard/sample was then frozen for 30 minutes, centrifuged and the supernatant used for analysis. The resulting internal standard concentration in the calibration standard and the sample was 120 ng/mL of serum.

Standards

Calibration standards were prepared as shown in Table 2
Where, A: 1ng/µL of ACE + HEPS in methanol
B: 0.1 ng/µL of ACE + HEPS in methanol

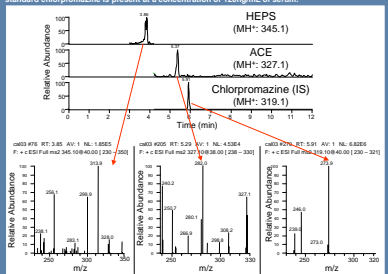
Table 2. Preparation of calibration standards

Calibration level	Volume of standard in 0.5 mL of serum (µL)	Concentration (ng/mL of serum)
Cal01	1.0 of B	0.2
Cal02	2.5 of B	0.5
Cal03	10 of B	2
Cal04	25 of B	5
Cal05	7.5 of A	15
Cal06	20 of A	40
Cal07	50 of A	100
Cal08	100 of A	200

Table 3. Top three product ions for HEPS, ACE and Chlorpromazine

Analyte	MHP	Product ions
HEPS	345.1	240+254+282
ACE	327.1	256+300+314
Chlorpromazine	319.1	239+246+274

Figure 1. Extracted ion chromatograms and MS/MS spectra for acepromazine (ACE) and 2-(1-hydroxyethyl) promazine sulfide (HEPS) at a concentration of 2ng/mL of serum. The internal standard chlorpromazine is present at a concentration of 120ng/mL of serum.



Results

Figure 1 shows extracted ion chromatogram from full scan MS/MS analysis of acepromazine (ACE), its major metabolite 2-(1-hydroxyethyl) promazine sulfide (HEPS) and the internal standard chlorpromazine. These chromatograms represent injection of horse serum containing 2 ng/mL of ACE and HEPS (40 pg on-column). The MS/MS spectra of the three analytes is also shown in Figure 1. The extracted ion chromatograms are generated by summing the three top intense ion in the MS/MS spectra as listed in Table 3.

Figure 2. Calibration curve for quantitation of acepromazine (ACE) in horse serum

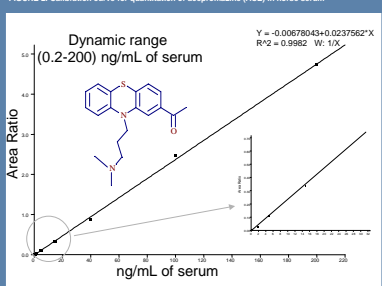


Figure 2 and 3 show standard curves for ACE and HEPS in horse serum with linearity over four orders of magnitude, i.e., 0.2 – 200 ng/mL. The R² value is 0.9982 for ACE and 0.9922 for HEPS. These calibration curves were generated with chlorpromazine (120 ng/mL) as the internal standard and illustrate that the wide linear dynamic range afforded by Deca XP Plus ion trap mass spectrometer.

Figure 3. Calibration curve for quantitation of 2-(1-hydroxyethyl) promazine sulfide (HEPS) in horse serum

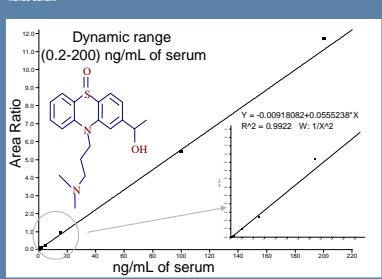


Figure 4. Extracted ion chromatograms for ACE and HEPS at a concentration of 0.5 ng/mL of horse serum. The internal standard chlorpromazine is present at a concentration of 120 ng/mL of serum

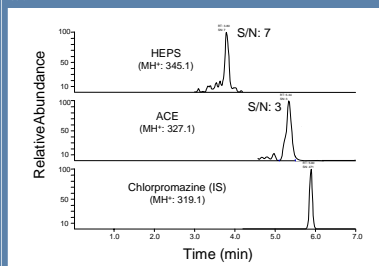


Figure 4 shows the extracted ion chromatograms for ACE and HEPS at a concentration of 0.5 ng/mL in serum. The signal to noise for ACE at 0.5 ng/mL of serum is 3:1 and hence represents the lower limit of detection for this compound. The signal to noise for HEPS at concentration of 0.5 ng/mL of horse serum is 7:1. This level thus represents the lower limit of quantitation for HEPS. Table 4 shows the lower limit of detection and quantitation for ACE and HEPS indicating that these analytes can be detected at reasonably low levels in horse serum.

Table 4. Limit of detection (LOD) and quantitation (LOQ) for ACE and its major metabolite, HEPS.

	LOD (Amount injected on column)	LOQ (Amount injected on column)
Acepromazine	5 pg	5 pg
2-(1-hydroxyethyl) promazine sulfide	2 pg	5 pg

Trademarks

Finnigan, Surveyor, and LCQ are trademarks of Thermo Electron.

Figure 5. Extracted ion chromatograms for ACE and HEPS obtained using full-scan MS/MS scans for analysis of horse serum sample obtained 4 hours post dosing.

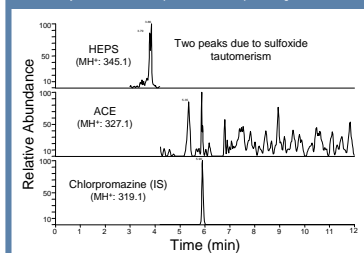


Figure 5 shows extracted ion chromatogram for ACE and HEPS obtained from full scan MS/MS analysis of horse serum sample obtained 4hr post administration of drug. No significant level of ACE could be detected in the horse at this time whereas its metabolite HEPS could be quantitated and its concentration determined as 1.3 ng/mL of serum. Determination of concentration of ACE and HEPS in ng/mL of horse serum drawn at different time points post administration is tabulated in Table 5. This shows that ACE can be detected in horse serum for up to 2 hrs after dosing whereas its metabolite can be detected for up to 6 hrs

Table 5. Determination of concentration of ACE and HEPS in ng/mL of horse serum drawn at different time points post administration of drug

Time	Amount (ng/mL of serum)	
	ACE	HEPS
30 min	2.3	1.8
45 min	1.6	1.7
1 hr	1.2	1.7
2 hr	0.6	1.3
4 hr	nd	1.3
6 hr	nd	1.4
24 hr	nd	nd

Conclusions

Full scan MS/MS analysis using a Finnigan Deca XP Plus ion trap mass spectrometer provides the selectivity and sensitivity necessary to support ADME studies of acepromazine in horse serum. Acepromazine was detected in horse serum up to 2 hrs after dosing whereas its metabolite was detected for up to 6 hrs. The limit of detection achieved for acepromazine in horse serum was determined to be 0.5 ng/mL, while the LOQ for its metabolite was 0.5 ng/mL.