Application Note: 318

Determination of Acepromazine and its Major Metabolite in Equine Serum by LC-MS/MS using the Finnigan LCQ Deca XP Plus Ion Trap Mass Spectrometer

Key Words

- Sensitivity
- Quantitation
- Finnigan™ LCQ™ Deca XP Plus
- Equine serum
- Acepromazine

Chromatography and Mass Spectrometry Application Report

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Introduction

Acepromazine is widely used as a sedative in horses. It serves as a tranquilizer to better facilitate 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 drugs in the Class 3 category can result in 2-6 months of suspension, up to $1500 fine, and loss of purse. Acepromazine has a slow elimination rate and stays in the body for a long time. This can be problematic if the drug is administered before a race event since its calming effect can lead to a better performance by the horse.

Goal

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

Experimental

HPLC

HPLC system:	Surveyor® MS pump with Surveyor autosampler
Column:	50 × 2.1 mm Hypersil® BDS C18 (Thermo Electron)
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

Mass spectrometer:	Finnigan LCQ Deca XP Plus
Ionization mode:	Positive ESI
Temperature:	300 °C
Spray voltage:	4.5 kV
Sheath gas:	45 units
Sweep gas:	6 units
Isolation width:	1.5

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

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Standards

Calibration standards were prepared as follows:

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

Where, A: 1 ng/µL of ACE + HEPS in methanol
B: 0.1 ng/µL of ACE + HEPS in methanol

Samples

Acepromazine was administered to the horse, and serum samples were drawn 30, 45 min and 1, 2, 4, 6 and 24 hrs after dosing.

Internal Standard

Chlorpromazine at a concentration of 100 ng/mL (9:1 ACN:1 M acetic acid) was used as the 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 refrigerated for 30 min, 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.

Results and Discussions

Full Scan LC-MS/MS Analysis

Figure 1 shows extracted ion chromatograms from full-scan MS/MS analysis of acepromazine (ACE), its major metabolite 2-(1-hydroxyethyl) promazine sulfoxide (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 intensities of the three most abundant ions in the MS/MS spectra as shown in Table 1.

Table 1. Top three product ions for ACE, HEPS and chlorpromazine

Analyte	MH^+	Product ions
Acepromazine (ACE)	327.1	240+254+282
2-(1-hydroxyethyl)promazine sulfoxide (HEPS)	345.1	256+300+314
Chlorpromazine (internal standard)	319.1	239+246+274

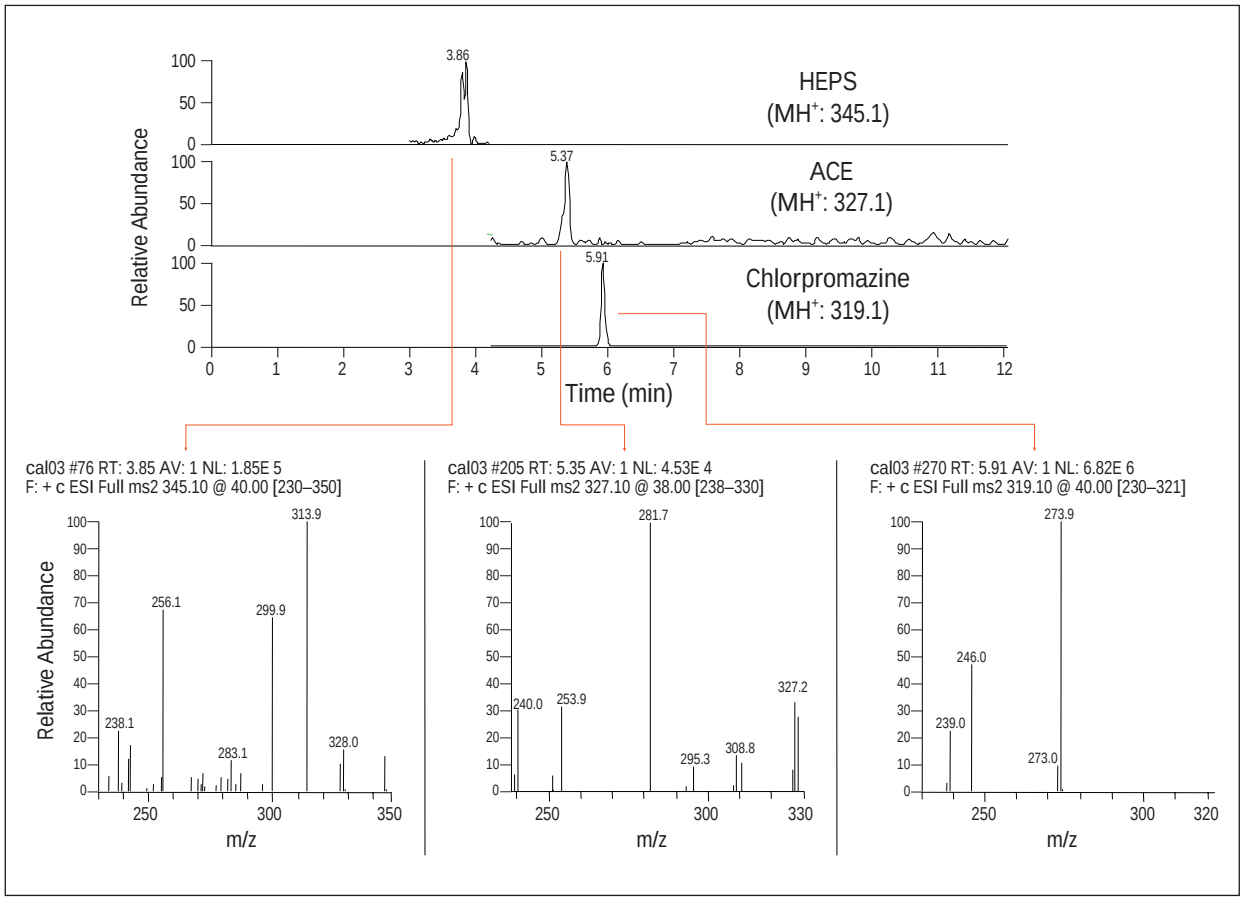

Figure 1. Extracted ion chromatograms and MS/MS spectra for acepromazine (ACE) and 2-(1-hydroxyethyl) promazine sulfoxide (HEPS) at a concentration of 2 ng/mL of serum. The internal standard chlorpromazine is present at a concentration of 120 ng/mL of serum.

Quantitation and Linear Dynamic Range

Figures 2 and 3 show calibration curves for ACE and HEPS in horse serum with linearity over four orders of magnitude, i.e., 0.2 – 200 ng/mL. The R^2 value is 0.9952 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 of the Finnigan LCQ Deca XP Plus ion trap mass spectrometer enables quantitation of analytes in biological matrices.

Limit of Detection and Quantitation

Figure 4 shows the extracted ion chromatograms for ACE and HEPS at a concentration of 0.5 ng/mL of 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 (LOD) for this compound. The signal to noise for HEPS at concentration of 0.5 ng/mL of horse serum is 7:1 and represents the lower limit of quantitation (LOQ) for HEPS. Table 2 shows the lower limits of detection and quantitation for ACE and HEPS indicating that these analytes can be detected at low levels in horse serum with minimal sample preparation.

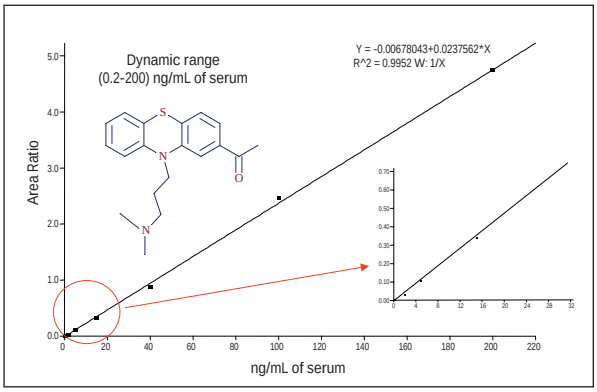

Figure 2. Calibration curve for quantitation of acepromazine (ACE) in horse serum.

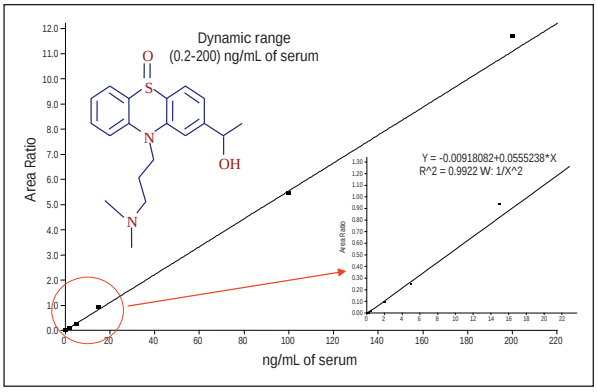

Figure 3. Calibration curve for the quantitation of 2-(1-hydroxyethyl) promazine sulfoxide (HEPS) in horse serum.

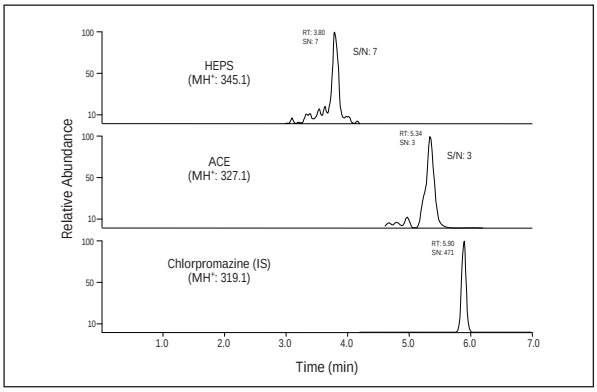

Figure 4. Extracted ion chromatograms for ACE and HEPS at a concentration of 0.5 ng/mL of horse serum. The internal standard chlorpromazine is present at a concentration of 120 ng/mL of serum.

Table 2. Limit of Detection (LOD) and Quantitation (LOQ) for ACE and its major metabolite, HEPS.

Analyte	LOD (on-column)	LOQ (on-column)
Acepromazine	5 pg	5 pg
2-(1-hydroxyethyl) promazine sulfoxide	2 pg	5 pg

Determination of ACE and HEPS in Samples from the Horse

Figure 5 shows extracted ion chromatograms for ACE and HEPS obtained by full-scan MS/MS analysis of serum sample obtained 4-hr 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 concentrations of ACE and HEPS in ng/mL of horse serum drawn at different time points post administration is tabulated in Table 3. It is seen that ACE could be detected in horse serum for up to 2 hr after dosing whereas its metabolite was detected for up to 6 hr.

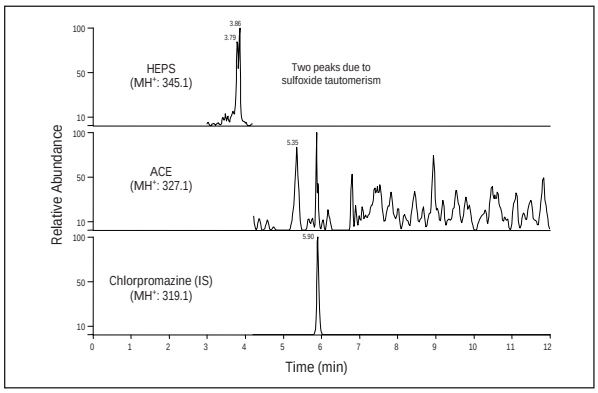

Figure 5. Extracted ion chromatograms for ACE and HEPS obtained by full-scan MS/MS analysis of horse serum sample obtained 4 hours post administration of drug.

Table 3. Determination of concentrations 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 with a Finnigan LCQ 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 for up to 2 hr after dosing whereas its metabolite was detected for up to 6 hr. The LOD 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.

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