MS/MS as an LC Detector for the Screening of Drugs and Their Metabolites in Race Horse Urine

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Introduction

Imipramine is a tricyclic antidepressant drug that is not classifed as a controlled substance by the Drug Enforcement Administration, but is classified by the Association of Racing Commissioners International Inc. as a class 2 drug in horses. Desipramine is a major metabolite of imipramine. Extracts of horse urine were screened for both analytes using on-line LC–PDA MS/MS. The urine sample was first treated with b-glucuronidase to hydrolyze glucuronide conjugates of imipramine and desipramine, followed by solid phase extraction. The concentration of imipramine and desipramine in the sample was determined by the internal standard method using the peak area ratio and linear regression analysis. This application note presents a rapid method for quantifying imipramine and desipramine in horse urine, illustrating the advantages of MS/MS detection in terms of specificity, sensitivity and unambiguous identification for the analysis of drugs and their metabolites.

Goal

1. Develop a rapid method to identify and quantitate tricyclic antidepressant imipramine and its major metabolite desipramine in horse urine.
2. Demonstrate the advantages of using MS/MS to identify and confirm the presence of imipramine and its metabolites.
3. Determine presence and structure of minor metabolites using Data Dependent™ LC–MS/MS analysis.

Experimental Conditions HPLC

LC System: Finnigan™ Surveyor™ MS pump with Surveyor autosampler and Surveyor PDA detector
Mobile phase: A: water containing 0.2% formic acid B: Acetonitrile containing 0.2% formic acid
Column: 50 × 2.1 mm ID packed with 5 µm BDS-Hypersil C18 stationary phase
Injection volume: 1 µL
Flow-rate: 200 µL/min

Finnigan Surveyor gradient pump programme.

Time (min)	% A	% B
0	98	2
0.2	98	2
8	25	75
9	10	90
10	10	90
10.01	98	2
15	98	2

Gradient

Mass Spectrometer

Mass spectrometer: Finnigan LCQ Advantage MAX™
Ionization mode: Positive electrospray ionization (ESI),
Capillary temperature: 275 °C
Spray voltage: 4.5 kV
Sheath gas: 30 units
Sweep gas: 8 units

Standards

Calibration and working standards used were solutions of imipramine, desipramine, and clomipramine.

Samples and Internal Standard

Imipramine was administered to the horse and a urine sample was drawn at 0, 2, 4, 8 and 24 hrs following the dose. 1 mL of the urine sample was spiked with 10 µL of 50 ng/µL Clomipramine internal standard.

Sample Preparation

The calibration standard and urine samples were treated with

Figure 1: LC–MS/MS analysis of Imipramine, Desipramine and Clomipramine (internal standard).

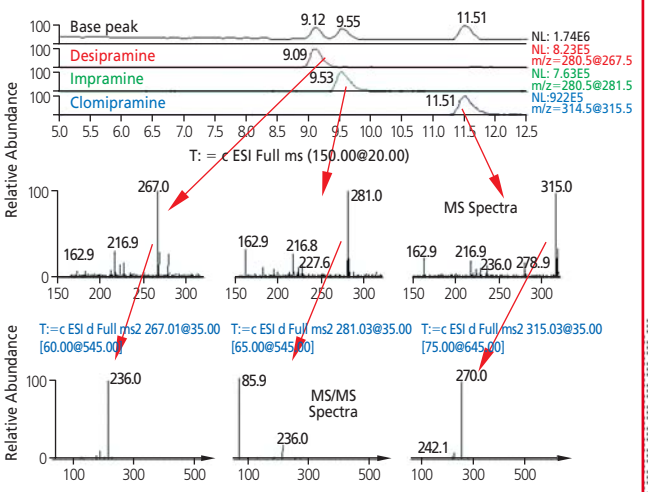


Figure 2: LC–UV analysis of Imipramine, Desipramine and Clomipramine (internal standard).

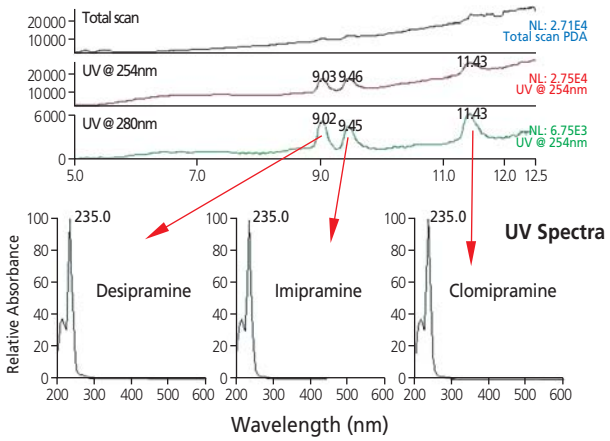
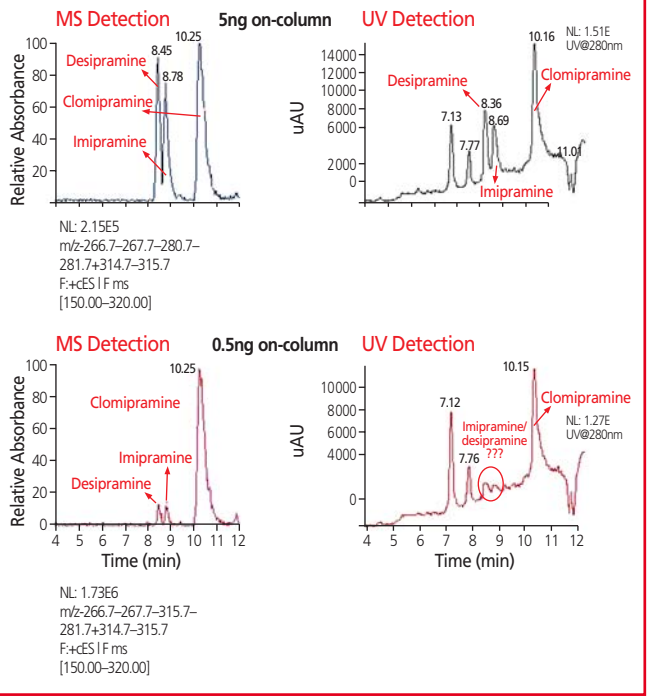


Figure 3: Comparison of MS and UV detection at two different concentrations.



b-glucuronidase to hydrolyze glucuronide conjugates of Desipramine and Imipramine, followed by solid-phase extraction.

Results and Discussions

LC-UV-MS/MS analysis of imipramine and desipramine:

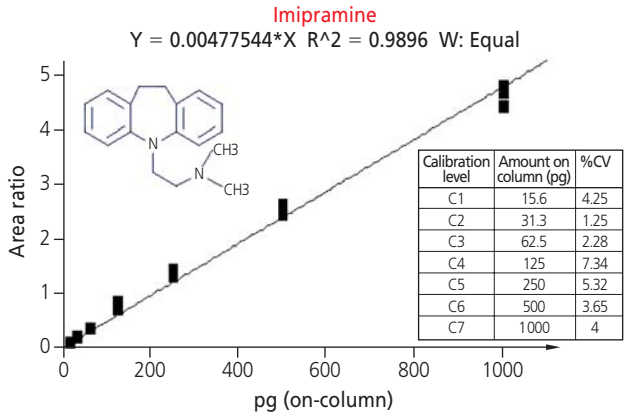
Figures 1 and 2 show the analysis of tricyclic antidepressant imipramine, its major metabolite desipramine, and the internal standard clomipramine by LC with MS/MS and UV detection, respectively. Figure 1 shows base peak and extracted ion chromatograms for the three analytes along with MS and MS/MS spectra. The MS and MS/MS spectra help in unambiguous identification of these analytes and represent the high specificity that can be obtained from such an analysis. Further, the MS/MS spectra can be stored in a library and used for rapid confirmation of the drug and its metabolite. Figure 2 shows total spectra obtained from a PDA detector as well as a UV trace at 254 nm and 280 nm. The position of elution of the three compounds had to be determined by sequential injections of individual analytes. As shown in Figure 2, the UV spectra for these compounds looks almost identical making their unambiguous identification difficult.

Figure 3 shows chromatograms obtained for the analysis of imipramine, desipramine and clomipramine (IS) with MS and UV detection at levels of 5 and 0.5 ng on-column. At 0.5 ng on-column, both imipramine and desipramine could be easily identified when MS was used as a detector whereas these analytes were hardly visible in the UV trace. The concentration of clomipramine is the same at both these levels. This illustrates the excellent sensitivity that can be obtained during analysis by LC-MS/MS.

Quantitation of Imipramine and Desipramine in Horse Urine

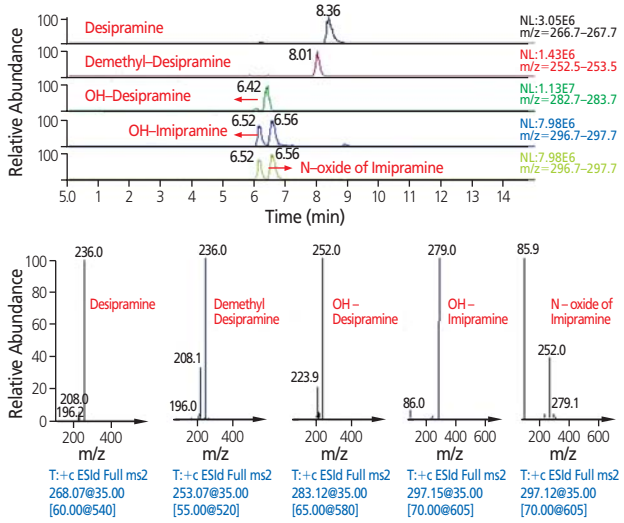
Figure 4 shows calibration curve obtained for imipramine in horse urine with clomipramine used as an internal standard. The coefficient of correlation for the calibration curve is 0.9896 with the %CV value of less than 7%. A similar calibration curve was also obtained for desipramine (data not shown). These calibration curves can be used for determining the amount of imipramine and desipramine in horse urine samples drawn at different times post

Figure 4: Calibration curve for Imipramine in horse urine.



Calibration level	Amount on column (pg)	%CV
C1	15.6	4.25
C2	31.3	1.25
C3	62.5	2.28
C4	125	7.34
C5	250	5.32
C6	500	3.65
C7	1000	4

Figure 5: Data Dependent LC–MS/MS analysis of metabolites of imipramine.



administration of the drug. Table 2 shows the amount of these two analytes as determined in horse urine at different times. For the sample drawn 2 hrs post administration of the drug, the amount of imipramine and desipramine was determined to be 28 and 1567 ng/mL, respectively. The amount of desipramine determined at this time is above the upper limit of quantitation for the calibration curve.

Identification of Metabolites of Imipramine

A urine sample from the race horse obtained 2 hrs after administration of the drug was also analysed by Data Dependent LC–MS/MS with MS/MS on the top two most intense ions to determine the presence of other metabolites. The extracted ion chromatograms in Figure 5 show the presence of 4 additional metabolites: desmethyl desipramine, OH desipramine, OH-imipramine, and N-Oxide of Imipramine, as well as their MS/MS fragmentation pattern. As indicated by the two peaks in the extracted ion chromatogram for *m/z* 297.2, imipramine is metabolized to two metabolites that have the same *m/z*. In this case, the MS/MS fragmentation pattern enables unambiguous distinction between the two metabolites.

Conclusions

Full scan MS/MS analysis using a Finnigan LCQ Advantage MAX ion trap mass spectrometer provides the selectivity and sensitivity necessary to support ADME studies of imipramine in horse urine.

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