Application Note: 315

Sensitivity and Quantitation using a Finnigan LCQ Deca XP Plus Ion Trap Mass Spectrometer

Gargi Choudhary, Tania A. Sasaki, and Julian J. Phillips, Thermo Electron Corporation, San Jose, CA

Key Words

- Sensitivity
- Quantitation
- LCQ Deca XP Plus
- Ion Trap Mass Spectrometer

Chromatography and Mass Spectrometry Application Report

Introduction

Ion trap mass spectrometry is widely recognized for solving analytical problems requiring high sensitivity MS^n such as for structural elucidation and identification of unknowns in complex matrices. Furthermore, its full-scan sensitivity is responsible for its utility in screening analysis. Although the popularity of ion trap mass spectrometry is concentrated mostly on qualitative analyses, these mass spectrometers perform well for quantitative analyses as well.

Goal

In this report we have used a full-scan MS/MS quantitative assay of antipsychotic drug alprazolam and its analog temazepam spiked in protein precipitated bovine plasma to evaluate the quantitative abilities of a Finnigan™ LCQ™ Deca XP Plus ion trap mass spectrometer.

Experimental

Compounds

Analytes: Temazepam and alprazolam
Internal Standard (ISTD): alprazolam-d5

Sample preparation

The proteins from a plasma matrix were precipitated by the addition of an equal volume of 80:20 acetonitrile: methanol containing 1% acetic acid. The plasma sample was then centrifuged for 5 minutes and supernatant decanted.

The supernatant was spiked with appropriate levels of analytes and the internal standard.

Sample solutions

Standard samples: 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 40, 100, 200, 400, 1000 pg/µL
Internal Standard (ISTD): 10 pg/µL

HPLC

HPLC system: Surveyor® MS pump with Surveyor autosampler
Column: 20 × 2.1 mm i.d. packed with 5 µm AQUASIL C18 stationary phase (Thermo Electron).
Injection volume: 5 µL
Flow Rate: 400 µL/min
Mobile phase A: water containing 10 mM ammonium acetate
B: acetonitrile containing 0.1 % formic acid
Gradient: 45% B for 3 minutes

Mass Spectrometer

Ionization mode: Positive electrospray ionization (ESI)
Capillary temperature: 350°C
Spray voltage: 4.5 kV
Sheath gas: 45 units
Sweep gas: 10 units
Maximum inject time: 400 ms
Isolation width: 1.0
Collision energy: 40%
Number of segments: 2
Segment 1:
Full-scan MS/MS
m/z 301.2 → m/z [100-305]
Segment 2:
Full-scan MS/MS
Scan event 1
m/z 309.2 → m/z [100-311]
Scan event 2
m/z 314.2 → m/z [100-320]

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Analysis

The standard samples were analyzed with five replicate injections at each concentration level and a blank (MeOH) injection between each level.

Results and Discussion

Eleven calibration standards over a concentration range of four orders were used to evaluate the quantitative performance of the LCQ Deca XP Plus mass spectrometer.

Figure 1 shows the extracted ion chromatograms from full-scan MS/MS of alprazolam (500 fg on-column) and alprazolam-d5 internal standard (50 pg on-column) in protein precipitated bovine plasma. These chromatograms were generated by summing the two most intense product ions observed in the full-scan MS/MS spectra (Figure 2) acquired for the analyte (m/z 274 + 281) and the internal standard (m/z 279 + 286). The LOD was determined to be 500 fg for alprazolam, with a signal-to-noise of 5 to 1.

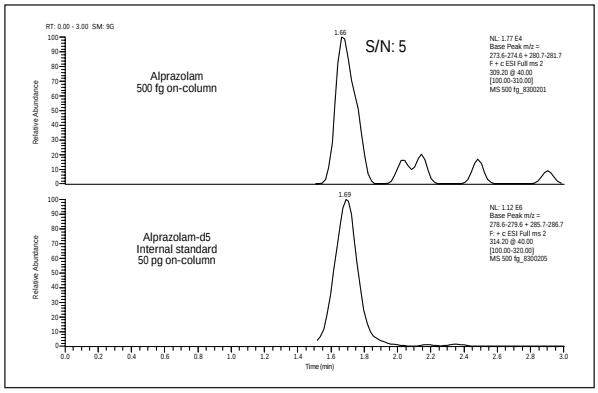

Figure 1. Extracted ion chromatograms of alprazolam (500 fg on-column) and alprazolam-d5 (50 pg on-column) internal standard in crashed bovine plasma.

Figure 3 shows the extracted ion chromatograms from full-scan MS/MS of temazepam and alprazolam spiked in protein-precipitated bovine plasma at 0.2 pg/µL (1 pg on-column) and the internal standard alprazolam-d5 at 10 pg/µL (50 pg on-column). Figure 1 shows extracted ion chromatograms of the most intense product ions observed in the full-scan MS/MS spectra for temazepam (m/z 255), alprazolam (m/z 274 + 281), and the internal standard, alprazolam-d5 (m/z 279 + 286). Figure 4 shows the full-scan MS/MS spectra of temazepam, and alprazolam at a concentration level of 1 pg on-column and the internal standard, alprazolam-d5 (50 pg on-column). This concentration level exhibited a signal-to-noise ratio of 7 to 1 for temazepam and 9 to 1 for alprazolam.

The calibration curve for alprazolam over the entire range of the assay is shown in Figure 5, with the linearity over the dynamic range from 500 fg to 5 ng on-column. The correlation coefficient is 0.9991. The precision values for the alprazolam assay are displayed in Table 1. The % CV or RSDs show that the LCQ Deca XP Plus ion trap mass spectrometer is capable of quantitating analytes at low picogram amounts in plasma. Table 2 shows that the limit of detection (LOD) and quantitation (LOQ) for the antipsychotic drug alprazolam are determined to be 500 fg and 1 pg, respectively, whereas that for its analog temazepam are 1 pg and 2.5 pg, respectively.

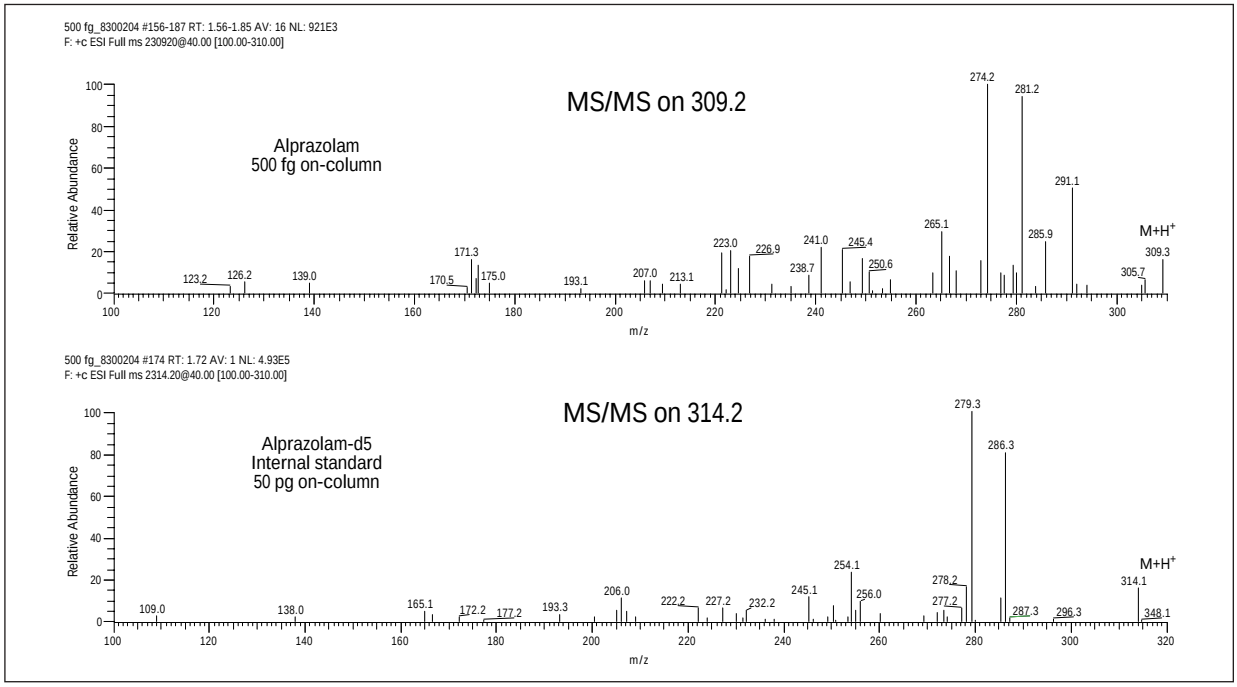

Figure 2. MS/MS spectra of alprazolam (500 fg on-column) and alprazolam-d5 internal standard.

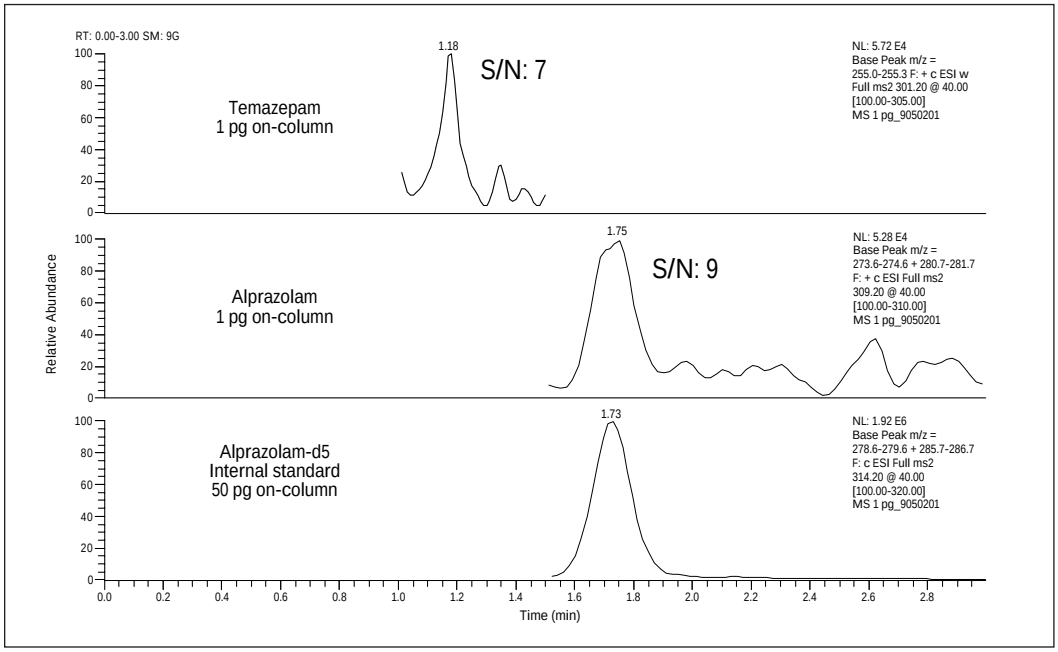

Figure 3. Extracted ion-chromatograms of temazepam and alprazolam spiked in bovine plasma at 0.2 pg/µL (1 pg on-column) and alprazolam-d5 at 10 pg/µL (50 pg on-column) internal standard.

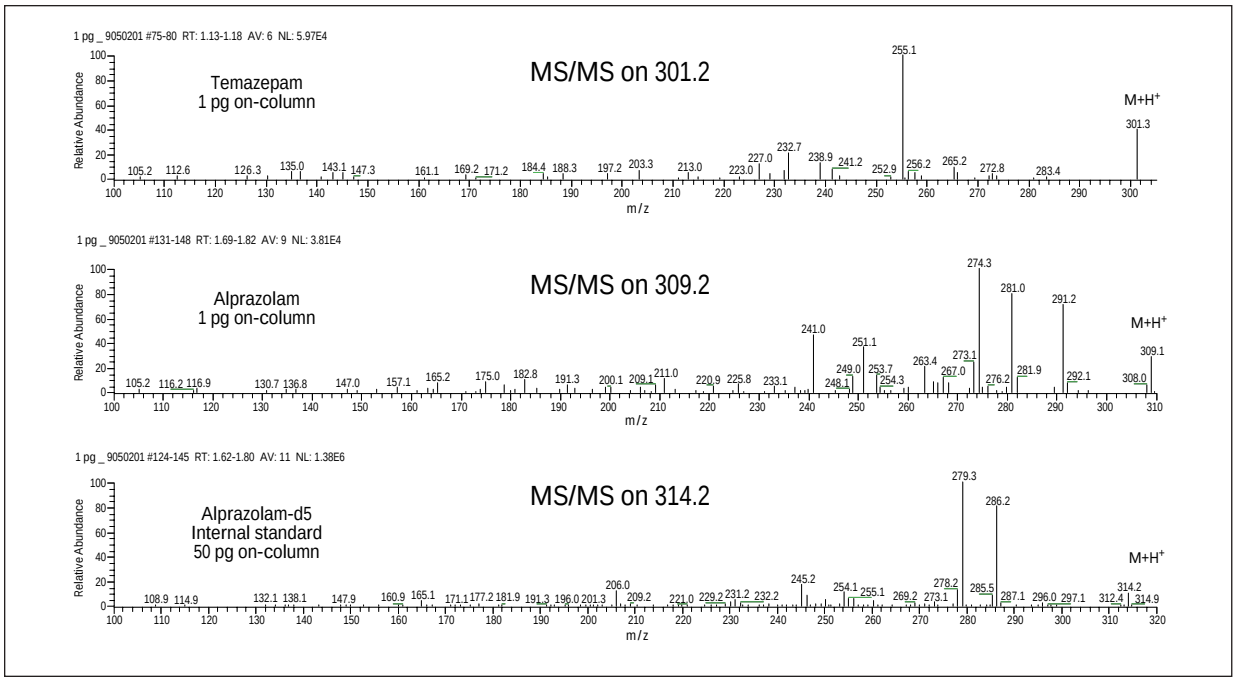

Figure 4. MS/MS spectra of temazepam and alprazolam (1 pg on-column) and alprazolam-d5 (50 pg on-column) internal standard.

Conclusions

These measurements demonstrate that the LCQ Deca XP Plus ion trap mass spectrometer can be used to effectively quantify compounds in biological matrices. Benzodiazapines were successfully quantified with linearity over four orders of magnitude for alprazolam. The CV was around 5-7% except near the LOQ, where the CV was 15%. The LOD and LOQ for alprazolam is 500 fg and 1 pg, respectively whereas that for temazepam are 1 pg and 2.5 pg, respectively.

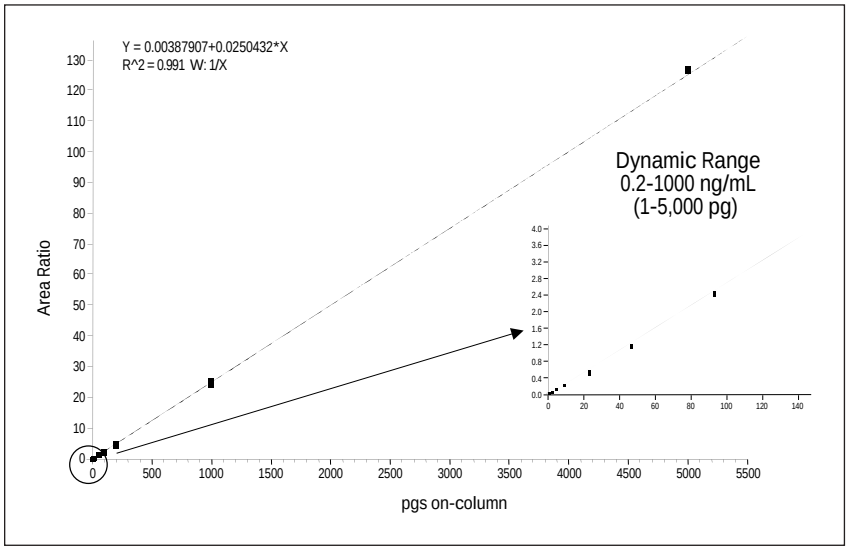

Figure 5. Calibration curve for quantitation of alprazolam in crashed bovine plasma.

Table 1

Within day precision (from five replicate injections) for assay of alprazolam in bovine plasma.

Amount on-column (pg)	% CV
0.5	13.9
1.0	14.9
2.5	7.3
5	6.4
10	6.5
25	5.3
50	3.2
100	4.8
200	1.3
1000	2.7
5000	1.8

Table 2

Amounts injected on-column for analytes in bovine plasma.

Analyte	LOD	LOQ
Alprazolam (309.2)	500 fg	1 pg
Temazepam (301.2)	1 pg	2.5 pg

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