Detection of Alprazolam and Temazepam in a Bovine Plasma Matrix Using the Agilent 500 Ion Trap LC/MS

Application Note

Pharmaceuticals

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Abstract

The Agilent 500 Ion Trap LC/MS detects, and accurately quantitates alprazolam (Xanax) and temazepam (Restoril) in the Bovine Serum Albumin (BSA) matrix, both before and after protein precipitation.

Introduction

Alprazolam (Xanax) and temazepam (Restoril) were analyzed in a Bovine Serum Albumin (BSA) matrix, both before and after protein precipitation using Liquid Chromatography Electrospray Ionization Mass Spectrometry (LC/ESI/MS/MS). The quantitative ability of the Agilent 500 Ion Trap LC/MS to detect, and accurately quantitate these drugs in the BSA matrix, is investigated here.

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Instrumentation

- Agilent ProStar 210 Solvent Delivery Module (2)
- Agilent 500 Ion Trap LC/MS equipped with an ESI source
- Agilent ProStar 420 Autosampler

Materials and Reagents

All solvents (reagent or HPLC Grade) and the Bovine Serum Albumin (p/n A4503-10G) were purchased from Sigma-Aldrich Corporation (St. Louis, MO). The analytes, temazepam (p/n T-907), alprazolam (p/n A-903), and alprazolam-d5 (Internal Standard, p/n A-910), were purchased from Cerilliant Corporation (Round Rock, TX).

Sample Preparation

Stock solutions were purchased at a concentration of 1 mg/mL in methanol. Further dilutions of the stock solutions were carried out in a 1:1 mixture of 2 mM ammonium acetate in 0.1 % aqueous formic acid: 0.1% formic acid in acetonitrile.

No matrix sample preparations

Solutions of 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10, 20, 40, 100, 200, 400, and 1000 pg/µL of alprazolam and temazepam were made from the stock solution.

Post-protein crash matrix preparation

Equal volumes of 0.3 mM Bovine Serum Albumin (BSA) in Phosphate Buffered Saline (PBS) and an 80:20 acetonitrile: methanol solution containing 0.1% acetic acid were centrifuged for 5 minutes at 12000 rpm and refrigerated for 1.5 hours. The supernatant was removed and spiked with the internal standard (10 pg/µL of alprazolam-d5) and the analytes in the same concentrations as used in the matrix preparation.

Pre-protein crash matrix preparations

The 0.3 mM BSA in PBS was spiked with the internal standard (10 pg/µL of alprazolam-d5) and the analytes in the same concentrations as the no matrix preparations. An equal volume of the 80:20 acetonitrile:methanol solution containing 0.1% acetic acid was added to the spiked BSA solutions. The mixtures were centrifuged for 10 minutes at 12000 rpm and then refrigerated for 1.5 hours. The supernatant was removed and subsequently analyzed. Each sample was run in triplicate for calibration curve purposes on a 500 Ion Trap.

Instrument Conditions

LC conditions

Column	Pursuit C18 050 × 020, 5 m (Agilent p/n 3000 - 050 × 020)	
Guard column	(Matrix runs only): Pursuit C18 10 × 2.0 mm, 5 m Direct Connect (Agilent p/n A3000-010D020)	
Solvent A	2 mM ammonium acetate in 0.1% aqueous formic acid	
Solvent B	0.1% formic acid in acetonitrile	
Flow rate	0.4 mL per min	
Injection volume	5 uL	
LC program	Time (min)	% B
	0	5
	0:30	95
	3:30	95
	3:40	5
	5:00	5

Mass spectrometry conditions

Ionization mode	ESI positive
Scan time	1.91 seconds/scan
Multiplier offset	100 V
Nebulizing gas pressure	49 psi
Drying gas pressure	39 psi at 350 °C
Needle (V)	5000
Shield (V)	400
Isolation window	5.0 *m/z*

Matrix scan conditions

	Alprazolam	Temazepam	Alprazolam-d5
Precursor ion (*m/z*)	309.2	301.2	314.2
Capillary (V)	60	70	100
Excitation amplitude (V)	1.0 Resonant	62 Non-resonant	0.5 Resonant
Product scan (*m/z*)	102–311	100–305	104–320
Product ions (*m/z*)	281.2 + 274	255.2	286.2 + 279.2

Each analyte was run individually with the internal standard in an MRM experiment for a total run time of 5 minutes. For the no matrix run, the analytes and internal standard were run individually in a MS/MS experiment with the product scan centered around the specific product ions.

Discussion

Calibration curves were run without the matrix, with the "after crash" spiked matrix, and the "before crash" spiked matrix with good linearity observed for each analyte. The extracted ion chromatogram of 500 fg (on column) of temazepam (*m/z* = 255.2) in the "before crash" matrix is shown in the top panel of Figure 1, while the bottom panel of Figure 1 is the extracted ion chromatogram of the internal standard, alprazolam-d5 (50 pg on column). The S/N (peak to peak) of temazepam in the spiked matrix was determined to be 16:1 at 500 fg on column.

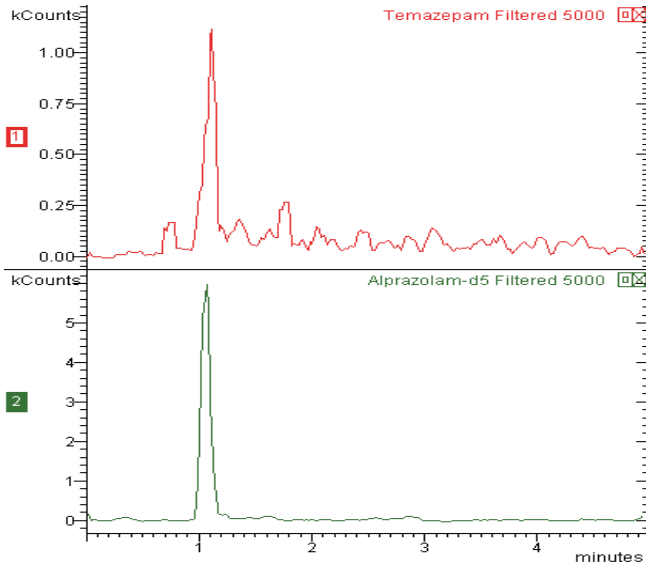

Figure 1. Extracted ion chromatogram of temazepam (500 fg on column) and alprazolam-d5 (50 pg on column) after being spiked in a BSA matrix.

The LOD (limit of detection) and LOQ (limit of quantitation) values for the three different types of matrix environments are summarized in Table 1. For both temazepam and alprazolam, the LOD and LOQ values are the same for all of the matrix conditions. A calibration curve of alprazolam in a "before crash" spiked matrix of these compounds is shown in Figure 2. An R^2 value > 0.99 was observed for both compounds in all of the matrices studied, over the calibration range of 0.2–1000 pg/µL (1–5000 pg on column).

Table 1. LOD and LOQ Values Calculated for the Three Matrix Conditions for both alprazolam and temazepam

	Alprazolam	Temazepam
LOQ (pg on column)		
No matrix	1.0	1.0
"after crash" spiked matrix	1.0	1.0
"before crash" spiked matrix	1.0	1.0
LOD (pg on column)		
No matrix	0.5	0.5
"after crash" spiked matrix	0.5	0.5
"before crash" spiked matrix	0.5	0.5

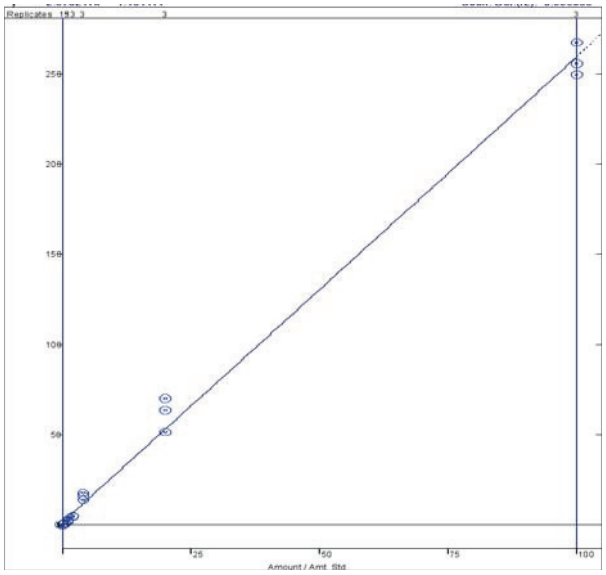

Figure 2. Calibration curve of alprazolam in a "before crash" spiked bovine matrix.

Even with the addition of the matrix, the range of quantitation did not change. Figure 3 shows the extracted ion chromatogram (*m/z* = 255.2) of temazepam in all three of the matrix conditions.

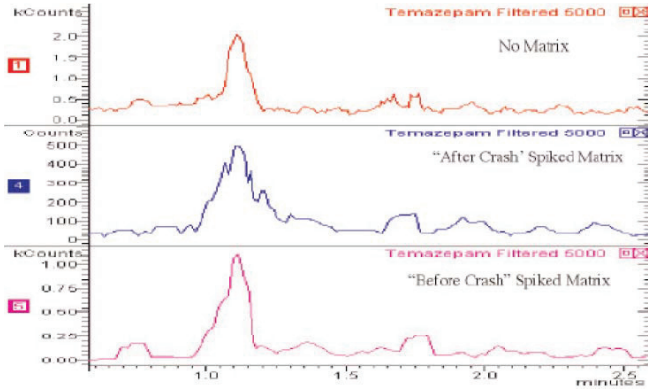

Figure 3. Extracted ion chromatogram (m/z = 255.2) of 500 fg of temazepam in no matrix, the "after crash" matrix, and the "before crash" spiked matrix.

It is evident from Figure 3 that the Agilent 500 Ion Trap LC/MS is capable of handling a biological matrix, and thus is still able to reach a low level of detection. These results show that the 500 Ion Trap is a sensitive and quantitative tool for the detection of target analytes in biological matrices.

Conclusion

The measurements taken with alprazolam and temazepam spiked into bovine plasma matrix illustrate that it is possible to reach low levels of quantitation (low- to sub-pg level) using the Agilent 500 Ion Trap LC/MS. Quantitaive analysis of temazepam and alprazolam in a biological matrix has been demonstrated in the 500 Ion Trap instrument for the first time, illustrating the use for this instrument in both quantitative and qualitative analysis.

References

G. Choudhary, T. A. Sasaki, and J. J. Phillips, "Sensitivity and Quantitation using a Finnigan LCQ Deca XP Plus Ion Trap Mass Spectrometer," Application Note 315, Thermo Electron Corporation, 2003.

For More Information

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