High-Throughput Quantitation of Midazolam in Human Serum by Ion Trap LC/MS

Application Note

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Introduction

Target analysis of pharmaceutical compounds in biological matrices is an important part of the drug development process. The analytical method must accommodate analysis of large numbers of samples and achieve the necessary sensitivity even though the analyte is in a complex matrix. Therefore, key factors for success are robustness, speed, and selectivity. With the use of MS/MS techniques and fast, isocratic chromatography, all of these goals can be met. Historically, triple-quadrupole systems were used for this type of analysis, but modern ion trap mass spectrometers are also suitable and have some advantages over traditional techniques.

This note demonstrates the use of an ion trap LC/MS/MS system for the fast, sensitive quantitation of a pharmaceutical compound in a biological matrix.

Experimental

All experiments were performed using an Agilent 1100 Series LC/MSD Trap coupled to an Agilent 1100 Series LC system consisting of a binary pump, autosampler, thermostatted column compartment, and vacuum degasser. The ion trap mass spectrometer was operated with an atmospheric pressure chemical ionization (APCI) source in the positive-ion mode.

Midazolam is a central nervous system depressant used for preoperative sedation and anesthesia. As a member of the widely used benzodiazepine group, it was chosen as a model compound for demonstrating the ability of an ion trap LC/MS/MS system to quantitate drugs in human serum.

Sample preparation was done by a simple precipitation step to remove proteins. A 40 µl aliquot of human serum was diluted with 100 µl acetonitrile that contained the internal standard, medazepam, at a concentration of 1000 ng/ml. After mixing,

the sample was centrifuged and the supernatant injected directly into the LC/MS/MS system. Isocratic chromatography kept the overall cycle time at only 2.0 minutes with a retention time of 1.1 minutes for both midazolam and medazepam. The ion trap was operated in full scan, manual MS/MS mode to achieve the highest specificity and sensitivity. The following transitions of midazolam and medazepam were used for quantitation:

Midazolam
MS/MS of *m/z* 326 → *m/z* 291

Medazepam
MS/MS of *m/z* 271 → *m/z* 242

Results and Discussion

With these experimental conditions, the limit of quantitation for midazolam in serum was 32 ng/ml (91.4 pg on-column) with a signal-to-noise ratio of about 20:1 (Figure 1). For quantitation only, the base peak of the product ion spectrum of midazolam (*m/z* 291) was chosen. This fragment corresponds to a radical cation generated by the loss of chlorine. Because the MS/MS spectrum of the internal standard showed a more complex fragmentation behavior, the sensitivity for the internal standard could be increased two-fold by the post-acquisition summing of multiple fragment ions (Figure 2).

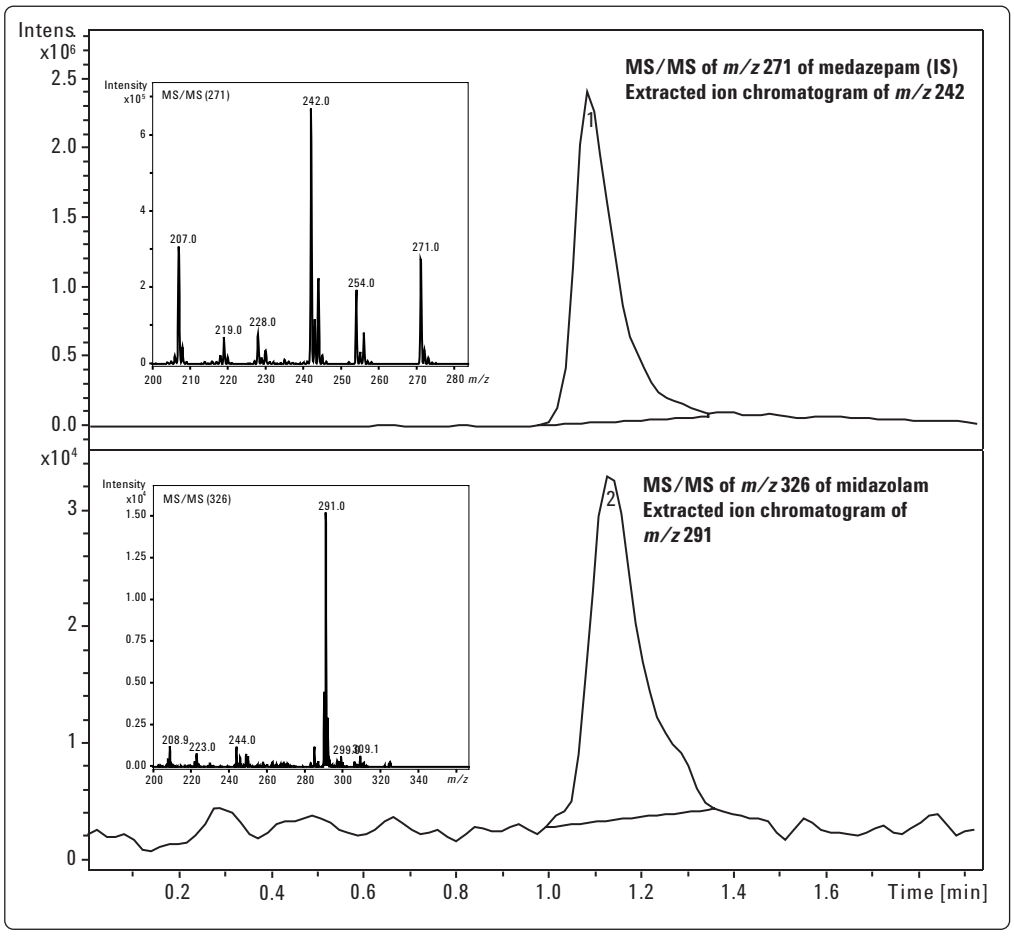

Figure 1. Extracted ion chromatograms of midazolam and its internal standard medazepam at the quantitation limit of 32 ng/ml; correlating full scan MS/MS spectra of midazolam and medazepam ISTD (inset) at peak apex

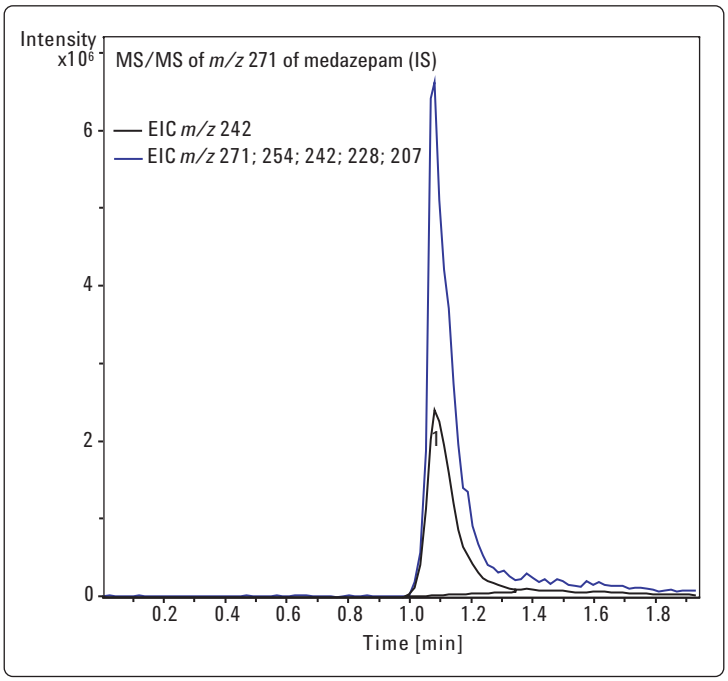

Figure 2. Overlay of extracted ion chromatograms of the internal standard medazepam at its working concentration; comparison of using one product ion (*m/z* 242) or sum of product ions (*m/z* 271, 254, 242, 228, 207)

The calibration curve was very linear from 32 ng/ml to 10500 ng/ml with a weighted (1/x) linear regression (Figure 3). The coefficient of correlation was 0.9999. The RSD of the quality control samples varied between 2.5% and 8.3% with a relative error below 7.9% (Table 1). The dynamic range and the precision demonstrated the good quantitation capabilities of the LC/MSD Trap, even when working with a crude sample matrix and rapid chromatography.

ANALYSIS METHOD

LC/MS/MS

Column:	ZORBAX Eclipse XDB-C8, 50x2.1 mm, 3.5 µm
Flow rate:	500 µl/min
Injection volume:	10 µl
Mobile phase:	A = 0.1% acetic acid in water B = methanol
Gradient:	Isocratic at 55% B

MS Conditions

Ionization mode:	Positive APCI
Drying gas flow:	4.0 l/min
Nebulizer pressure:	35 psig
Nebulizer temperature:	400°C
Drying gas temperature:	200°C
Corona:	3500 nA
Skim 1:	32.1 V
Capillary exit offset:	72.1 V
Averages:	2
ICC:	On
Maximum accumulation time:	120 ms
Target:	40000

Manual MS/MS:

Ion 1:	Isolation mass: *m/z* 326.1
Isolation width:	*m/z* 6.0
Fragmentation amplitude:	1.84 V
Fragmentation cutoff:	*m/z* 140
Ion 2:	Isolation mass: *m/z* 271.1
Isolation width:	*m/z* 6.0
Fragmentation amplitude:	1.80 V
Fragmentation cutoff:	*m/z* 140

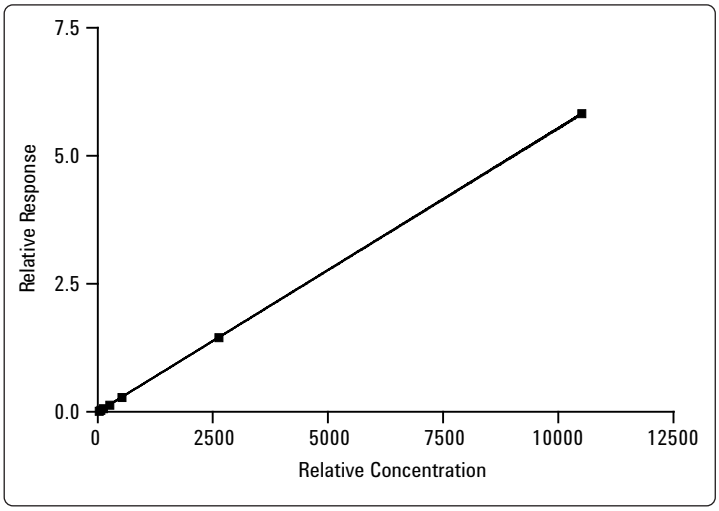

Figure 3. Calibration curve of midazolam from 32 ng/ml to 10500 ng/ml with weighted (1/x) linear regression, coefficient of correlation: 0.9999

Table 1. Statistics of QC samples for midazolam

Quality control sample	Measured conc. of midazolam (ng/ml) 1st	2nd	3rd	4th	Theor. conc. (ng/ml)	Mean conc. (ng/ml)	SD (ng/ml)	RSD (%)	Relative error (%)
7	33.2	34.0	28.9	33.7	32.8	32.4	2.4	7.4	–1.2
6	66.0	63.0	62.6	65.0	65.6	64.1	1.6	2.5	–2.3
5	119.2	109.2	111.5	121.0	121.3	115.2	5.8	5.0	–5.0
4	256.0	261.5	243.3	249.6	262.5	252.6	7.9	3.1	–3.8
3	446.6	477.8	524.3	486.4	525.0	483.8	32.0	6.6	–7.9
2	2395.9	2439.2	2853.8	2678.7	2625.0	2591.9	214.4	8.3	–1.3
1	11269.7	9905.1	9875.0	10008.5	10500.0	10264.6	672.5	6.6	–2.2

Conclusions

The data clearly show that the quantitative measurement of midazolam in a complex biological matrix can be done on the LC/MSD Trap system with high precision, high accuracy, and a fast cycle time. The sample preparation can be kept to a minimum. This data is comparable to data generated on triple-quadrupole instruments in terms of throughput, precision, and linearity. In contrast to triple-quadrupole instruments, full scan MS/MS spectra are always accessible on ion trap instruments without loss of sensitivity. These MS/MS spectra can be used for further confirmation of the target compound and the quantitation ions can be chosen post-acquisition. This significantly reduces method development time. Linearity and sensitivity are sufficient to perform pharmacokinetic studies.

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