Application Note 629

Quantitation of 122 Drugs in Urine by Triple Quadrupole Mass Spectrometry

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Key Words

TSQ Endura, TraceFinder, forensic toxicology, drugs of abuse, opiates, opioids, benzodiazepines, amphetamines, tricyclic antidepressants, barbiturates

Goal

To develop and analytically evaluate an HPLC-MS/MS method that employs a Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer for the quantitation of 122 pharmacologic agents in human urine for forensic toxicology.

Introduction

Forensic toxicologists face an ever-expanding list of compounds for analysis. Traditionally, compounds are analyzed in standard panels by immunoassay, GC, GC-MS, or LC-UV, depending on the compounds being targeted. LC-MS/MS can accommodate a wider variety of compounds on a single platform in a single analytical run, thereby saving time and money. In addition to the standard panels, forensic scientists need to frequently add new designer drugs to the list of targeted compounds. LC-MS/MS also has an advantage over other technologies in the ease by which new compounds can be added to existing methods.

In large panels, scan speeds of triple quadrupole mass spectrometers can limit the number of data points acquired, impacting sensitivity and quantitative performance. Performance can further deteriorate when an analysis involves polarity switching and very narrow peaks.

This note presents work done using a next-generation triple quadrupole mass spectrometer with fast SRM acquisition speed for quantitation of 122 analytes in a single chromatographic run, where scan speed does not impact sensitivity or quantitative performance. Compounds analyzed include opiates, opioids, benzodiazepines, barbiturates, amphetamines, tricyclic antidepressants, illicit compounds, and more.

Experimental

Sample Preparation

Samples were processed by enzymatic hydrolysis followed by liquid-liquid extraction (LLE). Briefly, an aliquot of urine was spiked with internal standard and incubated with ß-glucuronidase enzyme. The resulting mixture was extracted using Amtox A™ liquid-liquid extraction tubes (Ameritox Labs, Hilliard, OH). The organic layer was dried and reconstituted before an aliquot was analyzed by gradient HPLC and triple quadrupole mass spectrometry. Calibrators and controls were prepared by spiking compounds into blank synthetic urine in the range of 0.5 to 500 ng/mL. The upper calibration range was limited by the concentration of stock solutions used in making the multidrug spiking solutions.

Liquid Chromatography

Gradient elution was performed using a Thermo Scientific™ Dionex™ UltiMate™ 3000RS liquid chromatographic pump with an OAS autosampler.

Mobile phases consisted of 10 mM ammonium acetate in water and methanol (Fisher Scientific™ Optima™ grade) for solvents A and B, respectively. The column used was a Thermo Scientific™ Accucore™ PFP, 2.6 µm, 100 x 2.1 mm fused core (P/N 17426-102130). The gradient was run from 2 to 100% mobile phase B over 10 minutes at a flow rate of 0.5 mL/min (Figure 1). The injection volume was 10 µL and the total run time was 15 minutes.

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Mass Spectrometry

Compounds were detected on a TSQ Endura triple quadrupole mass spectrometer equipped with a Thermo Scientific™ Ion Max™ NG source and heated electrospray ionization sprayer. The TSQ Endura MS is designed with an active collision cell that speeds ion transmission, yielding industry leading scanning rates of up to 500 SRM/s with less than 0.005% cross talk. This makes it possible to analyze more compounds in a single run.

In this method, two selected-reaction monitoring (SRM) transitions were monitored for each of the 122 analytes to obtain ion ratio confirmation (IRC), and one SRM transition was monitored for each of the 84 stable isotope-labeled internal standards used. A total of 328 transitions were monitored in both positive and negative mode.

Data Analysis

Data were acquired and processed, including performing ion ratio calculations, by Thermo Scientific™ TraceFinder™ software.

Method Evaluation

Limits of detection, precision, and accuracy were evaluated by processing and analyzing calibrators and replicate controls. Matrix effects were determined by spiking 12 different lots of blank donor urine at 10 ng/mL and comparing results to that of a sample prepared in water.

Results

Limits of quantitation were defined as the lowest concentrations that had back-calculated values within 20%, ion ratios within defined tolerance, calibration curve with R^2 values >0.9, and quality controls with %RSD within 20%. Using these criteria, forensic cutoffs were met, and in many cases exceeded, for the compounds tested in this study (Table 1). Intra-assay precisions for quality control replicates were within 17% across all concentrations and all compounds, and most were within 10% (Table 1).

Passing matrix effects were defined as a back-calculated concentration of ±50% of nominal. Less than 2% of the results failed for compounds that had stable isotope-labeled analog internal standards, whereas 21% of the results were out of range for compounds without a stable isotope-labeled analog as the internal standard.

Figure 2 shows chromatograms with ion ratio calculations for selected compounds.

Figure 3 shows some representative calibration curves for selected compounds.

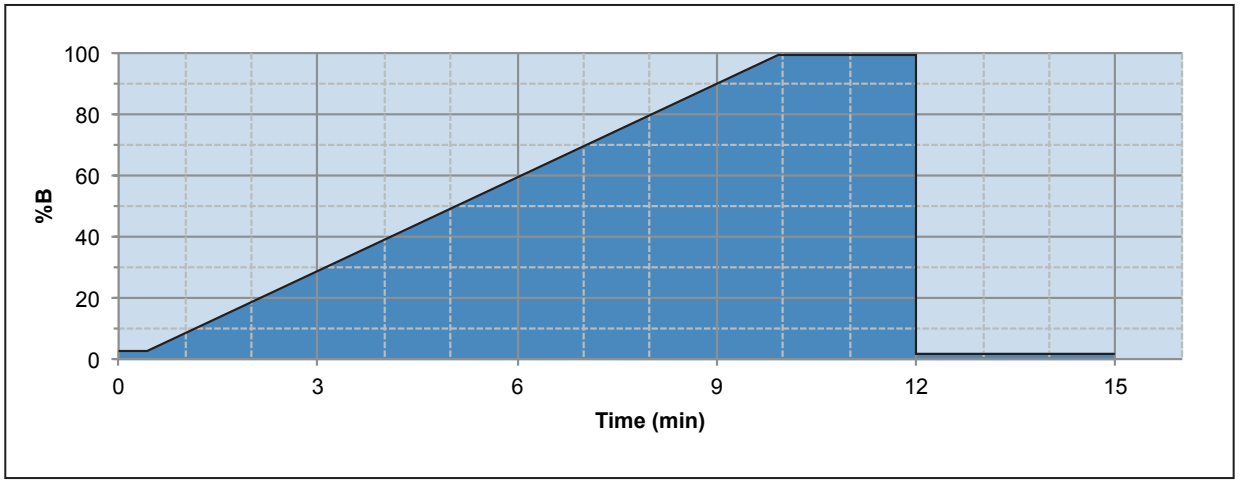

Figure 1. Liquid chromatography gradient used in this method. Mobile phases were 10 mM ammonium formate in water (A) and methanol (B).

Table 1. Evaluation data for 122 compounds showing LOQ and precision for quality control samples.

Compound	LOQ (ng/mL)	1 ng/mL %RSD	10 ng/mL %RSD	100 ng/mL %RSD
6-MAM	1	7.22%	5.71%	6.68%
7-aminoclonazepam	0.5	6.31%	4.62%	1.80%
7-aminoflunitrazepam	0.5	3.49%	1.20%	1.82%
Acetaminophen	50	BLQ	BLQ	3.89%
α-hydroxyalprazolam	1	1.57%	2.31%	2.61%
Alprazolam	0.5	2.71%	1.38%	9.56%
Amitriptyline	5	BLQ	9.10%	7.71%
Amphetamine	50	BLQ	BLQ	2.71%
Atenolol	1	5.58%	2.70%	9.70%
Atropine	0.5	3.44%	2.91%	4.23%
Benzoylecgonine	2	BLQ	5.00%	0.99%
Brompheniramine	2	BLQ	5.36%	7.81%
Buprenorphine	1	12.5%	11.7%	5.04%
Bupropion	2	BLQ	2.85%	1.30%
Butalbital	10	BLQ	BLQ	10.1%
Carbamazepine	2	BLQ	2.39%	2.71%
Carbamazepine-epoxide	0.5	1.93%	3.87%	5.03%
Carisprodol	0.5	3.66%	1.54%	4.04%
Chlordiazepoxide	0.5	10.3%	4.19%	3.53%
Chlorpheniramine	0.5	2.30%	2.40%	5.50%
Chlorpromazine	5	BLQ	13.8%	5.47%
Cimetidine	2	BLQ	5.83%	5.09%
Citalopram	5	BLQ	2.26%	9.39%
Clomipramine	2	BLQ	4.76%	4.94%
Clonazepam	1	4.78%	2.98%	5.75%
Clozapine	0.5	7.73%	2.76%	4.48%
Cocaethylene	1	13.6%	3.55%	4.24%
Cocaine	50	BLQ	BLQ	2.22%
Codeine	5	BLQ	5.15%	9.83%
Cotinine	0.5	5.09%	2.17%	2.75%
Cyclobenzaprine	2	BLQ	8.90%	4.27%
Desalkylflurazepam	0.5	11.7%	2.72%	4.89%
Desipramine	5	BLQ	3.11%	6.33%
Desmethylclomipramine	10	BLQ	7.63%	5.38%
Dextromethorphan	1	7.53%	11.5%	9.13%
Diazepam	5	BLQ	2.08%	5.05%
Digoxin	2	BLQ	10.1%	8.03%
Dihydrocodeine	1	11.6%	2.30%	4.34%
Diltiazem	1	8.00%	1.94%	3.04%
Diphenhydramine	0.5	3.09%	2.24%	3.29%
Doxepin	10	BLQ	2.70%	5.98%
Doxylamine	5	BLQ	3.40%	1.03%
Duloxetine	5	BLQ	5.79%	3.71%
Ecgonine ethyl ester	5	BLQ	3.40%	9.21%
Ecgonine methyl ester	2	BLQ	1.40%	1.97%
EDDP	2	BLQ	3.41%	10.3%
Ephedrine	0.5	7.26%	8.72%	8.34%

Table 1 continued. Evaluation data for 122 compounds showing LOQ and precision for quality control samples.

Compound	LOQ (ng/mL)	1 ng/mL %RSD	10 ng/mL %RSD	100 ng/mL %RSD
Fentanyl	0.5	4.66%	6.76%	3.25%
Flunitrazepam	1	6.98%	1.15%	2.16%
Fluoxetine	2	BLQ	3.34%	3.47%
Flurazepam	0.5	2.24%	2.05%	2.39%
Hydrocodone	2	BLQ	1.68%	3.27%
Hydromorphone	0.5	4.42%	11.3%	3.05%
Hydroxyzine	0.5	3.43%	2.75%	5.18%
Imipramine	1	11.2%	5.07%	3.31%
Ketamine	0.5	8.02%	4.11%	1.45%
Lamotrigine	1	3.47%	5.80%	1.80%
Lidocaine	0.5	3.26%	1.45%	4.68%
Lorazepam	0.5	7.34%	1.81%	2.46%
LSD	0.5	4.48%	4.25%	1.06%
Maprotiline	10	BLQ	2.86%	7.27%
MDA	0.5	4.52%	7.40%	3.23%
MDMA	0.5	6.47%	2.07%	4.76%
Meperidine	2	BLQ	7.00%	4.20%
Meprobamate	0.5	2.69%	7.52%	2.37%
Methadone	0.5	8.08%	6.75%	4.44%
Methamphetamine	50	BLQ	BLQ	12.9%
Methotrimeprazine	10	BLQ	3.82%	4.40%
Methylphenidate	2	BLQ	2.04%	5.05%
Metoprolol	5	BLQ	4.30%	4.51%
Mirtazapine	1	4.38%	1.90%	8.47%
Morphine	2	BLQ	7.78%	8.36%
Naproxen	2	BLQ	4.97%	2.80%
Nicotine	2	BLQ	1.92%	4.41%
Norbuprenorphine	1	11.8%	8.32%	9.04%
Norchlordiazepoxide	1	9.12%	4.06%	0.84%
Norcodeine	2	BLQ	7.97%	6.99%
Norcyclobenzaprine	2	BLQ	3.60%	7.79%
Nordiazepam	1	5.28%	4.17%	6.35%
Nordoxepin	0.5	6.35%	2.34%	1.78%
Norfentanyl	0.5	4.88%	1.64%	2.04%
Norfluoxetine	20	BLQ	BLQ	6.12%
Norketamine	0.5	4.38%	1.52%	1.75%
Normeperidine	0.5	5.28%	4.85%	1.91%
Norpropoxyphene	20	BLQ	BLQ	9.28%
Norsertraline	10	BLQ	6.23%	6.11%
Nortrimipramine	10	BLQ	4.40%	5.19%
Nortriptyline	0.5	8.80%	6.23%	5.76%
Norverapamil	0.5	1.60%	8.22%	8.18%
O-desmethyltramadol	1	1.43%	3.34%	6.99%
Olanzapine[1]	20	BLQ	BLQ	6.04%
Oxazepam	0.5	12.9%	3.60%	2.19%
Oxycodone	0.5	5.91%	NA	5.29%
Oxymorphone	0.5	15.8%	9.21%	5.41%
Paroxetine	1	13.8%	2.89%	3.37%

Table 1 continued. Evaluation data for 122 compounds showing LOQ and precision for quality control samples.

Compound	LOQ (ng/mL)	1 ng/mL %RSD	10 ng/mL %RSD	100 ng/mL %RSD
Phencyclidine	2	BLQ	11.8%	2.46%
Phenethylamine	2	BLQ	3.17%	6.56%
Pheniramine	0.5	4.87%	5.07%	4.74%
Phenobarbital	20	BLQ	BLQ	14.3%
Phentermine	10	BLQ	11.7%	7.51%
Phenylephrine	10	BLQ	2.04%	0.79%
Phenylpropanolamine	0.5	8.87%	2.66%	6.32%
Phenytoin	20	BLQ	BLQ	6.13%
Propoxyphene	50	BLQ	BLQ	2.19%
Propranolol	1	8.49%	2.13%	4.18%
Pseudoephedrine	10	BLQ	6.28%	3.15%
Quetiapine	0.5	4.36%	1.97%	8.07%
Quinidine	2	BLQ	5.51%	3.29%
Quinine	2	BLQ	6.29%	8.30%
Ranitidine	10	BLQ	5.46%	10.5%
Sertraline	5	BLQ	4.61%	4.80%
Strychnine	5	BLQ	3.51%	6.49%
Temazepam	0.5	2.79%	0.61%	5.20%
THC	2	BLQ	14.1%	14.2%
THC-COOH (neg)	1	10.6%	2.20%	2.19%
THC-COOH (pos)	1	8.04%	2.29%	2.28%
Theophylline	0.5	0.23%	0.67%	1.34%
Thioridazine[1]	100	1.36%	8.41%	5.30%
Tramadol	0.5	2.87%	3.68%	2.76%
Trazodone	0.5	3.49%	1.06%	1.18%
Trimipramine	0.5	3.75%	2.05%	4.10%
Verapamil	2	BLQ	4.99%	5.19%
Zolpidem	0.5	2.12%	1.70%	4.86%

[1] No internal standard would provide a good fit.
BLQ: below limit of quantitation.

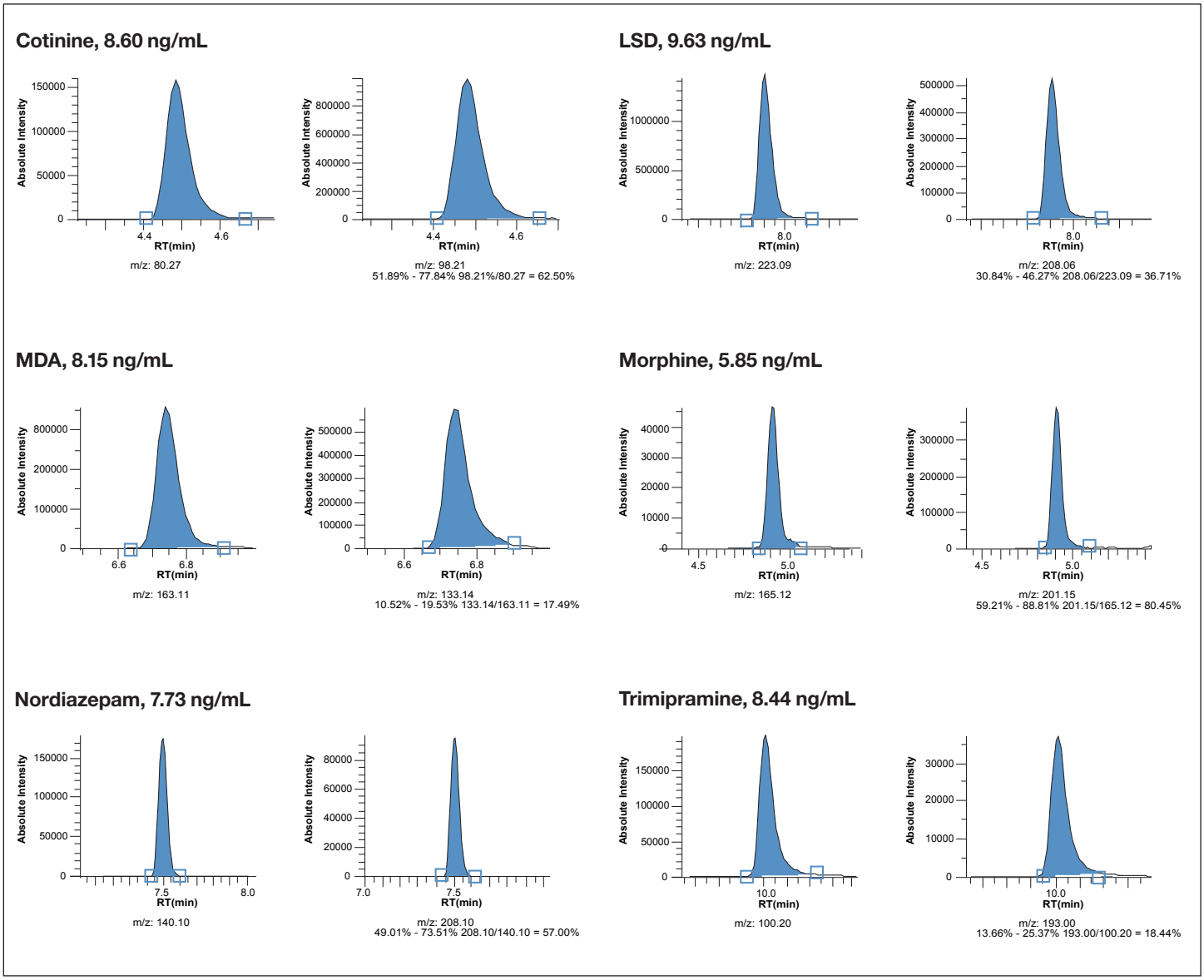

Figure 2. Representative chromatograms for selected compounds showing ion ratio confirmation.

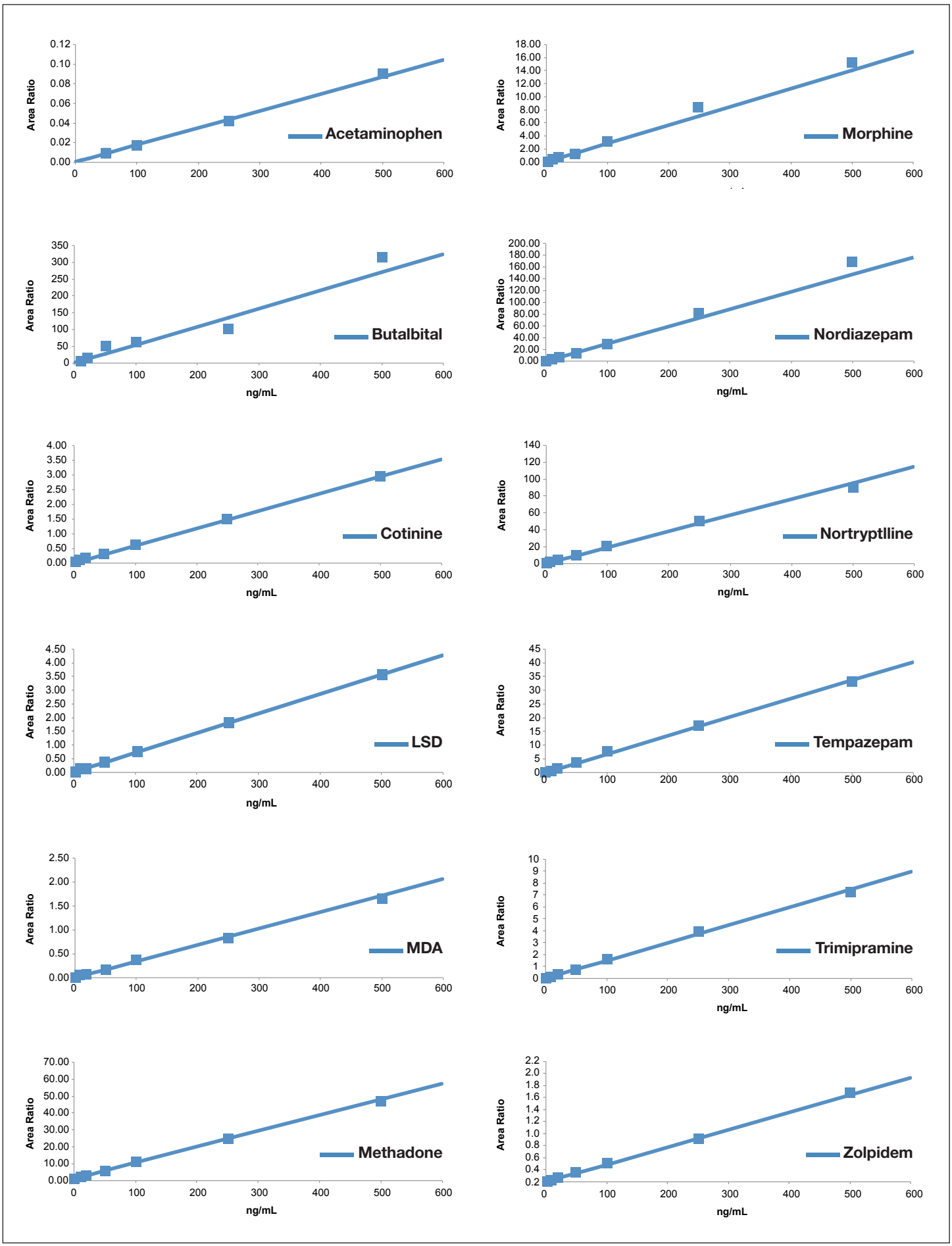

Figure 3. Representatiove calibration curves for select sompounds analyzed in this method. All back-calculated concentrations were within 20%.

Conclusion

- A single analytical HPLC-MS/MS method was developed for 122 chemically diverse compounds.
- The method includes both polar and non-polar analytes as well as positively and negatively ionizing compounds.
- Stable isotope-labeled analog internal standards are crucial to minimize matrix effects.
- The fast scanning speed and polarity switching of the TSQ Endura mass spectrometer enabled the analysis of all 122 compounds plus 84 stable-isotope labeled internal standards (328 total transitions) without a loss of signal intensity.
- A single sample processing scheme was used for all compounds, making the method efficient.
- Forensic toxicological limits of quantitation were met or exceeded.

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