Poster Note 64378

Evaluation of a Method for Forensic Quantitative Screening of Over 120 Drugs of Abuse on a Triple Quadrupole Mass Spectrometer

Kristine Van Natta and Marta Kozak
Thermo Fisher Scientific, San Jose, CA

Thermo SCIENTIFIC

Overview

Purpose: 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.

Methods: Enzymatic hydrolysis followed by liquid-liquid extraction prior to HPLC-MS/MS analysis.

Results: Limits of quantitation defined as acceptable back-calculated calibration curves, passing ion ration confirmation, and precise quality controls were met for 122 compounds.

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 constantly add new designer drugs. LC-MS/MS also has advantage over other technologies in the 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 poster presents work done using a next generation triple quadrupole mass spectrometer with fast SRM acquisition speed for quantitation of 122 analytes in single chromatographic run. Compounds analyzed include opiates, opioids, benzodiazepines, barbiturates, amphetamines, tricyclic antidepressants, illicit compounds, and more.

Methods

Sample Preparation

- Enzymatic hydrolysis
- Liquid-liquid extraction (LLE), Amtox A tubes (Ameritox Labs, Hilliard, OH)
- The organic layer was evaporated to dryness and reconstituted
- Calibrators and controls were prepared by spiking compounds into blank synthetic urine in the range of 0.5 to 500 ng/mL.

Liquid Chromatography

- Pump: Thermo Scientific™ Dionex™ Ultimate™ 3000RS with OAS autosampler.
- Mobile phases: 10 mM ammonium acetate in water(A) and methanol (B) (Fisher™ Optima™ grade)
- Column: Thermo Scientific ™ Accucore™ PFP, 2.6 µm, 100 x 2.1 mm fused core
- Gradient: 2 to 100% mobile phase B over 10 minutes.
- Total run time was 15 minutes

Mass Spectrometry

- Mass Spectrometer: TSQ Endura triple quadrupole mass spectrometer with a heated electrospray ionization (HESI II) sprayer.
- Two selected reaction monitoring (SRM) transitions were monitored for each analyte to obtain ion ratio confirmation (IRC) and one SRM transition was monitored for each of the 84 stable-labeled internal standards used.

Data Analysis

Data were acquired and processed including ion ratio calculations, using Thermo Scientific™ TraceFinder™ software. IRC tolerances used are given in Table 1.

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.

Table 1. Ion Ratio Tolerances.

Ion Ratio	Relative Tolerance
>50%	20%
>20-50%	25%
>10-20%	30%
≤10%	50%

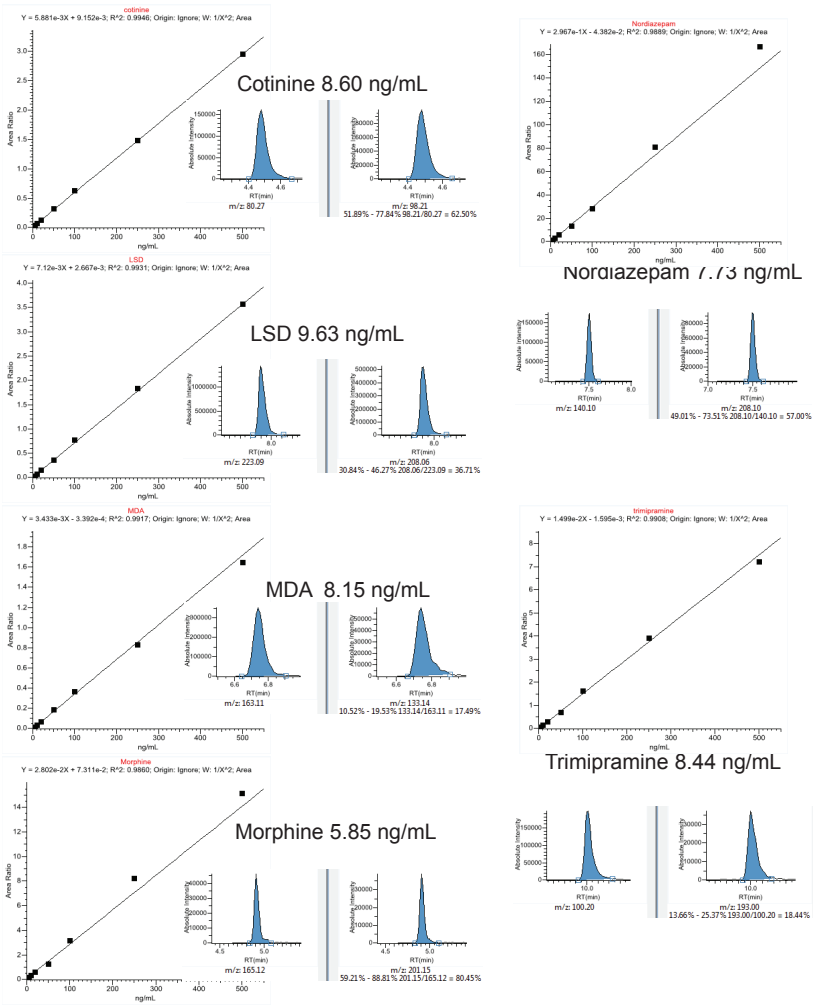
TABLE 2. Limits of quantitation in ng/mL for compounds analyzed with this method.

Compound	LOQ	Compound	LOQ
6-MAM	1	MDA	0.5
7-aminoclonazepam	0.5	MDMA	0.5
7-aminoflunitrazepam	0.5	Meperidine	2
Acetaminophen	50	Meprobamate	0.5
a-hydroxyalprazolam	1	Methadone	0.5
Alprazolam	0.5	Methamphetamine	50
Amitriptyline	5	Methotrimeprazine	10
Amphetamine	50	Methylphenidate	2
Atenolol	1	Metoprolol	5
Atropine	0.5	Mirtazapine	1
Benzoylecgonine	2	Morphine	2
Brompheniramine	2	Naproxen	2
Buprenorphine	1	Nicotine	2
Bupropion	2	Norbuprenorphine	1
Butalbital	10	Norchlordiazepoxide	1
Carbamazepine	2	Norcodeine	2
Carbamazepine-10,11-epoxide	0.5	Norcyclobenzaprine	2
Carisprodol	0.5	Nordiazepam	1
Chlordiazepoxide	0.5	Nordoxepin	0.5
Chlorpheniramine	0.5	Norfentanyl	0.5
Chlorpromazine	5	Norfluoxetine	20
Cimetidine	2	Norketamine	0.5
Citalopram	5	Normeperidine	0.5
Clomipramine	2	Norpropoxyphene	20
Clonazepam	1	Norsertraline	10
Clozapine	0.5	Nortrimipramine	10
Cocaethylene	1	Nortriptyline	0.5
Cocaine	50	Norverapamil	0.5
Codeine	5	O-desmethyltramadol	1
Cotinine	0.5	Olanzapine	10
Cyclobenzaprine	2	Oxazepam	0.5
Desalkylflurazepam	0.5	Oxycodone	0.5
Desipramine	5	Oxymorphone	0.5
Desmethylclomipramine	10	Paroxetine	1
Dextromethorphan	1	Phencyclidine	2
Diazepam	5	Phenethylamine	2
Digoxin	2	Pheniramine	0.5
Dihydrocodeine	1	Phenobarbital	20
Diltiazem	1	Phentermine	10
Diphenhydramine	0.5	Phenylephrine	10
Doxepin	10	Phenylpropanolamine	0.5
Doxylamine	5	Phenytoin	20
Duloxetine	5	Propoxyphene	50
Ecgonine ethyl ester	5	Propranolol	1
Ecgonine methyl ester	2	Pseudoephedrine	10
EDDP	2	Quetiapine	0.5
Ephedrine	0.5	Quinidine	2
Fentanyl	0.5	Quinine	2
Flunitrazepam	1	Ranitidine	10
Fluoxetine	2	Sertraline	5
Flurazepam	0.5	Strychnine	5
Hydrocodone	2	Temazepam	0.5
Hydromorphone	0.5	THC	2
Hydroxyzine	0.5	THC-COOH	1
Imipramine	1	Theophylline	0.5
Ketamine	0.5	Thioridazine	101
Lamotrigine	1	Tramadol	0.5
Lidocaine	0.5	Trazodone	0.5
Lorazepam	0.5	Trimipramine	0.5

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 cut-offs were met, and in many cases exceeded, for the compounds tested in this study (Table 2). Intra-assay precisions for quality control replicates were within 17% across all concentrations and all compounds, and most were within 10% (data not shown). 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-labeled analog internal standards whereas 21% of the results failed for compounds without a stable-labeled analog as the internal standard. Figure 1 shows representative calibration curves and chromatograms with ion ratio calculations for selected compounds.

FIGURE 1. Chromatogram of compounds in donor urine showing ion ratio.



Conclusion

- A single analytical HPLC-MS/MS method was developed for 122 chemically diverse compounds.
- The method includes both polar and non-polar as well as positively and negatively ionizing compounds.
- Stable-labeled analog internal standards are crucial to minimize matrix effects.
- The fast scanning speed and polarity switching of the TSQ Endura mass spectrometer enable the analysis of all 122 compounds plus 84 stable-labeled internal standards without 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.

For forensic toxicology use only.

www.thermofisher.com

Africa +43 1 333 50 34 0
Australia +61 3 9757 4300
Austria +43 810 282 206
Belgium +32 53 73 42 41
Brazil +55 11 3731 5140
Canada +1 800 530 8447
China 800 810 5118 (free call domestic) 400 650 5118
Denmark +45 70 23 62 60
Europe-Other +43 1 333 50 34 0
Finland +358 9 3291 0200
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9494
Italy +39 02 950 591
Japan +81 6 6885 1213
Korea +82 2 3420 8600
Latin America +1 561 688 8700
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Norway +46 8 556 468 00
Russia/CIS +43 1 333 50 34 0
Singapore +65 6289 1190
Sweden +46 8 556 468 00
Switzerland +41 61 716 77 00
Taiwan +886 2 8751 6655
UK/Ireland +44 1442 233555
USA +1 800 532 4752

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand

PN64378-EN 0616S