Challenges in Sample Preparation for Forensic Quantitative Screening of Over 120 Drugs of Abuse on a Triple Quadrupole Mass Spectrometer

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Overview

Purpose: To develop and analytically evaluate various sample preparation techniques along with 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

Rapid screening is a goal for many forensic toxicology laboratories. Newer, faster triple quadrupole mass spectrometers enable laboratories to include more compounds in one chromatographic run thereby saving analytical run time. The next challenge arises in finding a suitable sample processing technique that works for a variety of compounds across a wide chemical space with varying sensitivities and taking into account the different LOQ requirements. In this study, several liquid-liquid extraction (LLE) schemes were compared to see which method was a better fit for analyzing the wide range of compounds in human urine in a forensic toxicology setting.

Methods

Sample Preparation

- Enzymatic hydrolysis
- Liquid-liquid extraction (LLE)
  - Basic, Neutral, Acidic with EthylAcetate:Hexane (1:1 v/v),
  - Amtox A and B 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 Scientific™ Optima™ grade)
- Column: Thermo Scientific™ Accucore™ PFP, 2.6 µm, 100 x 2.1 mm
- Gradient: initial 0.5-min hold at 2% mobile phase B followed by 10-min ramp to 100% B.
- 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.
- Compounds are both positively and negatively ionized.

Data Analysis

Data was acquired and processed with Thermo Scientific™ TraceFinder™ software version 3.2. Calibration ranges, LODs, and LOQs were evaluated based on concentration accuracy; back-calculated concentrations had to be within 30%.

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.

The above methods were tested with over 100 compounds from a wide chemical space including amphetamines, antidepressants, barbiturates, benzodiazepines, drugs of abuse, and opioids, a space which includes polar and non-polar compounds as well as positively and negatively ionizing compounds.

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TABLE 1. Extraction recoveries for basic, neutral and acidic LLE and extraction tubes A and B. While LLE under basic conditions gave higher recoveries for a greater number of compounds than other extraction techniques, the AmtoxA LLE tubes gave the best compromise on recovery over the entire compound list.

Compound	Basic	Neutral	Acidic	TxA	TxB
6-MAM					
7-Aminoclonazepam					
7-Aminoflunitrazepam					
Acetaminophen					
a-Hydroxyalprazolam					
Alprazolam					
Amitriptyline					
Amphetamine					
Atenolol					
Atropine					
Benzoylecgonine					
Brompheniramine					
Buprenorphine					
Bupropion					
Carbamazepine					
Carbamazepine-epoxide					
Carisprodol					
Chlordiazepoxide					
Chlorpheniramine					
Chlorpromazine					
Cimetidine					
Citalopram					
Clomipramine					
Clonazepam					
Clozapine					
Cocaethylene					
Cocaine					
Codeine					
Cotinine					
Cyclobenzaprine					
Desalkylflurazepam					
Desipramine					
Desmethly-clomipramine					
Dextromethorphan					
Diazepam					
Dihydrocodeine					
Diltiazem					
Diphenhydramine					
Doxepin					
Doxylamine					
Duloxetine					
Ecgonine ethyl ester					
Ecgonine methylester					
Ephedrine					
Fentanyl					
Flunitrazepam					
Flurazepam					
Hydrocodone					
Hydromorphone					
Hydroxyzine					
Imipramine					
Ketamine					
Lamotrigine					
Lidocaine					
Lorazepam					
LSD					
maprotiline					
RECOVERY KEY	**greater**				**less**

Compound	Basic	Neutral	Acidic	TxA	TxB
MDA					
MDMA					
Meperidine					
Meprobamate					
Methadone					
Methamphetamine					
Methotrimeprazine					
Methylphenidate					
Metoprolol					
Mirtazapine					
Morphine					
Nicotine					
Norbuprenorphine					
Norchlordiazepoxide					
Norcodeine					
Norcyclobenzaprine					
Nordiazepam					
Nordoxepin					
Norfentanyl					
Norketamine					
Normeperidine					
Norpropoxyphene					
Norsertraline					
Nortrimipramine					
Nortriptyline					
Norverapamil					
O-Desmethyltramadol					
Olanzapine					
Oxazepam					
Oxycodone					
Oxymorphone					
Paroxetine					
Phencyclidine					
Phenethylamine					
Pheniramine					
Phentermine					
Phenylephrine					
Phenylpropanolamine					
Phenytoin					
Propoxyphene					
Propranolol					
Pseudoephedrine					
Quetiapine					
Quinidine					
Quinine					
Ranitidine					
Sertraline					
Strychnine					
Temazepam					
THC					
Theophylline					
Thioridazine					
Tramadol					
Trazodone					
Trimipramine					
Verapamil					
Zolpidem					

Results

Extraction Recoveries: While LLE under basic conditions gave higher recoveries for a greater number of compounds than other extraction techniques, the AmtoxA LLE tubes gave the best compromise on recovery over the entire compound list, taking into account required LOQs for all compounds (TABLE 1). LOQs met forensic toxicology requirements for 98% of the compounds tested (TABLE 2).

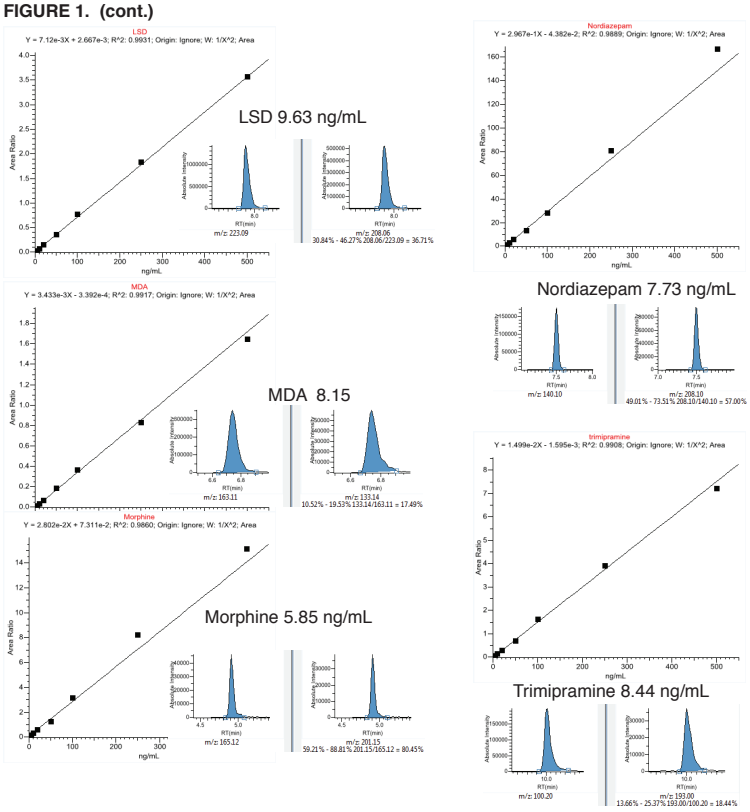
TABLE 2. Limits of Quantitation and QC Precision for Compounds Tested.

Compound	LOQ (ng/mL)	%RSD 1 ng/mL	%RSD 10 ng/mL	%RSD 100 ng/mL
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%	11.68%	5.04%
Bupropion	2	BLQ	2.85%	1.30%
Butalbital	10	BLQ	BLQ	10.14%
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	13.75%	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	13.64%	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	11.71%	2.72%	4.89%
Desipramine	5	BLQ	3.11%	6.33%
Desmethylclomipramine	10	BLQ	7.63%	5.38%
Dextromethorphan	1	7.53%	11.46%	9.13%
Diazepam	5	BLQ	2.08%	5.05%
Digoxin	2	BLQ	10.10%	8.03%
Dihydrocodeine	1	11.61%	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%	10.30%
Ephedrine	0.5	7.26%	8.72%	8.34%
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%	11.27%	3.05%
Hydroxyzine	0.5	3.43%	2.75%	5.18%
Imipramine	1	11.19%	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	12.90%
Methotrimeprazine	10	BLQ	3.82%	4.40%
Methylphenidate	2	BLQ	2.04%	5.05%

Table 2. (continued)

Compound	LOQ (ng/mL)	%RSD 1 ng/mL	%RSD 10 ng/mL	%RSD 100 ng/mL
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	11.81%	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	12.94%	3.60%	2.19%
Oxycodone	0.5	5.91%	NA	5.29%
Oxymorphone	0.5	15.76%	9.21%	5.41%
Paroxetine	1	13.75%	2.89%	3.37%
Phencyclidine	2	BLQ	11.76%	2.46%
Phenethylamine	2	BLQ	3.17%	6.56%
Pheniramine	0.5	4.87%	5.07%	4.74%
Phenobarbital	20	BLQ	BLQ	14.32%
Phentermine	10	BLQ	11.70%	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%	10.50%
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	14.09%	14.16%
THC-COOH (neg)	1	10.55%	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%

FIGURE 1. (cont.)



Matrix effects were determined by comparing concentration of analyte in spiked donor urine to a sample prepared in water. Calculated concentration within ±50% was considered passing. 8.3% of the individual analyte/donor results were outside of this range. However, less than 2% of those compounds that had a stable-labeled analog internal standard were out of range whereas 21% of those without an analog were out of range.

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.

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