Urine drug screening: using GC-MS/MS to augment LC-MS/MS screens

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Introduction

Clinical toxicology services, which encompass the detection of pharmaceuticals and drugs of abuse in biological samples, are routinely offered by hospital laboratories and can play an important role in patient management. In addition to immunoassay screening for routine drugs of abuse, our laboratory uses a liquid chromatography tandem mass spectrometry (LC-MS/MS) method to screen patient urine samples for a large number of clinically relevant compounds. Immunoassays are not available for many of the compounds in our method, which makes it a challenge to verify results by two techniques. Here we report the development and validation of a gas chromatography tandem mass spectrometry (GC-MS/MS) method for comprehensive urine drug screening with method performance evaluated in part by concordance with our LC-MS/MS method.

Sample preparation

GC-MS/MS samples were prepared by solid phase extraction (SPE) of 0.5 mL urine using UCT Clean Screen extraction columns. Acid/neutral drugs were eluted with hexane/ethyl acetate and basic drugs were eluted with methylene chloride/ isopropanol.
LC-MS/MS samples were prepared by a 1:5 dilution of urine into the starting condition of the chomatographic run.

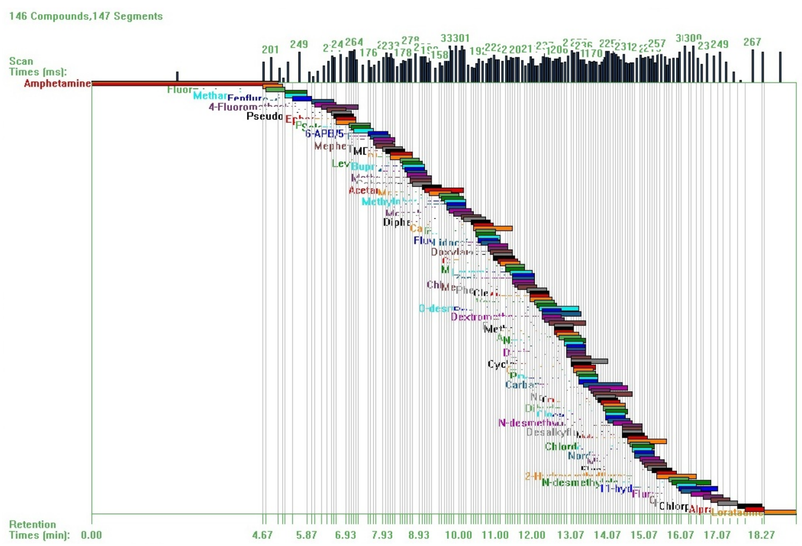
GC-MS/MS method

GC column:	30m BR-5ms column, 0.25 mm ID and 0.25 µm df (Bruker).
Instrumentation:	Bruker 436 GC and Scion TQ mass spectrometer with Bruker CP-8400 autosampler. Siltek-deactivated gooseneck inlet liner (Restek). Source held at 240°C and transfer line held at 280°C. Helium carrier gas flowed at 1 mL/min.
Oven Program:	Start 80°C hold for 1 minute 15°C/min 295°C hold for 5 minutes
Injection:	Injection port held at 280°C. 1 µL injections were conducted in split-splitless mode with pressure pulse (40 psi for 0.4 minutes, total 0.6 minute injection).
Data collection:	Data was collected using MSWS version 8.1 (Bruker) in SRM mode, monitoring 2 transitions per analyte. 20 minute runtime per injection. 3.5 minute solvent delay.
Data Analysis:	Data was analyzed using MS DataReview (Bruker). Positivity was determined on the basis of retention time and the presence and ratio of the two compound specific SRMs.

Reference LC-MS/MS method

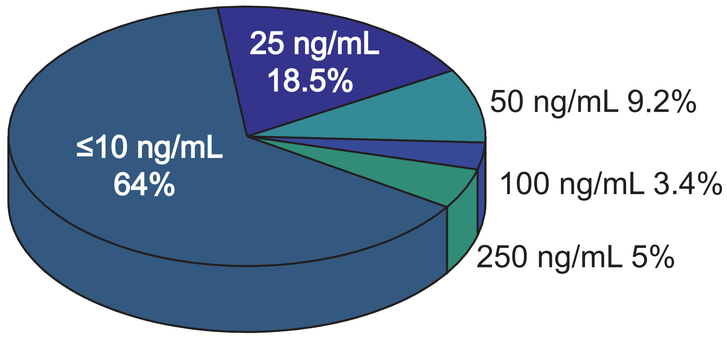
LC-MS/MS was performed using an Agilent HPLC with an ABSciex 3200 QTRAP mass spectrometer in positive-ESI mode. Mobile phase A consisted of water containing 5mM ammonium formate and 0.05% formic acid. 10µL of sample was injected onto a Kinetex C18 column (2.6µM, 50x3mm) held at 30°C. Compounds were eluted with a 2-100% gradient of mobile phase B (50:50 methanol:acetonitrile containing 0.05% formic acid) over 12 minutes at a flow rate of 400µL/min. Positivity was determined by retention time, presence of one SRM transition and a match between the collected product ion spectrum and an in-house built spectral library.

Scheduled SRM method



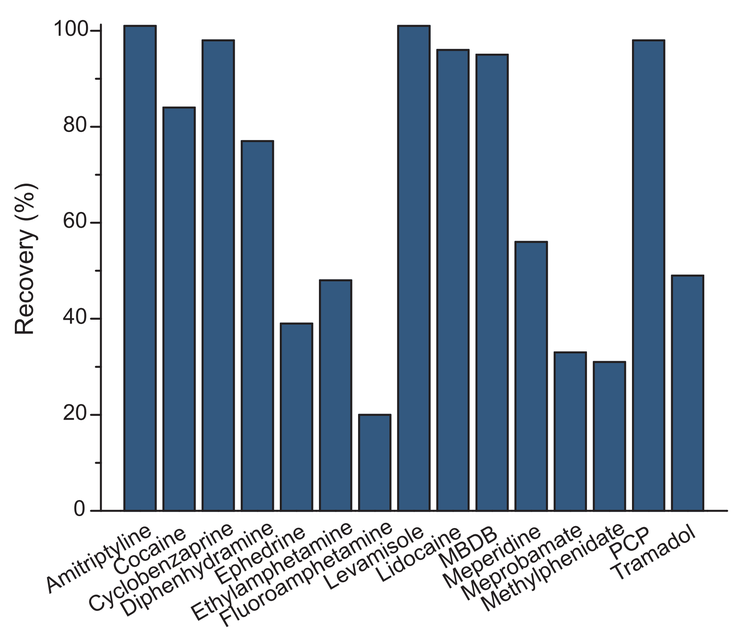
Lower limit of detection (LLOD)

The lower limit of detection was defined as the lowest concentration of analyte tested where the signal:noise ratio was ≥ 20. 146 total drugs tested.



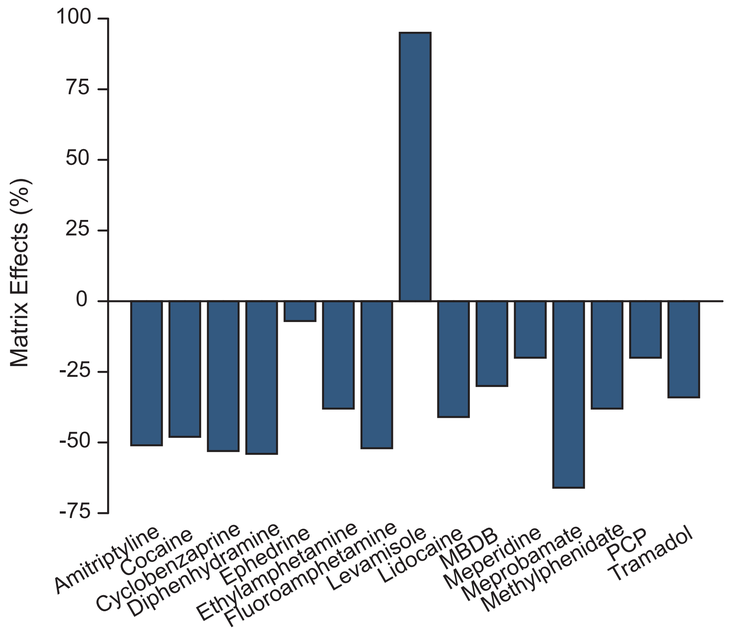
Recovery

Recovery was measured as the ratio of peak area of drug recovered from samples spiked pre-SPE extraction compared to drug recovered from samples spiked post SPE-extraction. Data are shown for a subset of drugs. Average recovery was 70%.



Matrix effects

Matrix effects were measured as the ratio of peak area between drug spiked into urine post-SPE extraction and drug spiked into water post-SPE extraction. Data are shown for a subset of drugs. Average matrix effect was ion suppression of 30%.



Method performance

Method performance was evaluated in part using 30 patient urine comparisons. Our LC-MS/MS method was used as the gold standard. Exclusion of drugs not in the GC-MS/MS method from the analysis substantially improved the sensitivity and NPV.

	Overall	Excluded
Sensitivity	25.5%	56%
Specificity	98.5%	98.5%
PPV	64%	64%
NPV	97%	99%

Conclusions

We present a method that could be used as an orthogonal technique to verify LC-MS/MS urine drug screening results and is compatible with a general unknown screening approach. The sensitivity of our GC-MS/MS method could be improved by the addition of a hydrolysis step prior to the extraction and/or derivitization to improve peak shape and lower the limits of detection for large/polar analytes. Use of both LC and GC technologies in our laboratory allows us to screen patient urine samples for a wider variety of compounds with a greater assurance of accuracy.

Acknowledgements

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