Overview

• A paper spray drug screening method was developed on a quadrupole-orbitrap mass spectrometer
• 125 target compounds were screened in MS/MS mode using an inclusion list
• 11 internal standards were monitored to perform semi-quantitative analysis
• 30 postmortem blood samples were analyzed by paper spray MS/MS. Results were compared to the screening and confirmatory results from a central toxicology lab.

Introduction

• Blood samples are analyzed as dried spots directly from paper
• Extraction solvent is added to the paper followed by application of a high voltage
• Ionization arises from electrospray from the sharp paper tip
• Screening for the complete drug panel requires about 2 minutes with no sample cleanup

Methods

Paper Spray MS
• Mass Spec: Thermo Q-Exactive Focus
• Paper Spray: Velox 360 paper spray interface with Velox paper
• Instrument mode: targeted MS/MS (PRM using an inclusion list)
  • 130 MS/MS scans for 125 targets and 11 internal stds.
• MS settings:
  • Isolation width: +/- 0.5 m/z
  • Resolution: 35,000
  • Polarity: positive ion mode
  • Spray voltage: 5000V
  • AGC target: 10^6
  • Max injection time: 50 ms
• Detection criterion: one fragment ion, 5 ppm m/z window
• Paper spray solvent: 85:10:5:0.01 ACN:acetone:water:acetic acid

Sample Preparation
• Blood sample was mixed 1:3 with an aqueous internal standard solution
• 12 µL of the blood/internal standard mixture was spotted on the cartridge and allowed to dry
• Internal standard solution:
  • 65 ng/mL alprazolam-d5
  • 650 ng/mL benzoylalanine-d8, cocaine-d3, and methamphetamine-d13
  • 260 ng/mL flurazepam-d7, hydrocodone-d3, trimipramine-d3
  • 1300 ng/mL gabapentin-d10
  • 2600 ng/mL methadone-d6
  • 325 ng/mL methamphetamine-d13
  • 130 ng/mL zolpidem-d6

Results

Solvent Optimization

• 95:5 Methanol:water with 0.01% acetic acid showed acceptable results in calibrators
• Significant matrix effects were observed in postmortem blood samples
• ACN based solvent eliminated relative matrix effects
• Dilution of the blood was required to allow solvent to penetrate into blood spot

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Tandem Mass Spectra Example

• Full MS/MS spectra were collected
• Paper spray MS/MS spectra are a composite of target compound and background
• Presence of one fragment ion used for detection
  • More can be used to improve selectivity

Electronic Ionization. Neat solvent
Cocaine 200 ng/mL

182.1173

ESI Infusion. Neat solvent
Cocaine 16.6 ng/mL. 3X below cutoff

182.1179

Paper Spray. Blood Spot

272.1550

150.0916

304.3002

119.0492
Paper Spray MS/MS on a Q-Orbitrap Mass Spectrometer
Michael Potter, and Nicholas E Manicke
University-Purdue University Indianapolis, Indianapolis, Indiana 46202, United States

Cross-Comparison With Independent Tox Lab Screening

30 post mortem samples were analyzed by both an independent toxicology lab and in-house by paper spray MS. The Tox Lab performed its normal screen and confirm workflow: a combination of HPLC-MS/MS and immunoassay screening followed by HPLC-MS/MS confirmation.

- **81**: Drug and drug metabolite targets detected by both
- **7**: Detected by Tox Lab but not by paper spray MS
  - 5/7 were below paper spray detection limit
  - 2/7 were not quantitated by the Tox Lab
- **16**: Detected by paper spray MS but not Tox Lab
  - 6/16 were not tested by Tox Lab (not ordered by customer)
  - 10/16 are likely false positives by paper spray method
  - 2 FP were opiates in the presence of other opiates
  - 6 were low levels near LDR

Paper Spray Semi-Quantitative Performance in Post-Mortem Samples

- In 30 post-mortem blood samples, 61 drug concentrations across all targets were obtained by both paper spray and the Independent Tox Lab HPLC-MS/MS confirmation method.
  - Results outside of the paper spray calibration range (<LQR) or above the ULOQ (N=3) were ignored.
- Paper spray correlated well with HPLC-MS/MS confirmation at the Tox Lab (R² > 0.99)
- Paper spray consistently over-estimated the concentration (slope = 1.13). Average deviation was +39%
- Paper spray quantitation could be improved by decreasing the number of targets or increasing the number of isotope labeled internal standards

HPLC-MS/MS Correlation Compared to Paper MS Spray Screening – All Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>92.0%</td>
<td>TP/(TP+FN)</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.8%</td>
<td>TN/(TN+FP)</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>89.0%</td>
<td>TP/(TP+FP)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>99.8%</td>
<td>TN/(TN+FN)</td>
</tr>
</tbody>
</table>

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