Using accurate mass LC/MS/MS in forensic toxicology screening

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Accurate Mass LC/MS has been around for a while but its use is now starting to increase rapidly:

- Can be used for a wide range of applications e.g. multi-drug analysis, proteins, environmental monitoring and biochemistry
- Has particular advantages for analysis of certain compounds
- Provides a complimentary method of analysis to existing techniques
- QTOF LC/MS with CID MS-MS can provide extremely selective and sensitive detection
Forensic Toxicological applications of QTOF LC/MS/MS

- **General drug screening**
  - Unknown screening
  - Targeted screening

- **Drug measurement**
  - selective and sensitive

Can be applied to various **specimens**; blood, plasma/serum, urine, oral fluid, hair, post-mortem fluid, tablets/solutions.

Can be applied for a wide range of **requests**; medico-legal, post-mortem and forensic toxicology.
Forensic drug screening

Advantages:
• Existing advantages of LC-MS (no derivatisation, sensitive, selective, etc). UHPLC provides rapid analysis. Can also couple to UV.
• Can detect a very wide range of analytes (depending on extraction used)
• Identification based on accurate mass molecular ion (searchable by library or e.g. ChemSpider or PubChem)
• Accurate mass MS-MS spectral matching
• Software assists with structural elucidation
• Can return to historic data to revisit possible presence of compounds

Disadvantages:
• More compounds are isobaric than you would think, even with accurate mass
• For absolute identification, still need a retention parameter (or if accurate mass CID MS-MS is available)
General screening
Agilent 2.1 mm x 100 mm Eclipse Plus C18 1.8 micron column
Acetonitrile and 0.1% formic acid mobile phase UHPLC gradient

System allows detection by accurate mass and automated MS-MS.
From the TIC, various filters can be used to identify potential peaks of interest, followed by searching against a database/library.
Use of QTOF LC-MS

Case Examples

Glucuronide metabolites (extended window of detection)

Temazepam glucuronide

+ESI Product Ion (2.553 min) Frag=110.0V ClD@32.6 (477.1063[z=1] -> **) 500std.d

Counts vs. Mass-to-Charge (m/z)

Counts vs. Acquisition Time

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Case Examples

Glucuronide metabolites (extended window of detection)

Use of QTOF LC-MS

For Forensic Use.
Case Examples
New “Designer Drugs” – 4-methylamphetamine, 2-aminoininan, MDAI

Use of QTOF LC-MS
Case Examples – 4-Methylamphetamines

Amphetamine

4-Methylamphetamines case possibility

4-Methylamphetamines reference standard

For Forensic Use.

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Case Examples

New “Designer Drugs” – 4-methylamphetamine, 2-aminoindan, MDAI
Case Examples – 2-Aminoindan

2-Aminoindan is isobaric with Tranylcypromine

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Formula</th>
<th>Mass</th>
<th>Delta Mass (ppm)</th>
<th>RT (min)</th>
<th>CAS</th>
<th>ChemSpider</th>
<th>IUPAC Name</th>
<th>Spectra #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tranylcypromine</td>
<td>C9H11N</td>
<td>133.08915</td>
<td>0.95</td>
<td>155.039</td>
<td>10393</td>
<td>(1R,2S)-2-Phenylethylcyclopentamine</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

2-Aminoindan is isobaric with Tranylcypromine
Case Examples

Re-examination of findings – **phenelzine** (following new evidence of use)
Use of QTOF LC-MS

- Complements existing forensic systems
  - Immunoassay
  - GC-MS
  - HPLC-DAD
  - LC-MS

- Replaces existing forensic systems
  - Immunoassay
  - GC-MS
  - HPLC-DAD
  - LC-MS
Complementary implementation

• Ensure you are aware of analytical coverage overlap or differences
• Possible to use same extraction procedures or extracts
• Provides additional confidence in results (particularly screening)
• Method can be validated and evaluated alongside existing systems

Replacement implementation

• Ensure it performs as well as if not better than the method it is replacing!
• Implementation should be carefully planned to fit in with the existing workflow
• The new method should be fully validated and evaluated
• The workforce should be trained appropriately for effective use
PROBLEMS AND PITFALLS

- If using sensitive QTOF MS for the first time, you will find even more things than traditional LC/MS-MS! This can alter detection window comments, operating cut-offs and other interpretative aspects.

- Be aware of in-source fragmentation this can affect identification of molecular ion.

- Experiment with positive and negative mode – some drugs can do both.

- Using deuterated internal standards or matrix dilution can minimise any ion suppression/enhancement effects.

- Don’t forget about U/HPLC – the better the chromatography, the better the MS data obtained.
CONCLUSIONS

• QTOF LC-MS provides a complementary method of analysis or can replace existing techniques

• Can be applied to a very wide range of analytes so has particular advantages for general screening (inc. ability to re-interrogate historic data)

• Need to be aware of interpretative and analytical issues

• Careful implementation and training can enhance workflow and significantly improve the laboratory service

THANK YOU FOR YOUR ATTENTION