Evolution of urine drug screening from immunoassay to LC MS/MS: Do we need accurate mass?

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• A bit of history
• Current setup
• Audit
• LC-MS/MS method
  • (Repeated a couple of times!)
• The future (with a Cost Improvement Programme).
Introduction

• This is very much work in progress
• It will be a little untidy round the edges and I don’t claim to have all the answers
• If you’ve got a better way of doing things, please let me know!
Introduction

• The ‘traditional’ drugs are thought to be not as popular with younger users.
• In Newcastle-upon-Tyne and Northumberland we’ve a big problem with gabapentin and pregabalin
  • Seems less of an issue (at PM) in Middlesbrough
• In Newbiggin, Lynemouth and Ashington big problem with diconal. Not seen 15 miles away in Newcastle.
• Folk may also be taking alternative drugs to avoid detection.
Introduction

• Numbers of compounds are escalating
Introduction

• Does this matter?
  • There is a study – plug at the end
    • Looking at NPS involvement in acute medical admissions / emergencies.
  • Chatting to a Forensic Psychiatrist in the pub:
    • “Acute / high dependency beds in Psychiatric Units are full of people with psychosis related to legal high use”
    • Have had a stillbirth involving NPS

• NPS is the preferred term
  • Novel Psychoactive Substances
  • Legal implies safe.
Introduction – UKAS

• For those of you with Toxicology services:
  • After discussion with UKAS we will be accredited to 15189 with additional requirements from ILAC-G19.
  • Those assays not fully validated for their intended purpose will not be added to our scope
  • QA is ‘interesting’ for the newer drugs, given the local variations.
    • Am recording agreement between the two LC-MS/MSs (different LC conditions) and clinical details
    • Also a few comparisons with Newcastle University
A bit of history

• Historically a single handed service
  • Shared NE England toxicology work with Newcastle General, latterly Freeman, Hospital.
• Took over service in 2011/12
  • Service in Newcastle now much reduced due to retirement of Toxicologist (very experienced BMS) and virtual collapse of two ancient Hewlett Packard GC-MSs
    • They seemed to be pre Agilent!
A bit of history

- **Workload**
  - Around 2000 drug screen urines a month (half needing confirmations)
  - Around 450 cases for HM Coroner
  - 7 day (not 24hr) urgent ethylene glycol and methanol service
  - 2 full time staff (me and a BMS2).
    - Soon to be joined by a band 3 (I hope).
    - Rotating BMS1 assistance for setting up routine screens.
Current setup

- Wansbeck Service
  - AU680 running CEDIA assays for the 6 groups
    - Amphetamines, BDZ, cannabis, cocaine, EDDP and opiates. Positive amphetamine and opiate screens confirmed on LC-MS/MS
  - 2 LC-MS/MS (Thermo Quantum Ultra, with Accella LC [turboflow] and CTC autosampler).
  - Routine assays using Tracefinder (relatively easy for rotating BMS to use)
  - Non-routine and more complex still using Xcalibur.
  - Should be interfaced to lab IT, but ‘issues’
    - Moving swiftly on…
Current setup

- Urine LC-MS/MS assays
  - Abused Prescription Drugs
  - Amphetamine confirm / stimulant NPS screen
  - Buprenorphine
  - BDZ (extended)
  - Big screen (everything we’ve got tuned up, 40 minute run)
- Cannabis confirm
- Methadone confirm
- Opiate confirm
- Employment screen extra drugs
Audit

- This concentrated on the amphetamine screening assay as false positives common
  - Other screens not too bad – running at around about 1-5% false positive rate.

- Amphetamine audit:
  - 948 requests (269 from women), from 1/6/14 to 31/12/14
  - Audit included Freeman (Roche Integra).
  - In this time period:
    - 201 requests for NPS
    - 135 requests for synthetic cannabinoids / Spice
The difference was significant between the proportions of male and female false positives

- P = 0.0005

Clinically this matters as false positives in pregnancy can lead to child protection procedures being instigated in entirely innocent cases.

- Very difficult to remove investigation from the records
- Almost guilty until proven innocent, but it is a nightmare for the social workers – who will be blamed no matter what happens.

<table>
<thead>
<tr>
<th></th>
<th>Positive immunoassay and confirmation</th>
<th>Positive immunoassay and negative confirmation</th>
</tr>
</thead>
<tbody>
<tr>
<td>pregnant</td>
<td>15</td>
<td>29</td>
</tr>
<tr>
<td>non pregnant</td>
<td>70</td>
<td>88</td>
</tr>
<tr>
<td>male</td>
<td>285</td>
<td>208</td>
</tr>
</tbody>
</table>

Audit – false positives
## Audit – drugs detected

<table>
<thead>
<tr>
<th>Drug</th>
<th>Total</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>283</td>
<td></td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>8</td>
<td>Only found in amphetamine positive samples. Presence due to impurities in the amphetamine</td>
</tr>
<tr>
<td>Ethylamphetamine</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>p-methoxyamphetamine</td>
<td>2</td>
<td>Only found in amphetamine positive samples. Amphetamine metabolite (?), but also known to be abused</td>
</tr>
<tr>
<td>Methiopropamine</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>MDMA</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>12</td>
<td>Metabolite of MDMA</td>
</tr>
<tr>
<td>Ritalinic acid</td>
<td>31</td>
<td>Metabolite of methylphenidate and its illicit analogue</td>
</tr>
<tr>
<td>Trazodone and mCPP</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Ketamine</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Cathine</td>
<td>4</td>
<td>May be an endogenous compound or final metabolite of cathinones</td>
</tr>
<tr>
<td>Mephedrone</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Methylethcathinone</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Pentedrone</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Levamisole</td>
<td>101</td>
<td>Cocaine contaminant.</td>
</tr>
<tr>
<td>July Powder</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>20</td>
<td>Antihistamines that can be abused</td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Procyclidine</td>
<td>15</td>
<td>A prescribed drug that can be abused</td>
</tr>
</tbody>
</table>

*Not much Ecstasy – is the population using it not accessing treatment services?*
The assay

- 20 μL urine + 1 mL of internal standard
  - IS amphetamine D6, PCP D5 and Methaqualone D7 in 5% MeOH
  - Gives IS peaks at a range of retention times.
- Kinetix biphenyl (Phenomenex)
- Methanol / Water with 0.1% formic acid
- Gradient of 2 – 100% MeOH
- 62 drugs in screen (slowly increasing)
  - In audit over half of these not detected in 6 months.
The assay

- All compounds have two transitions whenever possible.
- Standards of 0.1 and 1.0 mg/L for all analytes
  - Allows for approximate quantification if needed.
Issues

• Drug substance handed to the service in July 2014.
• Tuned up in tandem in an attempt to identify it
  • Client had hoped for mephedrone, but this was more ‘ketamine like’
    • Can’t trust drug dealers these days
• In time passed to Clinical Pharmacology, Newcastle University
  • Either ethylphenidate or methylmethphenidate.
Issues

- Sometimes ‘July Powder’ detected with ritalinic acid.
- So likely to be ethylphenidate
- Now have tuned up system with ethylphenidate
- Retention time very similar to July Powder
Issues

• Fluoroamphetamine
  • Fluoromethamphetamine detected in a post mortem where we got the powder as well as the patient samples.
  • Metabolised to fluoroamphetamine.
  • Fluoromethamphetamine not detected in any sample since then
  • Fluoroamphetamine has turned up regularly since then
• Or has it?
Issues

• 2-Fluoroamphetamine
  • 154>83, 109

• Peak with slightly different retention time.

• Extracted one sample into MTBE, dried down and reconstituted in methanol. Infused via T piece
  • 154>67, 94

• Gave enough of the 154>83 and or 109 to be detectable.
  • ?3- or 4-fluroamphetamine
But one ethylphenidate this month...
Time scale

- We could remove drugs from the screen once they are no longer detected.
- But what happens if they come back?
- Some drugs (e.g. methiopropamine) aren’t picked up by the CEDIA assay.
- So we’re stuck with ever increasing numbers of compounds
  - Full revalidation on each occasion??
    - Aarrgghh.
Opiate confirmation

- Morphine, morphine 3- and 6-glucuronide
- Codeine and glucuronide
- Dihydrocodeine and glucuronide
- 6-monacetyl morphine and 6-acetylcodine
- Oxycodone
- Pholcodine
- Fentanyl and norfentanyl, alfentanil and sufentanil
  - Fentanyl does occasionally appear
  - But of course CEDIA assay does not detect it.
**Issues**

- Morphine and norcodeine are isobaric
  - So LC method optimised to provide good separation
  - Also have morphine D3 as internal standard to line morphine peak up with.
- But these optimal conditions mean noroxycodone \((\text{C}_{17}\text{H}_{19}\text{NO}_4)\) and dihydrocodeine \((\text{C}_{18}\text{H}_{23}\text{NO}_3)\) co-elute
  - They are isobaric
Issues

• About once or twice a fortnight have samples that contain just 6-monoacetyl morphine and 6-acetylcodineine
  • We interpret these as spiking of the sample with heroin
  • A few of them have clearly been spiked with buprenorphine
    • Massive buprenorphine peak (incompatible with life), no metabolites.
BDZ (benzodiazepine)

- Generally not much of an issue as diazepam is widely available
- False positive screens known with sertraline
  - We also suspect nefopam
    - As an aside we are seeing more of this drug
    - Used as an alternative to tramadol
    - Illicit users are catching on it.
BDZ (benzodiazepine)

• Recent one
  • Had hoped to have the full story for you, but waiting for standard from Sigma.
• Patient admitted having taken ‘diazepam’
• BDZ screen positive, but symptoms not typical for diazepam overdose.
• Urine positive for zopiclone (and other bits n bobs)
  • Reason for positive screen suggested in literature?
BDZ (benzodiazepine)

- Were passed one of the tablets.
  - Broke it up and dissolved in acetonitrile
- Infused through T-piece
- $351 > 206, 179, 186$ and $104$
- Matched phenazepam standard
  - According to Toxicology at Glasgow University it is known to be passed off as diazepam…
BDZ (benzodiazepine)

- Did the patient take it?
  - Attempted to guess settings for main reported analyte (3-hydroxy) (i.e. added 16 to the transitions).
  - Didn’t work (to be fair works 50% of the time)
  - Urine negative for phenazepam itself
    - Little human data on metabolism (unless can read Russian)
  - So need standard (Sigma usually pretty quick).
BDZ (benzodiazepine)

- Some structures…

Diazepam

Phenazepam

3-hydroxyphenazepam

Sertraline

Nefopam
BDZ (benzodiazepine)

- The other ‘designer’ BDZ
  - Flubromazolam, flubroazepam etc
  - Seem to be detected by immunoassay screens.
  - But of course we won’t see anything on the LC-MS/MS
  - Parent compound may be largely absent.
Big screen
• Currently at 250+ drugs and metabolites
• 7 screens in Xcalibur for the results
  • Can only be read by the two of us in Toxicology
  • C18-PFP column (ACE/Hichrom) with 2mmol/L ammonium acetate + 0.1% formic acid in methanol water. Gradient of 2-100% methanol over 40 mins
• Used as an initial screen
Big screen

- Adding lots of compounds to the big screen reduces sensitivity of MS to individual compounds
  - For compounds with standards just look for it at the known retention time +/- 2.5 mins
  - So only a small part of a 40 min run time
  - Diazepam and metabolites in particular badly affected
Big screen

• With this column a peak isobaric with amphetamine has the same RT as amphetamine D6. Fortunately completely resolved on the biphenyl column.

• Common interferant for cyclizine with the same RT.
  • Disappears completely with the biphenyl column

• But biphenyl column gives poorer peak shapes for a number of drugs
  • No way could we set up the method with a new column type in under a fortnight…
Summary
• The immunoassay screens are OK
• Amphetamine is barely fit for purpose
• EDDP and cocaine appear to be specific
• Opiate will always need confirmation to distinguish between heroin and therapeutic opiate use
  • Abuse of diamorph etc not unknown.
• The elephant in the corner of the room are the synthetic cannabinoids
  • No chance. Thankfully neither had a referral lab with an accurate mass MS…
Summary

• Workload too much to allow transfer of all the work to the LC-MS/MSs.
• AU is fast and efficient
  • MLA staff occasionally mix up urines when decanting from universal to analyser tube.
  • Rotating MLA staff can’t cope with toxicology booking in. Samples too diverse.
The future

- Accurate mass MS not cheap.
  - Cost similar to a mid-range tandem
  - Does not fit well with the CIP
  - Though the income from toxicology does…

- A new technique to get used to.

- In the interim:
  - Am in the process of buying some software – ACD – MS Fragmenter.
    - Enter the structure and it will calculate the MS fragments. Only £2300 odd for one user

- Hopefully get one with the new MSC due for the main lab in 2018.
The future

• Tandems have the specificity and the higher end ones are more sensitive
• Scanning accurate mass (ToF or Orbitrap) can (in theory) detect anything.
  • But what about compounds that don’t ionise well
  • Or positive / negative switching?
• Can we really spend 30 or so mins on each sample reading the results?
A plug...

- IONA study.

Identification and characterization of the clinical toxicology of novel psychoactive substances (NPS) by laboratory analysis of biological samples from recreational drug users.

Short title

Identification Of Novel psychoActive substances (IONA)
IONA

• There are 4 studies
  – 1. Analysis of NPIS enquiry data
  – 2. Collation of toxicology data provided by participating NHS laboratories
  – 3. Further analysis of samples already collected as part of clinical care
  – 4. Collection of samples for research purposes from people attending participating emergency departments
IONA Study Overview

National Poisons Information Service
- Edinburgh
- Newcastle
- Birmingham
- Cardiff

NHS Toxicology labs
- Wansbeck
- Birmingham
- Cardiff

Forensic Toxicology labs
- Scottish Police Authority (Edinburgh)

Clinical data from cases of suspected NPS exposure

HPRU Newcastle
- Laboratory analysis
- Data processing

Samples and clinical data from patients with severe NPS toxicity

Samples for identification of NPS

NHS Hospitals
- Bart’s, London
- Blackpool
- Edinburgh
- Liverpool
- London Hospital
- Newcastle
- Northumbria
- North Manchester
- Southport & Omskirk
- St Thomas’, London
- ..more to follow
IONA

• Led by Newcastle University / Newcastle Hospitals.
• Study website: www.ncl.ac.uk/hpru/research/project/5099
• 0191 222 8094
• Simon.thomas@newcastle.ac.uk
• For the lab side of things, nigel.brown1@nhs.net
• No grant money unfortunately. But am combining with audit and QA requirements.
  – 3 for the price of 1. 😊
Acknowledgements

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- Structures obtained from ChemSpider
  - Royal Soc Chem database – very useful