LC-MS for Pain Management Support

Gwen McMillin, PhD, DABCC(CC,TC)
University of Utah
ARUP Laboratories
Outline

- Overview of drug testing, as a component of the therapeutic plan, in the management of chronic pain
- A mini-SWOT analysis for application of LC-MS to pain management drug testing
- Considerations for optimizing utility of LC-MS results
Drug testing in pain management

- Baseline testing, before initiating opioid therapy
- Routine testing
  - Periodic, based on patient risk assessment
  - To evaluate changes
    - Therapeutic plan (drugs, formulations, dosing)
    - Clinical response (poor pain control, toxicity)
    - Clinical events (disease, surgery, pregnancy)
    - Patient behavior
Objectives of drug testing

Detect and encourage appropriate drug use

Detect and discourage inappropriate drug use

Non-Adherence

Adherence
Traditional approach

- Immunoassay-based screen
- Confirm screen positive results with mass spectrometric method (GC-MS, LC-MS)

*Not appropriate for pain management*

- Reflex testing leads to unnecessary expenses if the results are consistent with expectations, or if results are not used to make patient care decisions
- Confirmation of negative results may be more important than confirmation of positive results
- Immunoassay-based screens may not be available for specimens and drugs of interest
Drugs monitored for pain management represent ~25% of “Top 200” prescriptions filled, 2011

Concentrations (ng/mL) required to trigger a positive benzodiazepine (300 ng/mL cutoff)

<table>
<thead>
<tr>
<th>Substance</th>
<th>EMIT</th>
<th>Nex Screen</th>
<th>Triage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alprazolam</td>
<td>79</td>
<td>400</td>
<td>100</td>
</tr>
<tr>
<td>Alpha-OH-alprazolam</td>
<td>150</td>
<td>N/A</td>
<td>100</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>500</td>
<td>5,000</td>
<td>650</td>
</tr>
<tr>
<td>7-amino-clonazepam</td>
<td>11,000</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Diazepam</td>
<td>120</td>
<td>2,000</td>
<td>200</td>
</tr>
<tr>
<td>Nordiazepam</td>
<td>140</td>
<td>500</td>
<td>700</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>350</td>
<td>300</td>
<td>3,500</td>
</tr>
<tr>
<td>Temazepam</td>
<td>210</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>890</td>
<td>4,000</td>
<td>200</td>
</tr>
</tbody>
</table>

False negatives likely
Concentrations (ng/mL) required to trigger a positive opiate (300 ng/mL cutoff)

<table>
<thead>
<tr>
<th>Opiate</th>
<th>EMIT</th>
<th>CEDIA</th>
<th>Triage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Codeine</td>
<td>247</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>6-monoacetylmorphine</td>
<td>1088</td>
<td>300</td>
<td>400</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>364</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Hydromorphone</td>
<td>498</td>
<td>300</td>
<td>500</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>5,388</td>
<td>10,000</td>
<td>20,000</td>
</tr>
<tr>
<td>Oxymorphone</td>
<td>&gt;20,000</td>
<td>20,000</td>
<td>40,000</td>
</tr>
<tr>
<td>Noroxymorphone</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

False negatives likely
Percent of “missed” positive results comparing immunoassay with LC-MS/MS

<table>
<thead>
<tr>
<th>Compound</th>
<th>Immunoassay cutoff (ng/mL)</th>
<th>LC-MS/MS cutoff (ng/mL)</th>
<th>% missed by immunoassay (total n ~8000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codeine</td>
<td>300</td>
<td>50</td>
<td>29.6% (45)</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>50</td>
<td>50</td>
<td>23.3% (701)</td>
</tr>
<tr>
<td>Hydromorphone</td>
<td>50</td>
<td>50</td>
<td>69.3% (1878)</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>200</td>
<td>20</td>
<td>53.3% (646)</td>
</tr>
<tr>
<td>Nordiazepam</td>
<td>40</td>
<td>40</td>
<td>40.0% (320)</td>
</tr>
</tbody>
</table>

Mikel et al., *TDM* 31(6):746-8, 2009
Immunoassays that might be required to detect opioids

“Opiates”
- Codeine
- Morphine
- Hydrocodone
- Hydromorphone

Opioid antagonists
- Methadone
- Propoxyphene

Opioids
- Oxycodone
- Oxymorphone
- Fentanyl
- Tapentadol
- Tramadol
- Meperidine
- Buprenorphine

Heroin
Evolving approach

- Understand needs
- Understand testing options
- Select tests based on needs and options
- Evaluate results
- Follow-up testing for unexpected or inadequate results

- HUGE opportunities for MS-based approaches
MS in pain management

- Confirmation testing
  - Resolve unexpected negatives/positives
  - Determine compound(s) responsible for a positive screen

- Quantitative testing
  - Concentrations of specific analytes, and patterns (e.g. metabolic ratios) guide interpretation for some drug classes
  - May help construct longitudinal patient profiles

- Multi-drug detection panels
  the “new” approach to “screening” that could eliminate the need for confirmation testing
Strengths

- Specificity
- Sensitivity
- Flexibility

Weaknesses

- Specificity
- Sensitivity
- Flexibility

Opportunities

- Specificity
- Sensitivity
- Flexibility

Threats

- Specificity
- Sensitivity
- Flexibility
Considerations

- Performance of multi-analyte panels
- Specific analytes detected
- Cutoffs and required dynamic range
- Carryover
- Isobaric interferences
- Ion suppression
- Reimbursement potential
- Challenges with interpretation
Performance of multi-analyte panels

- Standardization is currently lacking among laboratories
- No single extraction method can recover all drugs of interest with equivalent performance
- Chromatographic resolution of all analytes is tough to achieve within a ‘reasonable’ run time
- Not all analytes ionize adequately to achieve desired sensitivity; may require different modes, techniques, etc.
- Many isobaric compounds exist
Examples of isobaric opioid species (nominal mass)

<table>
<thead>
<tr>
<th>m/z</th>
<th>Opioid compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>286.1</td>
<td>Morphine, Hydromorphone, Norcodeine, Norhydrocodone</td>
</tr>
<tr>
<td>300.3</td>
<td>Codeine, Hydrocodone</td>
</tr>
<tr>
<td>302.2</td>
<td>Oxymorphone, Noroxycodone, Morphine n-oxide, Dihydrocodeine</td>
</tr>
<tr>
<td>328.2</td>
<td>6-monoacetylmorphine, Naloxone</td>
</tr>
</tbody>
</table>
Examples of isobaric opioid species (exact M+H mass)

<table>
<thead>
<tr>
<th>m/z</th>
<th>Molecular formula</th>
<th>Opioid compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>285.13649</td>
<td>C$<em>{17}$H$</em>{19}$NO$_3$</td>
<td>Morphine, Hydromorphone, Norcodeine, Norhydrocodone</td>
</tr>
<tr>
<td>299.15214</td>
<td>C$<em>{18}$H$</em>{21}$NO$_3$</td>
<td>Codeine, Hydrocodone</td>
</tr>
<tr>
<td>301.13141</td>
<td>C$<em>{17}$H$</em>{19}$NO$_4$</td>
<td>Oxymorphone, Noroxycodone, Morphine n-oxide, Dihydrocodeine</td>
</tr>
<tr>
<td>301.16779</td>
<td>C$<em>{18}$H$</em>{23}$NO$_3$</td>
<td></td>
</tr>
<tr>
<td>327.14706</td>
<td>C$<em>{19}$H$</em>{21}$NO$_4$</td>
<td>6-monoacetylmorphine, Naloxone</td>
</tr>
</tbody>
</table>
Exact mass depends on specific ions of interest, and isotope abundances
Isobaric pairs in an assay designed to detect 67 drug analytes (= 45 parent drugs)

- Codeine and Hydrocodone
- Morphine and Hydromorphone
- 6-monoacetylmorphine and Naloxone
- Methylphenidate and Normeperidine
- Methamphetamine and Phentermine
- Amobarbital and Pentobarbital
Example EIC

Stimulants (Amps, Cocaine)

Naloxone, Naltrexone

Morphine, Oxymorphone, Hydromorphone...

Benzos

THC

Marin et al, JAT, 2012 Jul 15 [Epub ahead of print]
Performance vs. ELISA (n = 119)

ELISA:
- 55 positive samples = 46%;
- 78 positive results, 10 drug classes

TOF:
- 71 positive samples = 60%;
- 200 results, 36 drug analytes

48 negative by both methods
Benzodiazepines detected by traditional vs. LC-MS/TOF methods (n=493)

Does this suggest false negatives by the traditional approach? False positives by TOF? Combination?

161 positives by traditional reflex
591 positives by LC-MS/TOF screen

Alprazolam  Clonazepam  7-aminoCLZ  Diazepam  Temazepam  Oxazepam  Nordiazepam  Midazolam  Lorazepam

LC-MS/MS  LC-MS/TOF
### Proposed ‘cutoff’ and ranges of opioids in pain management patients

<table>
<thead>
<tr>
<th>Opioid</th>
<th>Range (ng/mL)</th>
<th>Opioid</th>
<th>Range (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codeine</td>
<td>25-30,000+</td>
<td>Fentanyl</td>
<td>1-600+</td>
</tr>
<tr>
<td>Morphine</td>
<td>25-150,000+</td>
<td>Buprenorphine</td>
<td>1-40,000+</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>25-15,000+</td>
<td>Meperidine</td>
<td>25-200,000+</td>
</tr>
<tr>
<td>Hydromorphone</td>
<td>25-5,000+</td>
<td>Tapentadol</td>
<td>25-40,000+</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>25-50,000+</td>
<td>Methadone</td>
<td>50-25,000+</td>
</tr>
<tr>
<td>Oxymorphone</td>
<td>25-30,000+</td>
<td>Tramadol</td>
<td>50-125,000+</td>
</tr>
</tbody>
</table>

Theoretical carryover

- High Positive
- Low Positive
- Negative
- Negative
Possible contributing factors to carryover

- High concentration samples mixed with low concentration samples
- 96-well or other close-proximity sample arrangements
- Sample preparation (e.g. dry-down steps)
- Human error (e.g. pipetting)
- Automation
- Column overload
- Instrumentation

- Random blanks
- Group high concentration samples together, if possible
- Random repeat testing
<table>
<thead>
<tr>
<th>Active pharmaceutical compound</th>
<th>Process impurities</th>
<th>Allowable pharmaceutical impurity limit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codeine</td>
<td>Morphine</td>
<td>0.15</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>Codeine</td>
<td>0.15</td>
</tr>
<tr>
<td>Hydromorphone</td>
<td>Morphine Hydrocodone</td>
<td>0.15 0.1</td>
</tr>
<tr>
<td>Morphine</td>
<td>Codeine</td>
<td>0.5</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>Hydrocodone</td>
<td>1.0</td>
</tr>
<tr>
<td>Oxymorphone</td>
<td>Hydromorphone Oxycodone</td>
<td>0.15 0.5</td>
</tr>
</tbody>
</table>

MRO Alert XXI, No. 3, 2010
Utility of quantitative results

- Helps identify carryover
- May identify pharmaceutical impurities

- Determine patterns of parent drug and metabolite(s)
  - May help determine what parent drugs were taken
  - May estimate chronology of last dose
  - May determine metabolic phenotype for a patient
  - May identify drug-drug or food-drug interactions
  - May identify changes in clinical status that affect pharmacokinetics
  - May identify adulteration intended to mimic adherence
Opioids can be both parent drugs and drug metabolites.
Simplified metabolism of oxycodone and hydrocodone

Norhydrocodone, $R = H$
Noroxycodone, $R = OH$

Hydrocodone, $R = H$
Oxycodone, $R = OH$

CYP3A4

Conjugates

Hydromorphone, $R = H$
Oxymorphone, $R = OH$

CYP2D6

Norhydromorphone
Noroxymorphone

Heltsley et al, JAT 34:32-8, 2010
Urine oxycodone data ‘clusters’ (n = 396)

May represent patients with impaired CYP2D6

May represent patients with accelerated CYP2D6 or direct addition of parent drug to urine
Buprenorphine metabolism

Picard et al. Drug Metab and Dispill 33:689-95, 2005
Summary of urine buprenorphine patterns (n = 1,946)

<table>
<thead>
<tr>
<th>Analyte pattern</th>
<th>Number of samples</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bup-gluc + Norbup + Norbup-gluc</td>
<td>956</td>
<td>49%</td>
</tr>
<tr>
<td>Bup + Bup-gluc + Norbup + Norbup-gluc</td>
<td>809</td>
<td>42%</td>
</tr>
<tr>
<td>Norbup + Norbup-gluc</td>
<td>68</td>
<td>4%</td>
</tr>
<tr>
<td>Bup + Norbup</td>
<td>58</td>
<td>3%</td>
</tr>
<tr>
<td>Norbup-gluc</td>
<td>24</td>
<td>1%</td>
</tr>
</tbody>
</table>

~91% of positives

Bup: Buprenorphine (free)
Norbup: Norbuprenorphine (free)
Bup-gluc: Buprenorphine-3-glucuronide
Norbup-gluc: Norbuprenorphine-3-glucuronide
# Summary of urine buprenorphine patterns (n = 1,946)

<table>
<thead>
<tr>
<th>Analyte pattern</th>
<th>Number of samples</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bup-gluc + Norbup + Norbup-gluc</td>
<td>956</td>
<td>49%</td>
</tr>
<tr>
<td>Bup + Bup-gluc + Norbup + Norbup-gluc</td>
<td>809</td>
<td>42%</td>
</tr>
<tr>
<td>90th percentile of Bup = 16 ng/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bup &gt; 1000 ng/mL</td>
<td>78</td>
<td>3%</td>
</tr>
</tbody>
</table>

Bup: Buprenorphine (free)  
Norbup: Norbuprenorphine (free)  
Bup-gluc: Buprenorphine-3-glucuronide  
Norbup-gluc: Norbuprenorphine-3-glucuronide
Results suggest drug was added

<table>
<thead>
<tr>
<th></th>
<th><strong>BUP (ng/mL)</strong></th>
<th><strong>NORBUP (ng/mL)</strong></th>
<th><strong>Naloxone (ng/mL)</strong></th>
<th><strong>BUP: Naloxone Ratio</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39,400</td>
<td>24</td>
<td>6,690</td>
<td>5.9</td>
</tr>
<tr>
<td>2</td>
<td>39,200</td>
<td>36</td>
<td>9,560</td>
<td>4.1</td>
</tr>
<tr>
<td>3</td>
<td>31,100</td>
<td>20</td>
<td>8,500</td>
<td>3.7</td>
</tr>
<tr>
<td>4</td>
<td>20,200</td>
<td>23</td>
<td>5,160</td>
<td>3.9</td>
</tr>
<tr>
<td>5</td>
<td>19,300</td>
<td>11</td>
<td>4,470</td>
<td>4.3</td>
</tr>
<tr>
<td>6</td>
<td>18,800</td>
<td>31</td>
<td>4,430</td>
<td>4.2</td>
</tr>
<tr>
<td>7</td>
<td>15,000</td>
<td>7</td>
<td>2,300</td>
<td>6.5</td>
</tr>
<tr>
<td>8</td>
<td>12,100</td>
<td>14</td>
<td>3,110</td>
<td>3.9</td>
</tr>
<tr>
<td>9</td>
<td>11,100</td>
<td>12</td>
<td>2,920</td>
<td>3.8</td>
</tr>
<tr>
<td>10</td>
<td>10,900</td>
<td>7</td>
<td>3,010</td>
<td>3.6</td>
</tr>
</tbody>
</table>

NOTES:

Expected ratio of BUP:Naloxone for Suboxone® = 4

Average ratio of BUP:Naloxone for these patients: 4.4

McMillin et al., *JAT* 36(2):81-7, 2012
Variables with metabolic ratios

- Patient phenotype
  - Genetics
  - Co-medications
  - Clinical status

- Drug
  - Formulation
  - Route of administration
  - Dose and dosing pattern

- Sample preparation method
  - Hydrolysis, and associated efficiency
  - Extraction method, and associated recoveries

- Analytical method and reporting
  - Cutoff and reportable range
  - Normalization of results
  - Inaccuracies due to ion suppression
Hydrolysis efficiency for morphine

<table>
<thead>
<tr>
<th>Morphine Metabolite</th>
<th>Chemical (acid)</th>
<th>Enzyme (P. vulgar, 2 hrs)</th>
<th>Enzyme (H. pomatia, 16 hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine-3-glucuronide</td>
<td>100 ± 4</td>
<td>94 ± 2</td>
<td>50 ± 13</td>
</tr>
<tr>
<td>Morphine-6-glucuronide</td>
<td>98 ± 5</td>
<td>12 ± 1</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Patient urine</td>
<td>100 ± 0</td>
<td>64 ± 19</td>
<td>35 ± 20</td>
</tr>
</tbody>
</table>

Percent (%) recovery of opioids using different hydrolysis methods

Ion suppression comparison

SPE

NOC

OC

OC-d6

M

Dilute & Shoot

NOC

OC-d6

OC
Alternate specimens: another opportunity for MS…

Oral fluid

Blood (serum/plasma)
Utility of oral fluid and blood

- When adulteration or substitution of urine is suspected
- Dialysis patients
- Physician/clinic preference

- Timed blood samples are foundation of pharmacokinetic evaluations and therapeutic drug monitoring
- Oral fluid patterns and expectations are not well characterized (compared to blood and urine) for all drugs of interest in pain management
Oral fluid vs. urine

- 15% disagreement with urine; oral fluid poor for
  - Hydromorphone and oxymorphone
  - Benzodiazepines
- Sensitivity 69% versus urine
- Specificity 92% versus urine
- Concentrations between the two matrices do not correlate

Challenges

Blood
- Requires phlebotomy
- Requires processing

Oral fluid
- Many patients have dry mouth
- Specimen volume limited
- Presence depends on drug and metabolite pKa, protein binding

- Concentrations are 10-100 times lower than in urine
- Commercially available tests are limited

Great opportunity for MS-based methods
Conclusions

- MS-based methods have great potential to improve patient care in the area of pain management, through strengths in sensitivity, specificity, and flexibility.

- MS-based methods apply well to alternative matrices such as oral fluid and blood.

- Standardization of cutoffs, analytes detected, and approach to metabolic ratios is needed.

- Methods should be carefully scrutinized for potential of carryover, contribution of isobaric interferences, and ion suppression.
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