AACC Online Conference

The Laboratory's Role in Drug Monitoring for Pain Management

Analytical Methods III

Mass Spectrometry

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Learning Objectives

After this presentation, you should be able to:

1. List the reasons for confirming immunoassay positive results
2. Describe the advantages of mass spectrometry assays over immunoassays
3. Describe the advantages of direct testing by mass spectrometry without initial testing by immunoassays
4. List the disadvantages of mass spectrometric assays

Mass Spectrometry in Pain Management Drug Testing

Uses of Mass Spectrometry (MS)

I. Confirmation of immunoassay test results

II. Direct to MS testing without initial screen by immunoassays

I. Confirmation Of Immunoassay Test Results

Confirmation of immunoassay positives is neither mandatory nor always necessary

- Provider may forego confirmation test if immunoassay result is consistent with clinical expectation
- Confirmation test often ordered when:
  - Specimens screen negative for prescribed drugs
  - Specimens screen positive for non-prescribed drugs
  - Specimens screen positive for illicit drugs
  - Unusual patient behaviors are observed

I. Confirmation Of Immunoassay Test Results

What is an acceptable Confirmation Test? Does MS meet the following criteria?

- Confirmation test should be
  - Based on an analytical principle different from that of the immuno-reaction ✓
  - More specific than immunoassay to eliminate false positive ✓
  - Equal to or more sensitive than immunoassay ✓

Typical Confirmation Test Menu for Pain Management

Amphetamines	Amphetamine, methamphetamine, MDMA, MDA
Buprenorphine	Buprenorphine, norbuprenorphine
Benzodiazepines	α-OH-alprazolam, 7-aminoclonazepam, lorazepam, nordiazepam, oxazepam, temazepam
Cocaine	Benzoylecgonine
Cannabinoids	Δ^9-THC-carboxylic acid
Fentanyl	Fentanyl, norfentanyl
Opiates	6-acetylmorphine, codeine, dihydrocodeine, hydrocodone, hydromorphone, morphine, oxycodone, oxymorphone

I. Confirmation Of Immunoassay Test Results

Confirmation tests overcome limitations of immunoassays by being able to:

1. Confirm the presence of true positives and eliminate false positives
2. Give specific identification of drug or drugs present in positive urine
3. Quantitative measurements of drug concentrations
4. Have better analytical sensitivity and lower reporting cutoffs

I. Confirmation Of Immunoassay Test Results

MS confirmation test can:

1. Confirm true positive and eliminate false positive results

   - False positives (interferences) are caused by drugs that are structurally unrelated to target drugs, e.g.,
     - Ofloxacin and opiates assays
     - Bupropion and amphetamines assays
     - Oxaprozin and benzodiazepines assays
   - Assays from different manufacturers have different profiles of interfering substances – consult package inserts and technical support services

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I. Confirmation Of Immunoassay Test Results

MS confirmation test can:

2. Provide specific identification of drugs/metabolites, including those which are immunoassay false negatives due to low immuno-reactivity

   - Immunoassays are class assays. Combinations below give same ‘opiates’ positive result:

     morphine

     morphine + hydromorphone

     morphine + hydrocodone + hydromorphone

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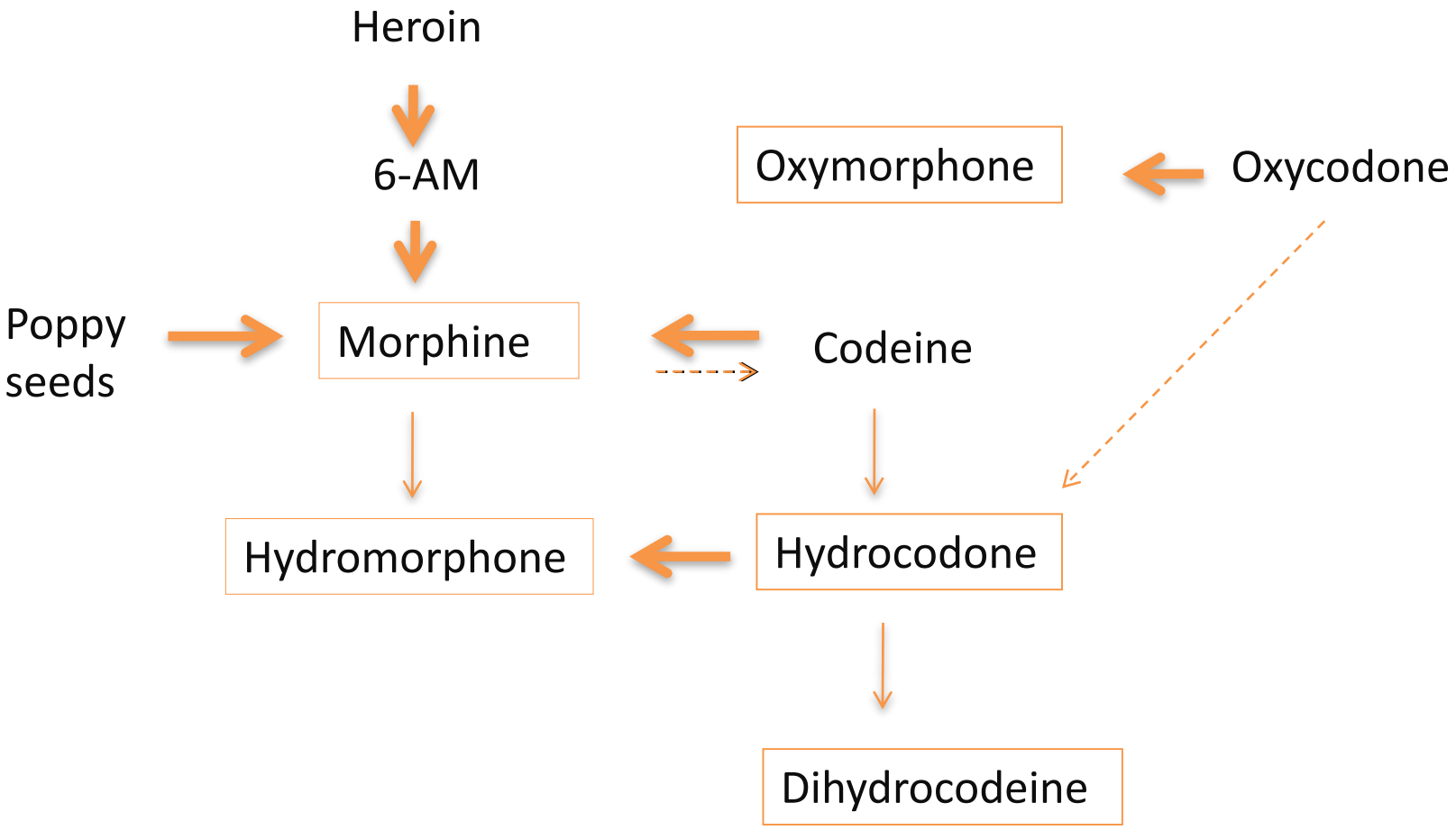
I. Confirmation Of Immunoassay Test Results

MS confirmation test can:

3. Measure quantitatively drug/metabolite concentrations
   - Knowing drug concentrations can help interpretation:
     - How high is the concentration of a positive?
     - How low is the concentration of a negative?
     - Where did an unexpected opiate come from?
       - Is it a metabolite of the prescribed opiate?
       - Due to non-prescription use of the opiate?
       - Is it a contaminant of the prescribed opiate

Opiates Metabolic Pathways



Kwong 2012

Interpretation of Opiates Test Result : Need for Specific Drug Identification and Quantitation

Patient on MSContin

Opiate(s) Present (ng/ml)	Opiates IA Result*	Source of Morphine
Morphine 500	POS	MSContin Poppy seeds
Morphine 50,000	POS	MSContin
Morphine 50,000 Hydromorphone 500	POS	MSContin
Morphine 50,000 Hydromorphone 5,000	POS	MSContin Dilaudid®

*300 ng/ml cutoff

Haddox, Kupper, Cone. 2010 AAPM Annual Meeting Poster

Ranges of Acceptable Opioid Process Impurities In Commercial Drug Substances

NB: New Methods Eliminate These Impurities for Hydrocodone and Hydromorphone; Both Varieties Are Currently Available

Commercial Active Pharmaceutical Ingredient (API)	Process Impurities	Allowable Limit (%)	Typical Observed (%)
Codeine	Morphine	0.15	0.01 – 0.1
Hydrocodone	Codeine	0.15	0 – 0.1
Hydromorphone	Morphine Hydrocodone	0.15 0.1	0 – 0.025 0 – 0.025
Morphine	Codeine	0.5	0.01.– 0.05
Oxycodone **	Hydrocodone	1.0	0.02 – 0.12
Oxymorphone	Hydromorphone Oxycodone	0.15 0.5	0.03 – 0.1 0.05 – 0.4

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Assay Cutoffs vs. Positivity Rates

Compound	Cutoffs, ng/ml MS/MS	EIA	MS/MS Pos (%)	EIA Pos (%)	% Missed Samples
Cocaine Metab	25	300	297 (4.1)	171 (2.3)	42.4
Hydromorphone	50	(300)*	2709 (33)	831 (10)	69.3
Hydrocodone	50	(300)*	3005 (37)	2304 (28)	23.3
Oxycodone	50	100	2129 (27)	2028 (25)	4.7

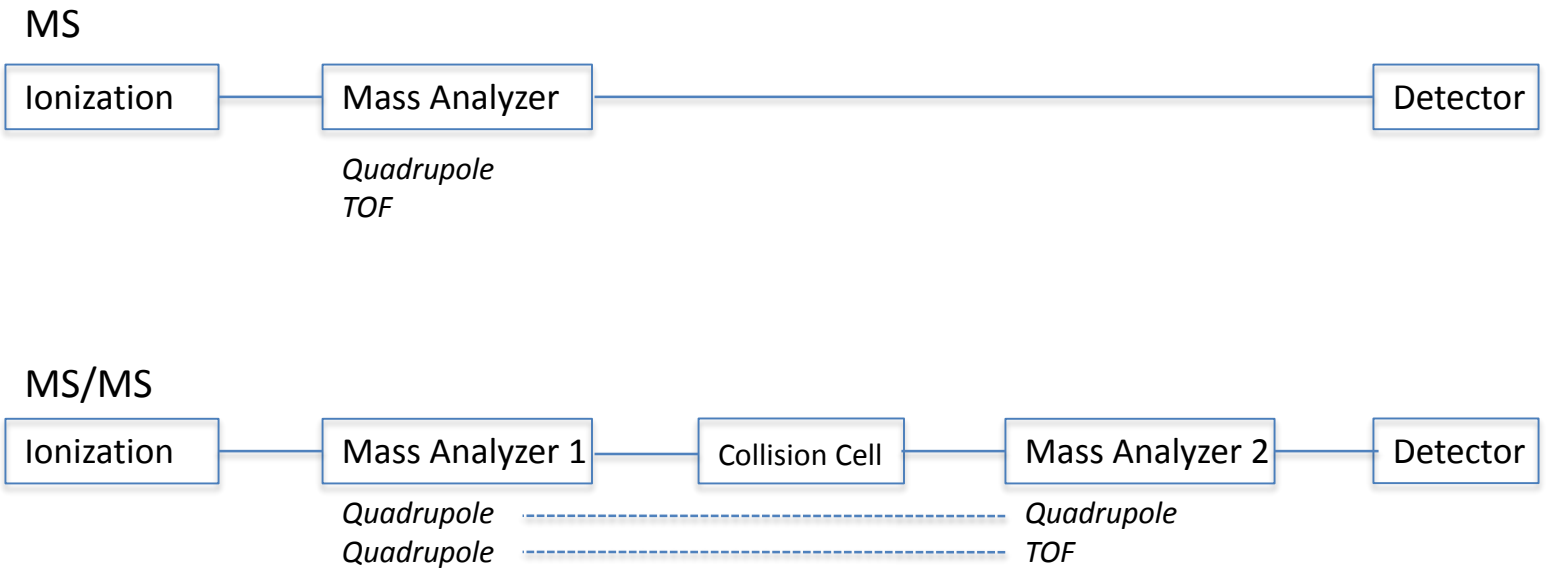
* Opiates cutoff of 300 ng/mL

Modified from Mikel et al. Ther Drug Monit 2009;31:746

Mass Spectrometry

- Analytes are separated by chromatography before MS: GC-MS and LC-MS
- In a mass spectrometer, the three components are:
  1. Ion source: ionizes analytes
  2. Mass analyzer: sorts ions based on mass-to-charge ratios
  3. Detector: measures abundance of separated ions
- Two or more mass analyzers in sequence: tandem mass spectrometry (MS/MS)

Mass Spectrometry (MS) and Tandem Mass Spectrometry (MS/MS)



Quadrupole Tandem Mass Spectrometry

- Most commonly used mass spectrometers: quadrupole MS/MS
  1. Ion source: ionizes analytes
  2. Quadrupole mass analyzer 1 (Q1): sort ions based on ions mass-to-charge ratios (‘precursor’ ions)
  3. Collision Cell (Q2): Collision-induced-dissociation of ‘precursor’ ions into ‘daughter’ ions
  4. Quadrupole mass analyzer 2 (Q3): sort ‘daughter’ ions based on ions mass-to-charge ratios
  5. Detector: measures abundance of separated ions

Time-of-flight (TOF) Mass Spectrometry

- Recent development: use Liquid Chromatography-Time-Of-Flight-MS (LC-TOF-MS) for broad-based drug screen
- TOF mass analyzer sort ions based on the time required to travel a fixed distance. The 'flight time' is proportional ion mass
- High resolution TOF-MS is capable of very accurate mass determination
- Identification is based retention time, accurate mass determination, isotopic pattern of ions
- False positive possible. Confirmation with fragmentation spectra quadrupole-TOF(Q-TOF)-MS

Mass Spectrometric Assay Validation

Validation of a MS-based assay should include the following:

- Accuracy (bias) and precision
- LOD and LOQ
- Linearity and AMR
- Interference studies including evaluation of matrix effects
- Sample Stability
- Dilution integrity (if applicable)
- Carry-over

Mass Spectrometric Assays - Summary

Advantages

- Direct specific identification of drug(s) present:
  - Immunoassays are class-specific, whereas MS assays are analyte-specific (positive for benzodiazepines vs. positive for clonazepam)
  - Identification of multiple analytes – having metabolite pattern is an added advantage

Mass Spectrometric Assays - Summary

Advantages

- Better analytical sensitivity lower cutoffs. For example, opiates assays:
  - Immunoassay: 300 ng/ml of calibrator drug–equivalent
  - LC-MS/MS: 50 ng/ml or lower for each opiate, including those missed by immunoassay due to low reactivity (e.g., hydromorphone)
- Quantitative measurement and wide analytical measuring range
- Range of drugs/metabolites detected, including emerging drugs with no immunoassays

Mass Spectrometric Assays - Summary

Disadvantages

- Sample preparation labor-intensive
- Slow throughput
  - Several class-specific assays may be needed for different drug groups
  - Multi-drug assay of different drug classes are technically more challenging

Mass Spectrometric Assays - Summary

Disadvantages

- Automation not easy
- Lack of MS-LIS interface to improve transcription accuracy and efficiency
- High capital cost for instrumentation
- Advanced technical expertise
- Analytical sensitivity: picks up contaminants

Thank you for attending!

Please join me in the Networking Lounge for an online Q&A session.

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Self-Assessment Questions

1. Which of the following statements on confirmation testing on an immunoassay positive result is INCORRECT?

    a. Confirmation testing should be performed on a fresh aliquot obtained from the original container

    b. A different immunoassay with better analytical sensitivity can be used as the confirmation test

    c. A gas chromatographic assay can be used as the confirmation test

    d. A clinician can forgo confirmation test if the immunoassay positive result is consistent with clinical expectation

Self Assessment Questions

2. Which of the following statements on mass spectrometry confirmation is INCORRECT?

    a. Drug(s) in a positive urine can be specifically identified

    b. Quantitative measurement of drug concentrations from a patient on opiate therapy can help to assess the origin of an unexpected opiate

    c. A disadvantage of the analytical sensitivity of mass spectrometry assay is the detection of contaminants

    d. Matrix effects refers to interfering substances reducing, not enhancing the efficiency of ionization of analytes

Self-Assessment Questions

3. Which of the following statements on mass spectrometry testing is INCORRECT?

    a. Bypassing initial immunoassay and adopting direct mass spectrometry testing should be considered if the positivity rate for the analyte is low

    b. Throughput of mass spectrometric assays is a limiting factor for switching to directing testing without initial immunoassay testing

    c. In using high resolution liquid chromatography-time of flight mass spectrometry (LC-TOF-MS), the two major criteria for analyte identification are accurate mass determination and retention time

    d. Matrix effects affects both quadrapole and time-of-flight mass spectrometers