LC-MS for Pain Management Support

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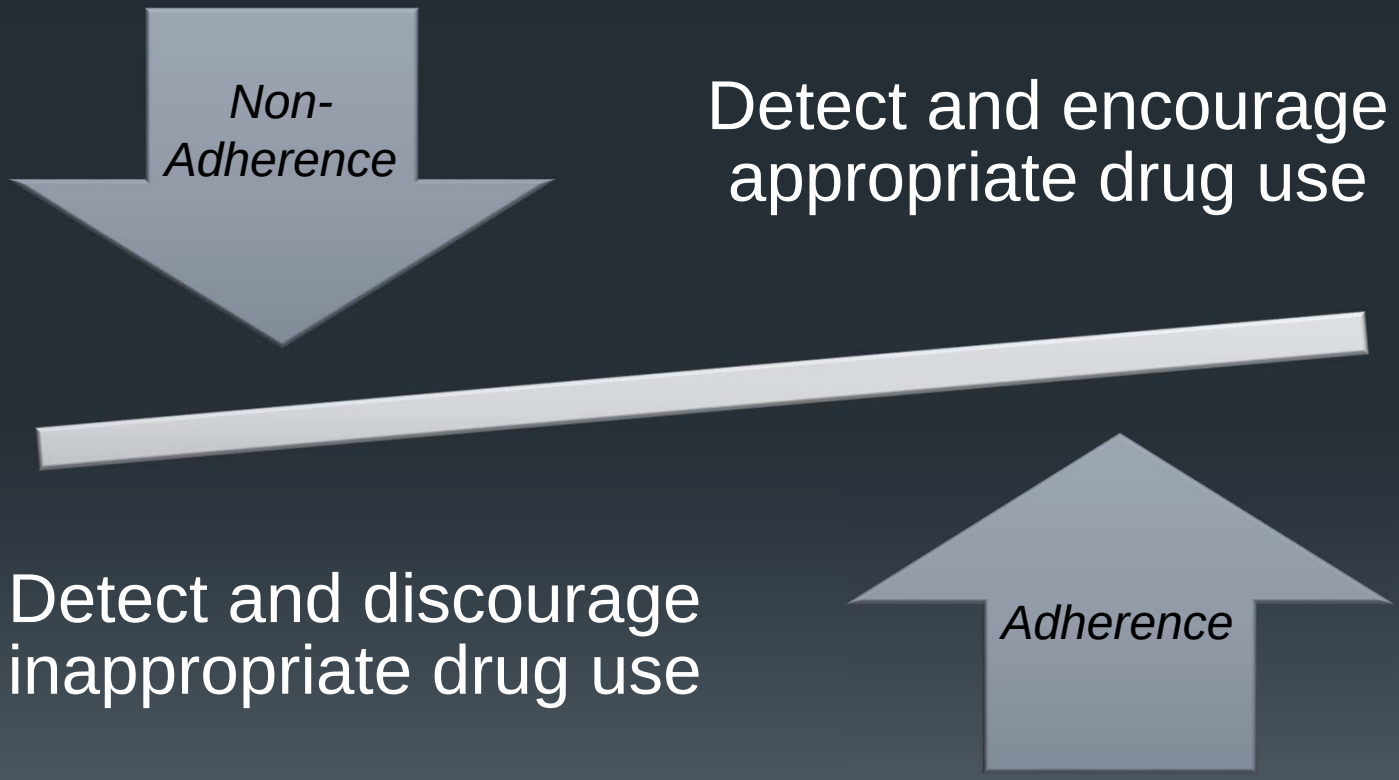
Outline

- 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



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

Rank #s from
http://www.pharmacytimes.com/publications/issue/2012/July2012/Top-200-Drugs-of-2011

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

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

False negatives likely

Concentrations (ng/mL) required to trigger a positive opiate

(300 ng/mL cutoff)

	EMIT	CEDIA	Triage
Morphine	300	300	300
Codeine	247	300	300
6-monoacetylmorphine	1088	300	400
Hydrocodone	364	300	300
Hydromorphone	498	300	500
Oxycodone	5,388	10,000	20,000
Oxymorphone	>20,000	20,000	40,000
Noroxymorphone	-	-	-

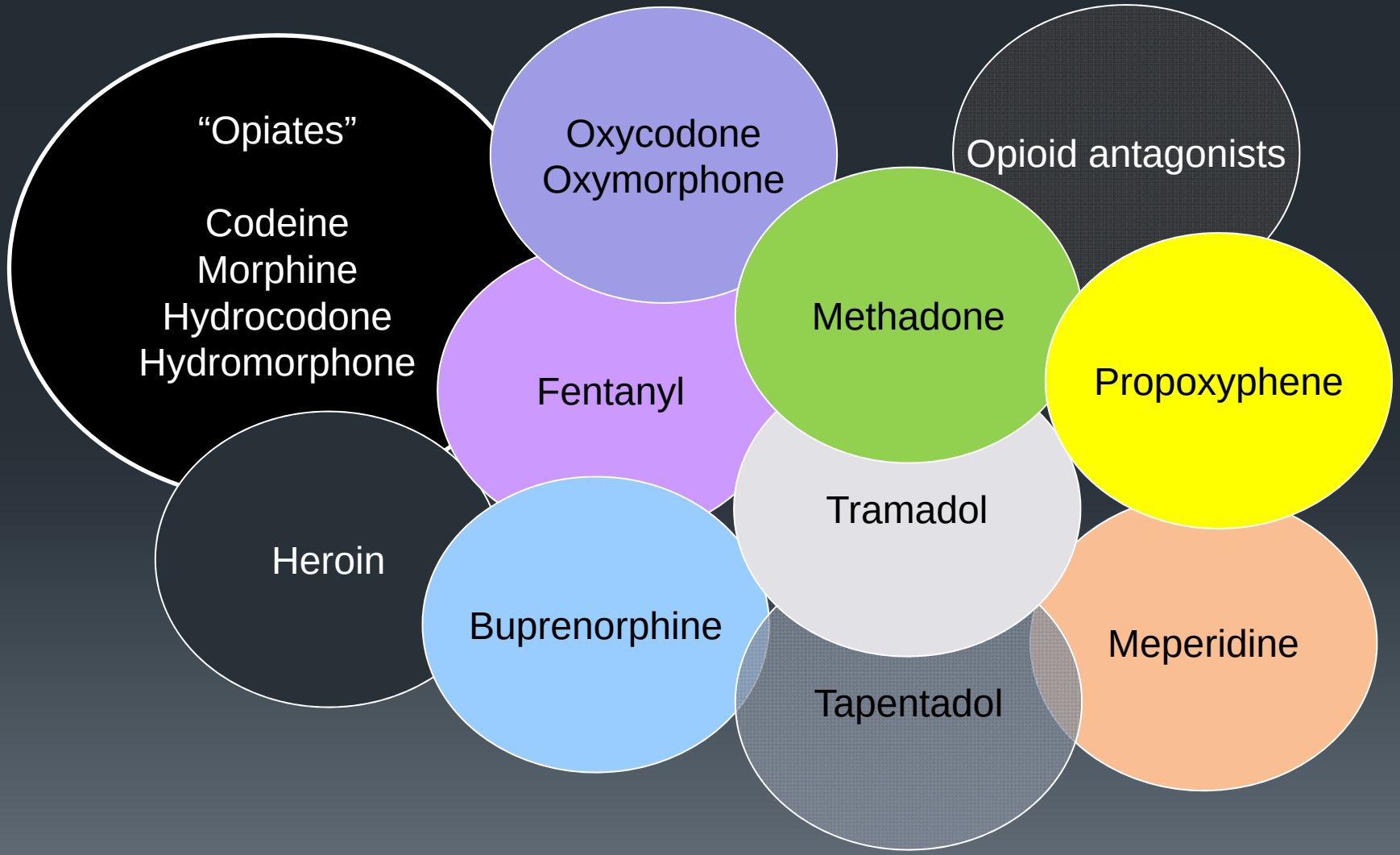
False negatives likely

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

Compound	Immuno-assay cutoff (ng/mL)	LC-MS/MS cutoff (ng/mL)	% missed by immunoassay (total n ~8000)
Codeine	300	50	29.6% (45)
Hydrocodone		50	23.3% (701)
Hydromorphone		50	69.3% (1878)
Alprazolam	200	20	53.3% (646)
Nordiazepam		40	40.0% (320)

Mikel et al., *TDM* 31(6):746-8, 2009

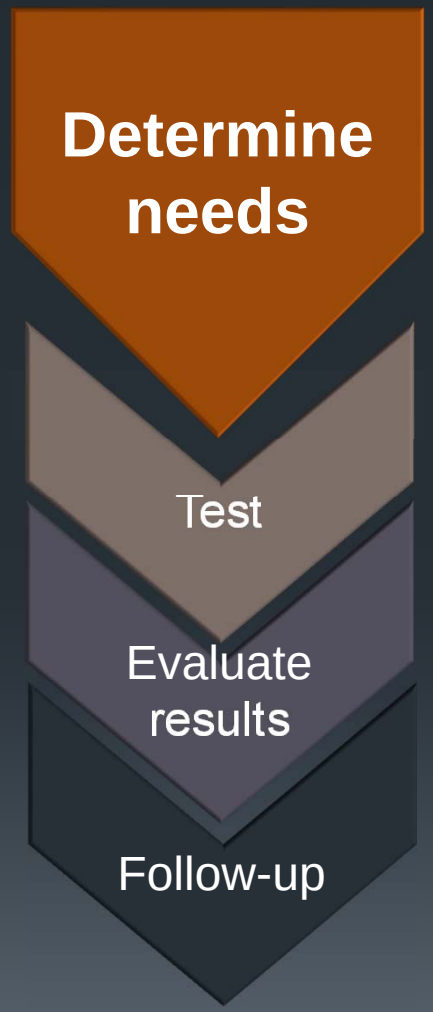
Immunoassays that might be required to detect opioids



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

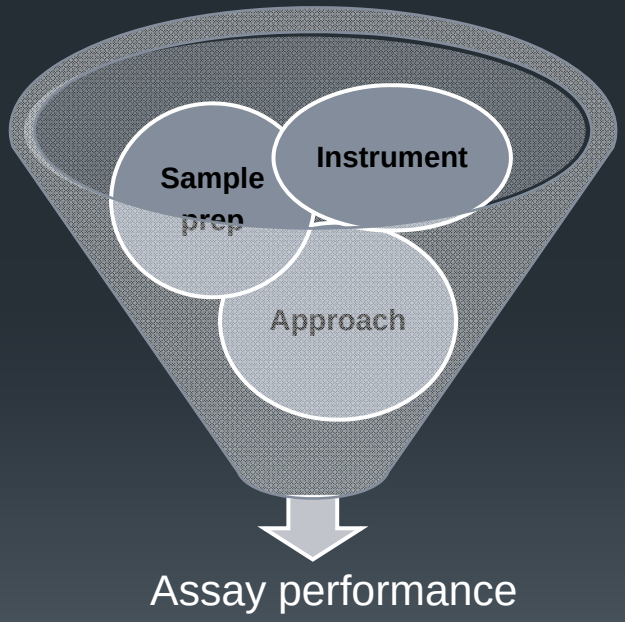
- Specificity
- Sensitivity
- Flexibility

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)

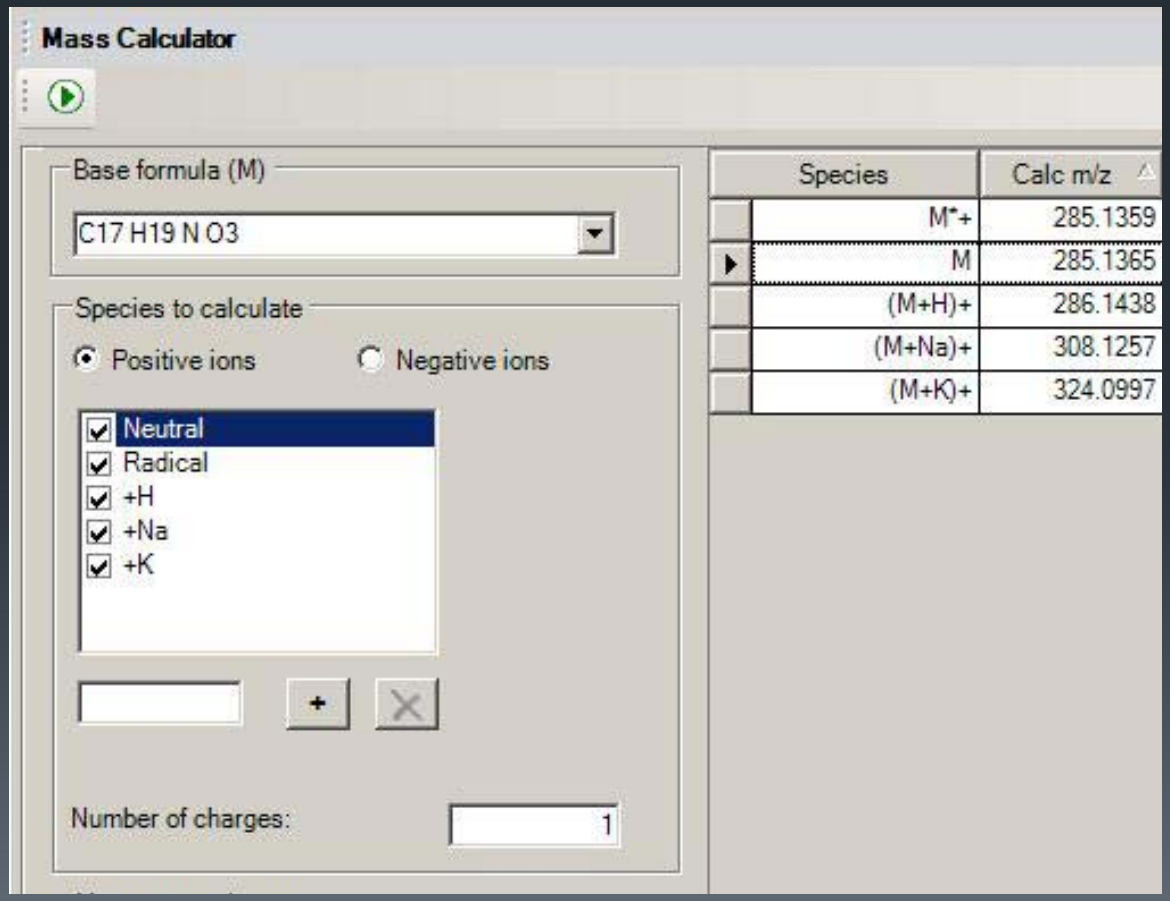
m/z	Opioid compound
286.1	Morphine, Hydromorphone, Norcodeine, Norhydrocodone
300.3	Codeine, Hydrocodone
302.2	Oxymorphone, Noroxycodone, Morphine n-oxide, Dihydrocodeine
328.2	6-monoacetylmorphine, Naloxone

Examples of isobaric opioid species (exact M+H mass)

m/z	Molecular formula	Opioid co
285.13649	$C_{17}H_{19}NO_3$	Morphin Hydr Norc Norhy
299.15214	$C_{18}H_{21}NO_3$	Codein Hydrocodon
301.13141	$C_{17}H_{19}NO_4$	Oxymorphone Noroxycodone Morphine n-oxide
301.16779	$C_{18}H_{23}NO_3$	Dihydrocodeine
327.14706	$C_{19}H_{21}NO_4$	6-monoacetylmorphine Naloxone

Chromatographic separation may be required…

Exact mass depends on specific ions of interest, and isotope abundances

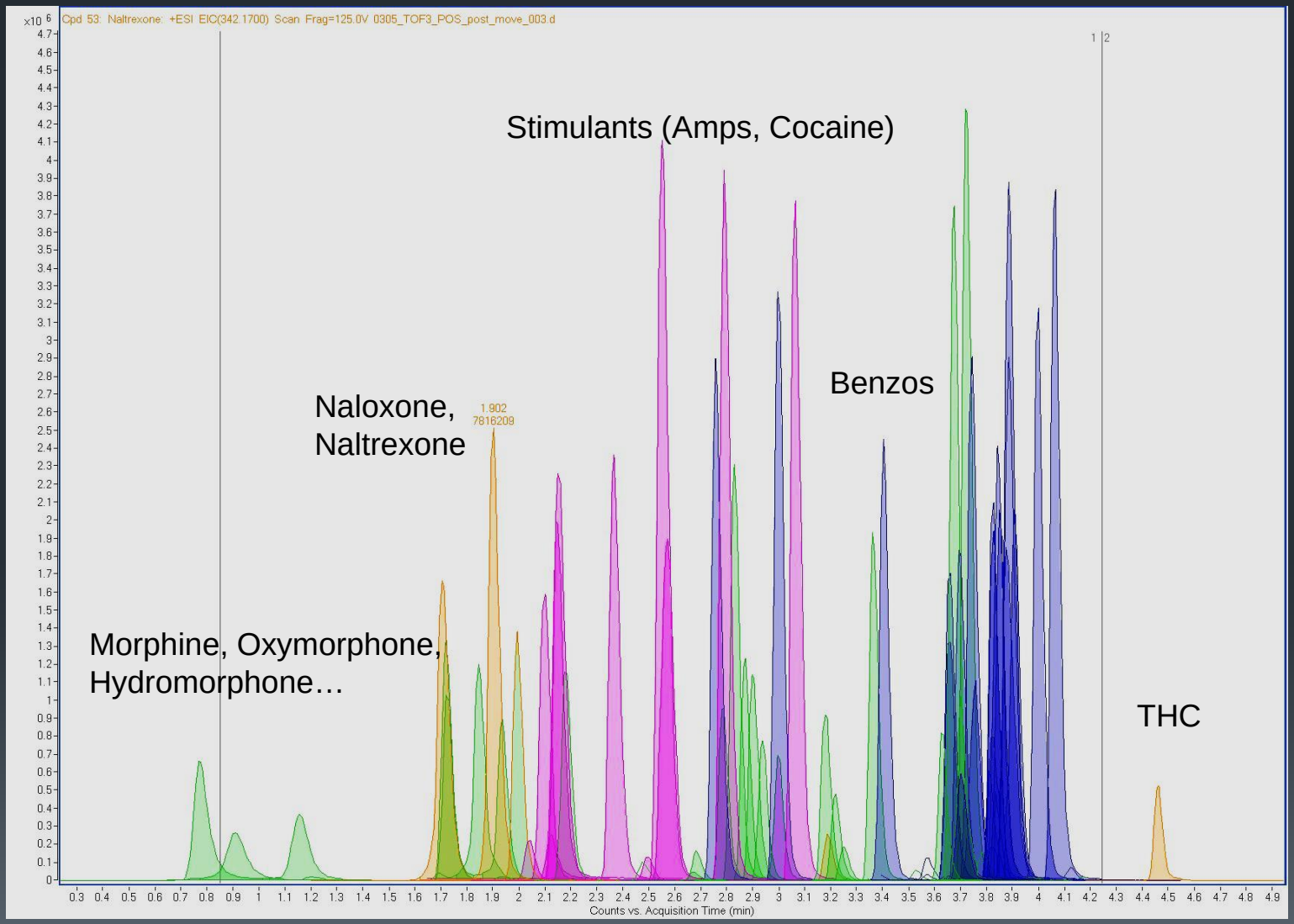


Isobaric pairs in an assay designed to detect 67 drug analytes (= 45 parent drugs)

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

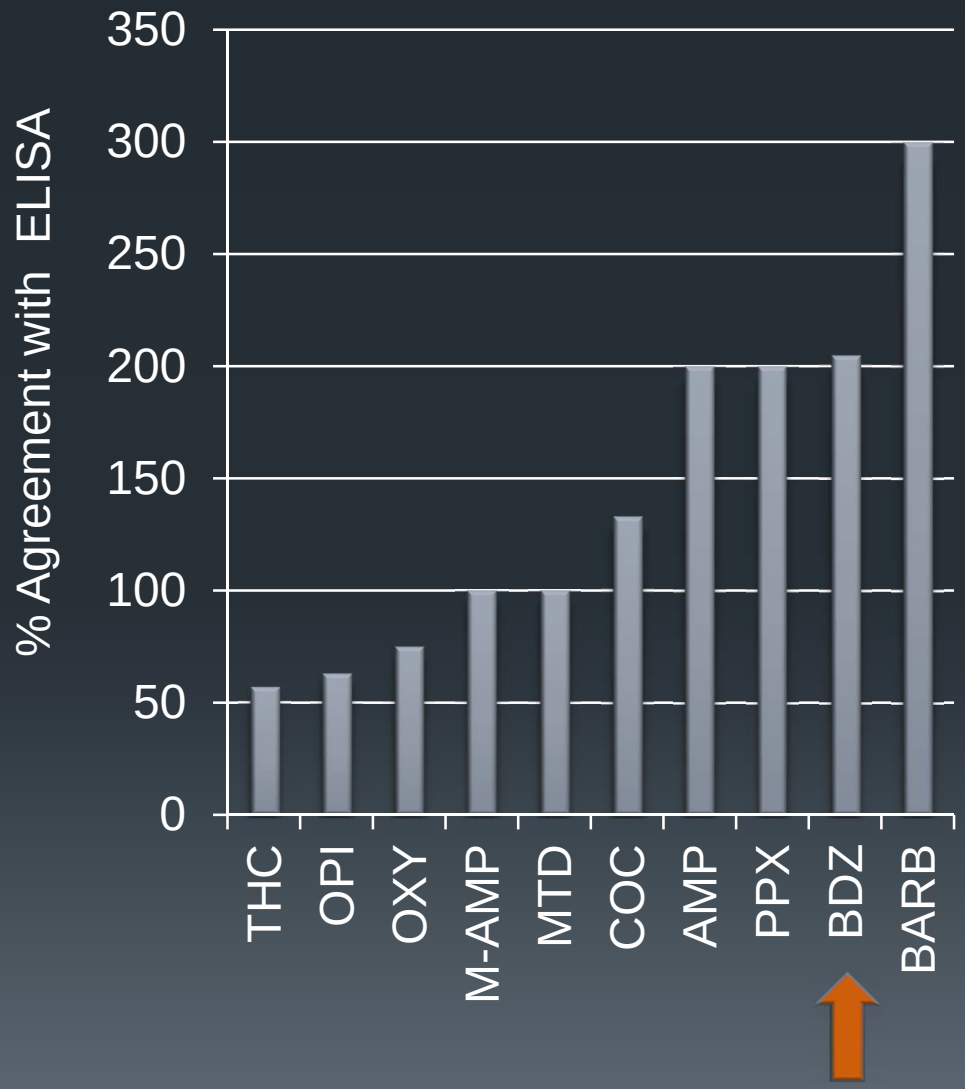
Resolved with chromatography

Example EIC



Marin et al, *JAT*, 2012 Jul 15 [Epub ahead of print]

Performance vs. ELISA (n = 119)



48 negative by both methods

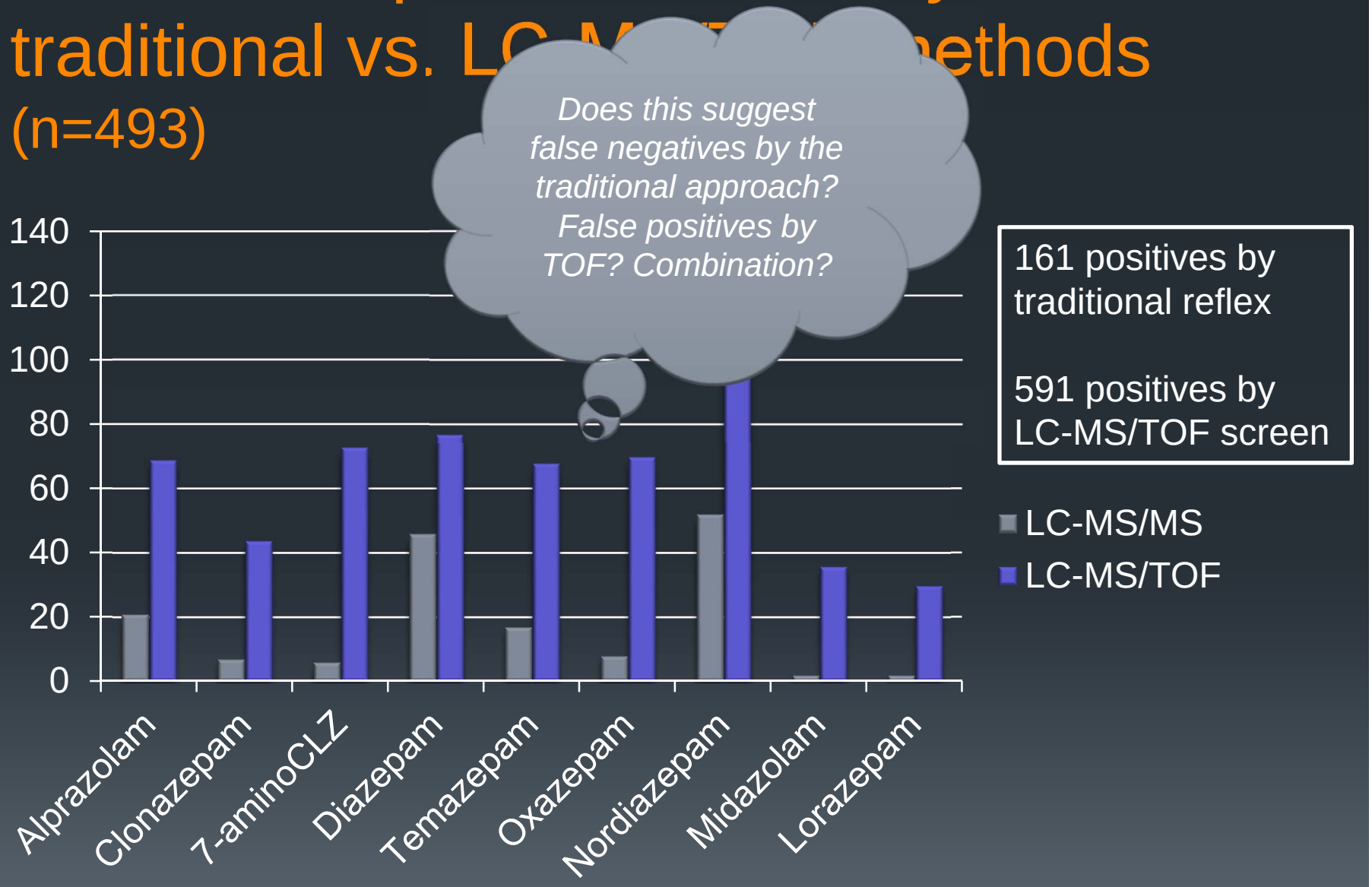
ELISA:

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

TOF:

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

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

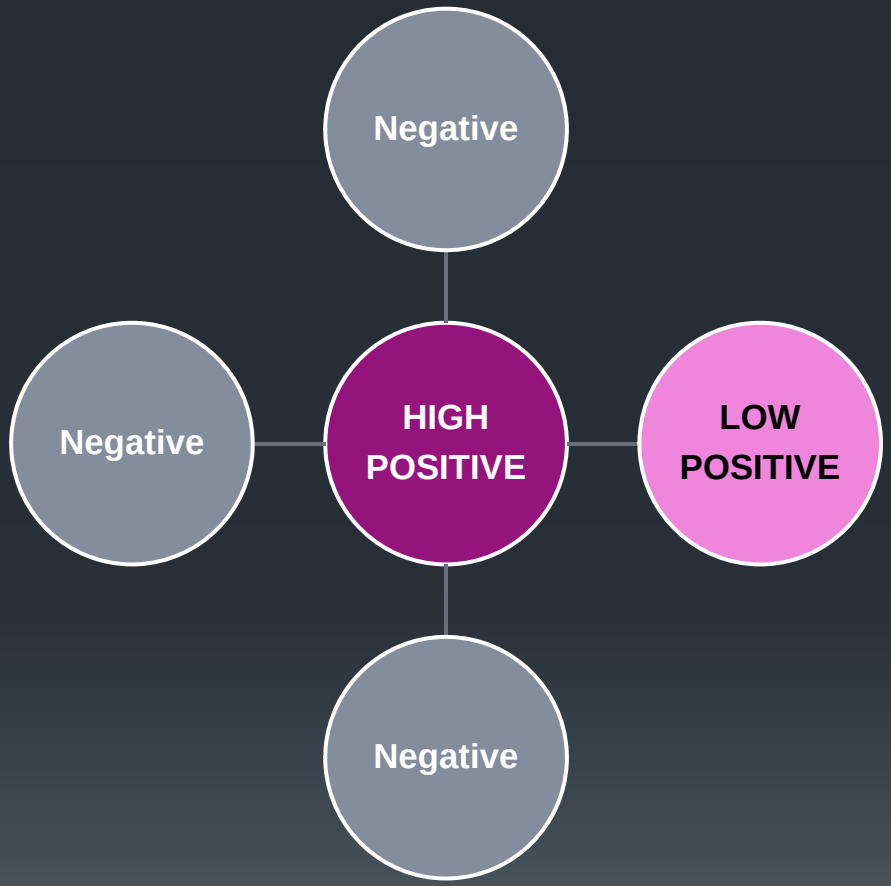


Proposed ‘cutoff’ and ranges of opioids in pain management patients

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

Adapted from Pesce et al, *J Opioid Manag* 7(2):117-22, 2011 and McMillin et al, *JAT* 36(2):81-7, 2012

Theoretical carryover



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

Opioid process impurities

Active pharmaceutical compound	Process impurities	Allowable pharmaceutical impurity limit (%)
Codeine	Morphine	0.15
Hydrocodone	Codeine	0.15
Hydromorphone	Morphine Hydrocodone	0.15 0.1
Morphine	Codeine	0.5
Oxycodone	Hydrocodone	1.0
Oxymorphone	Hydromorphone Oxycodone	0.15 0.5

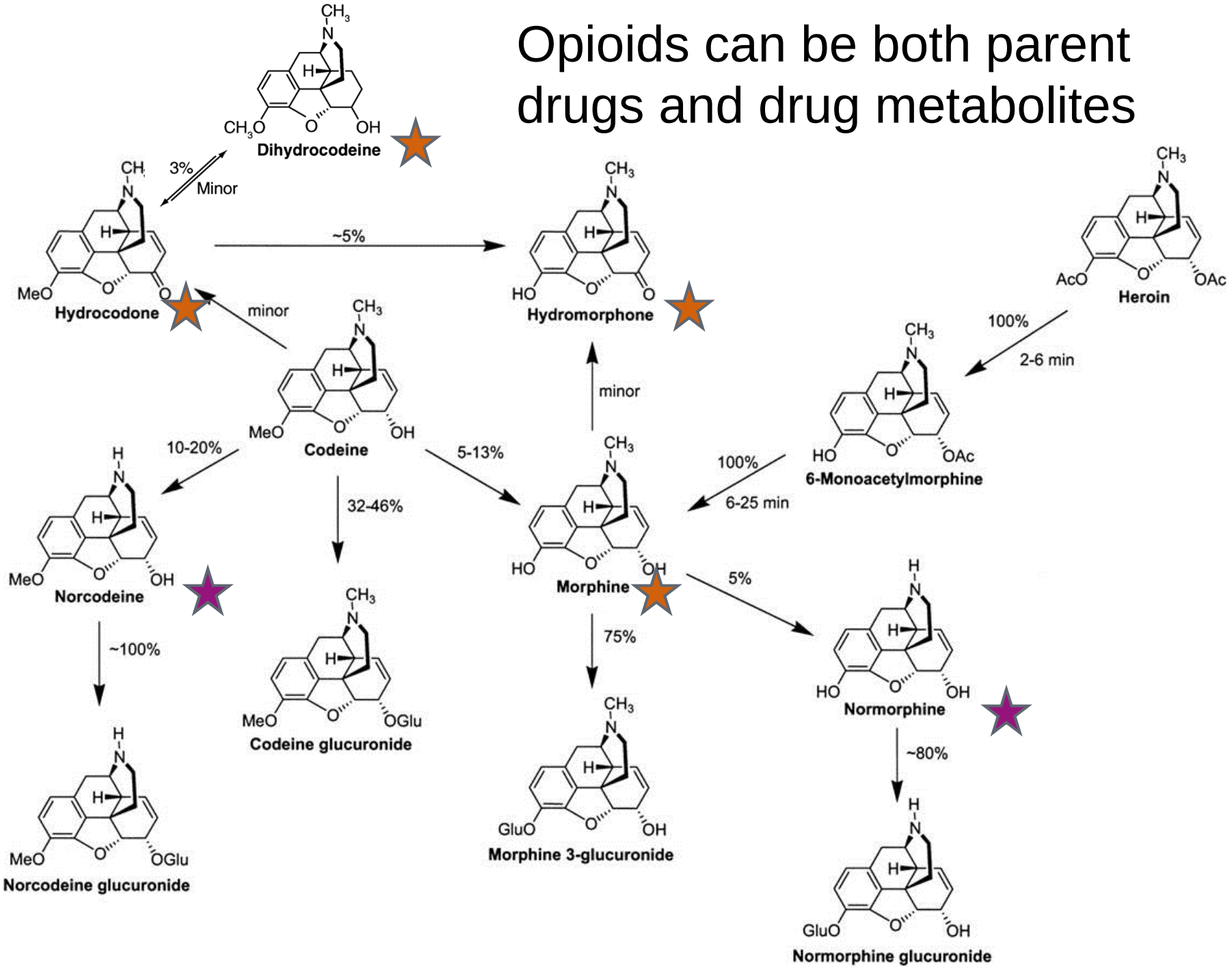
MRO Alert XXI, No. 3, 2010

Utility of quantitative results

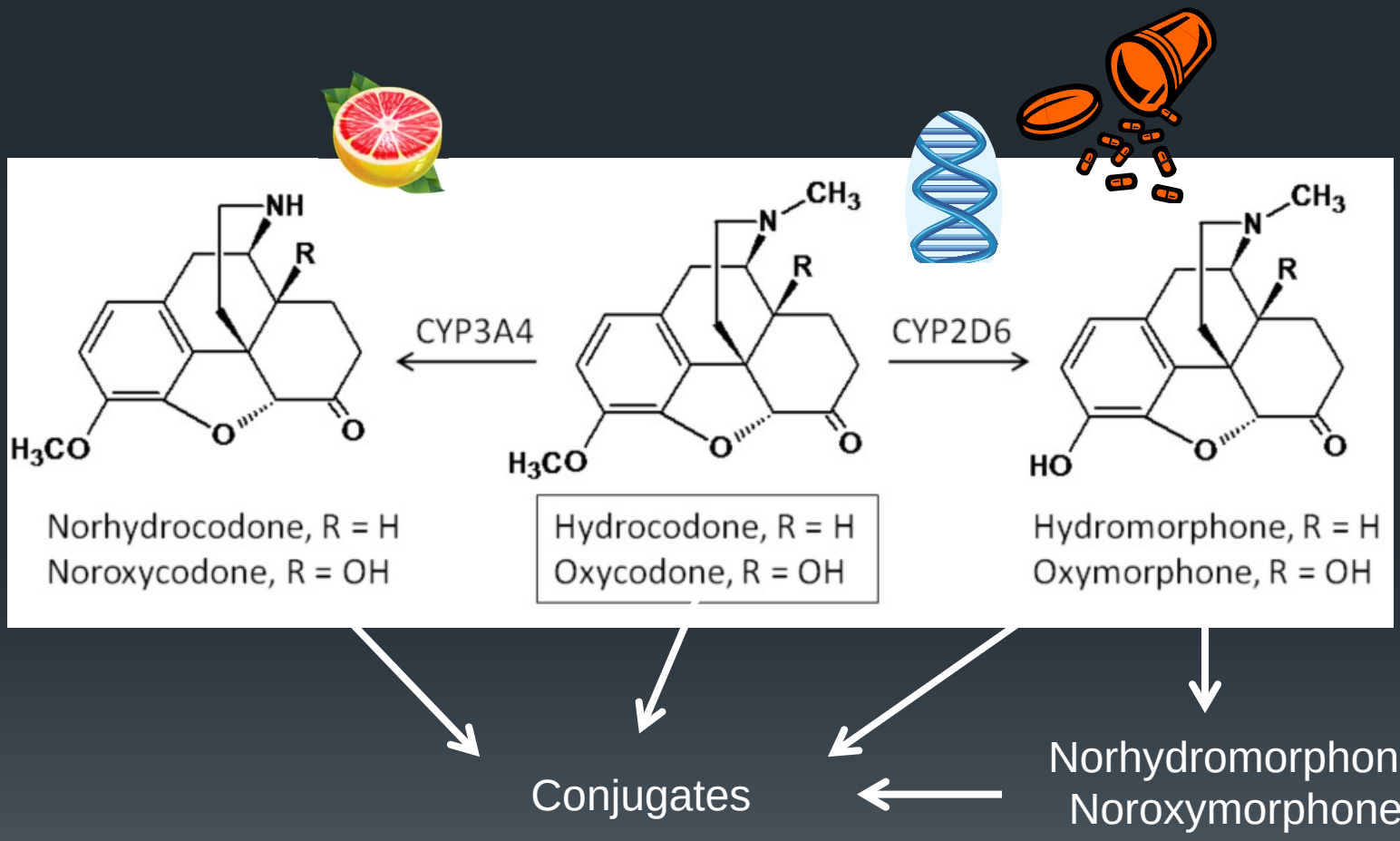
- Helps identify carryover
- May identify pharmaceutical impurities

- 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

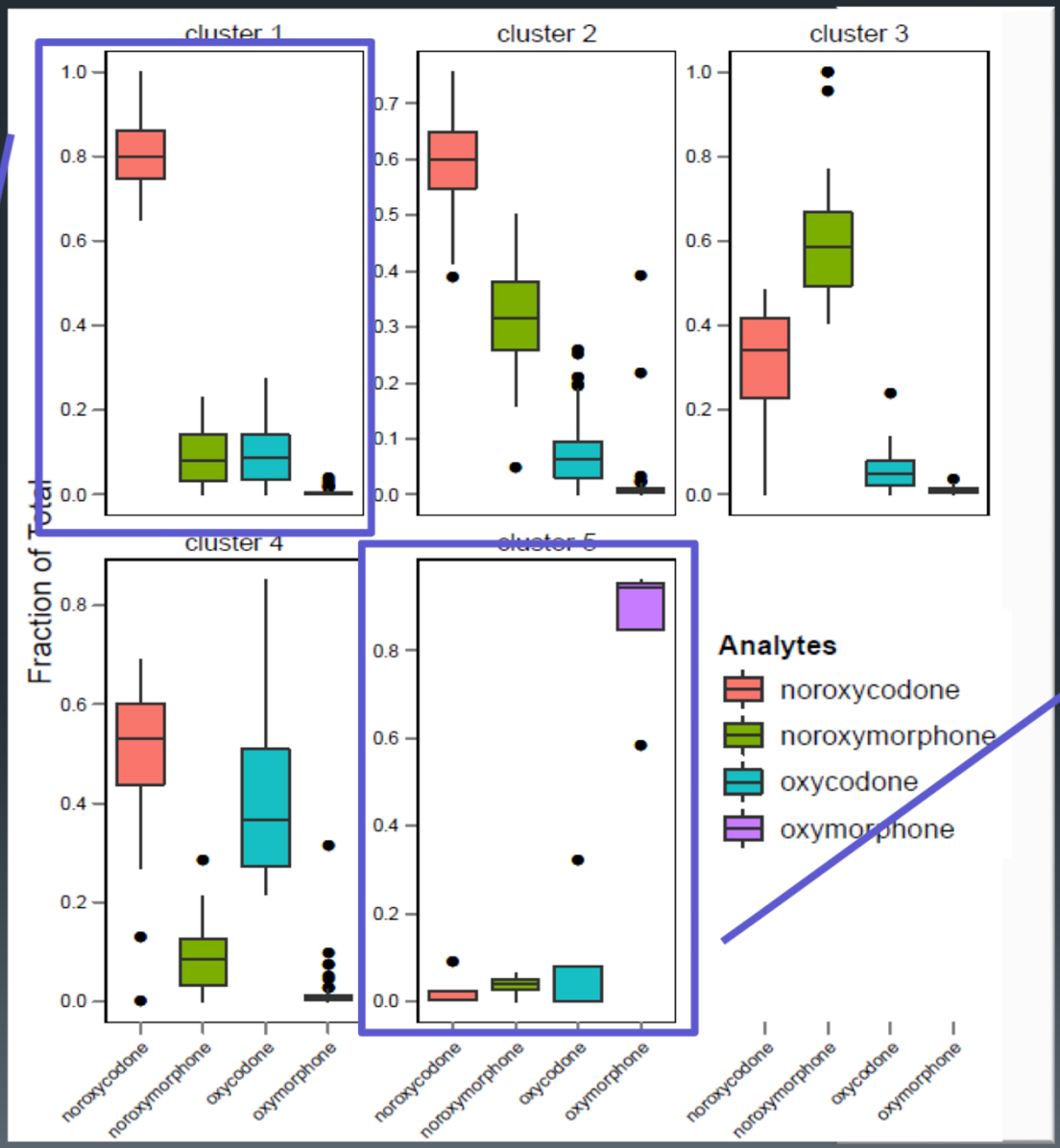


Simplified metabolism of oxycodone and hydrocodone



Heltsley et al, *JAT* 34:32-8, 2010

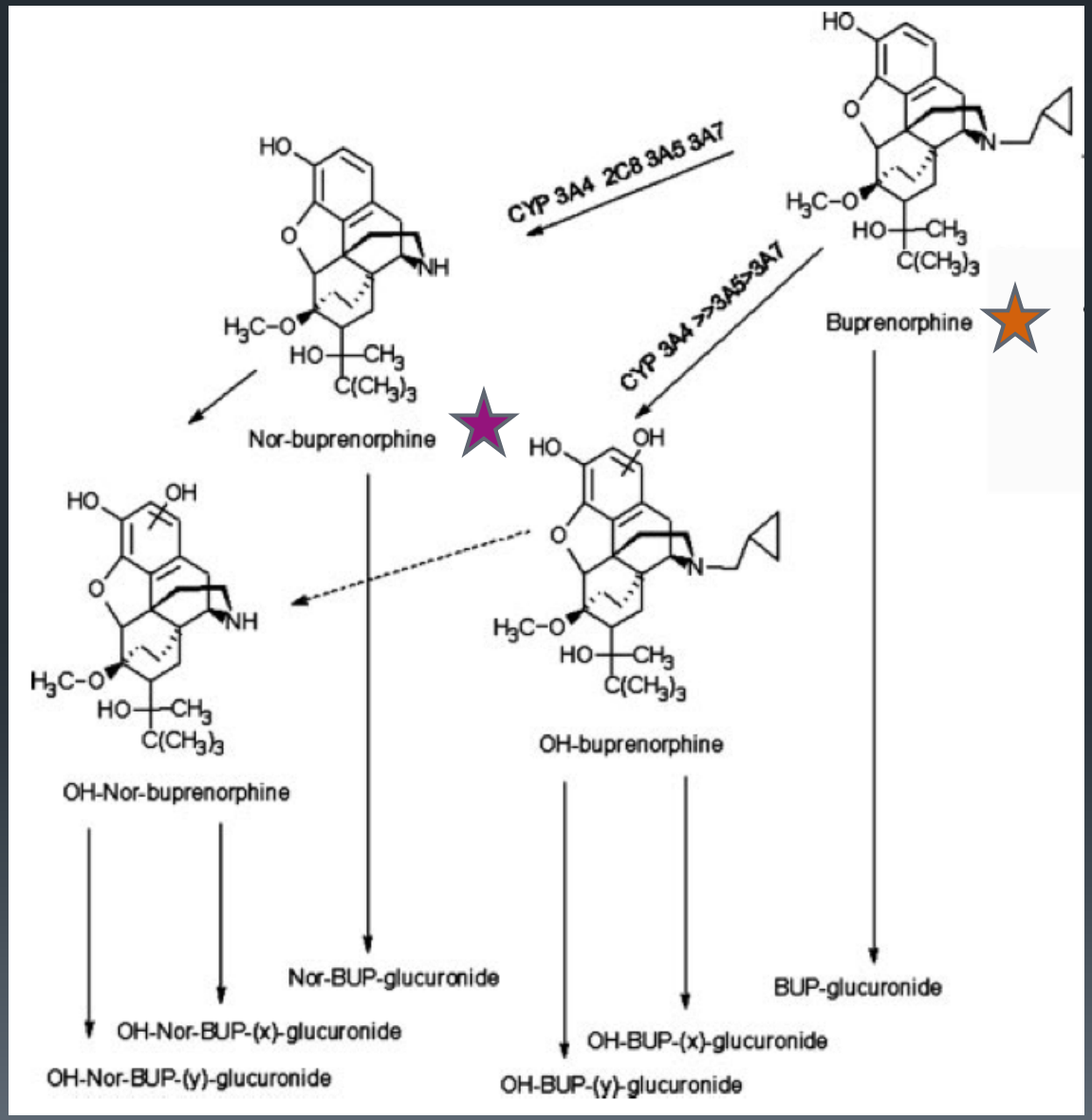
Urine oxycodone data ‘clusters’ (n = 396)



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 Disp* 33:689-95, 2005

Summary of urine buprenorphine patterns (n = 1,946)

Analyte pattern	Number of samples	% of total
Bup-gluc + Norbup + Norbup-gluc	956	49%
Bup + Bup-gluc + Norbup +Norbup-gluc	809	42%
	~91% of positives	
Norbup + Norbup-gluc	68	4%
Bup + Norbup	58	3%
Norbup-gluc	24	1%

Bup: Buprenorphine (free)
Norbup: Norbuprenorphine (free)
Bup-gluc: Buprenorphine-3-glucuronide
Norbup-gluc: Norbuprenorphine-3-glucuronide

Summary of urine buprenorphine patterns (n = 1,946)

Analyte pattern	Number of samples	% of total
Bup-gluc + Norbup + Norbup-gluc	956	49%
Bup + Bup-gluc + Norbup +Norbup-gluc	809	42%
90th percentile of Bup = 16 ng/mL		
Bup > 1000 ng/mL	78	3%

Bup: Buprenorphine (free)
Norbup: Norbuprenorphine (free)
Bup-gluc: Buprenorphine-3-glucuronide
Norbup-gluc: Norbuprenorphine-3-glucuronide

Results suggest drug was added

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

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

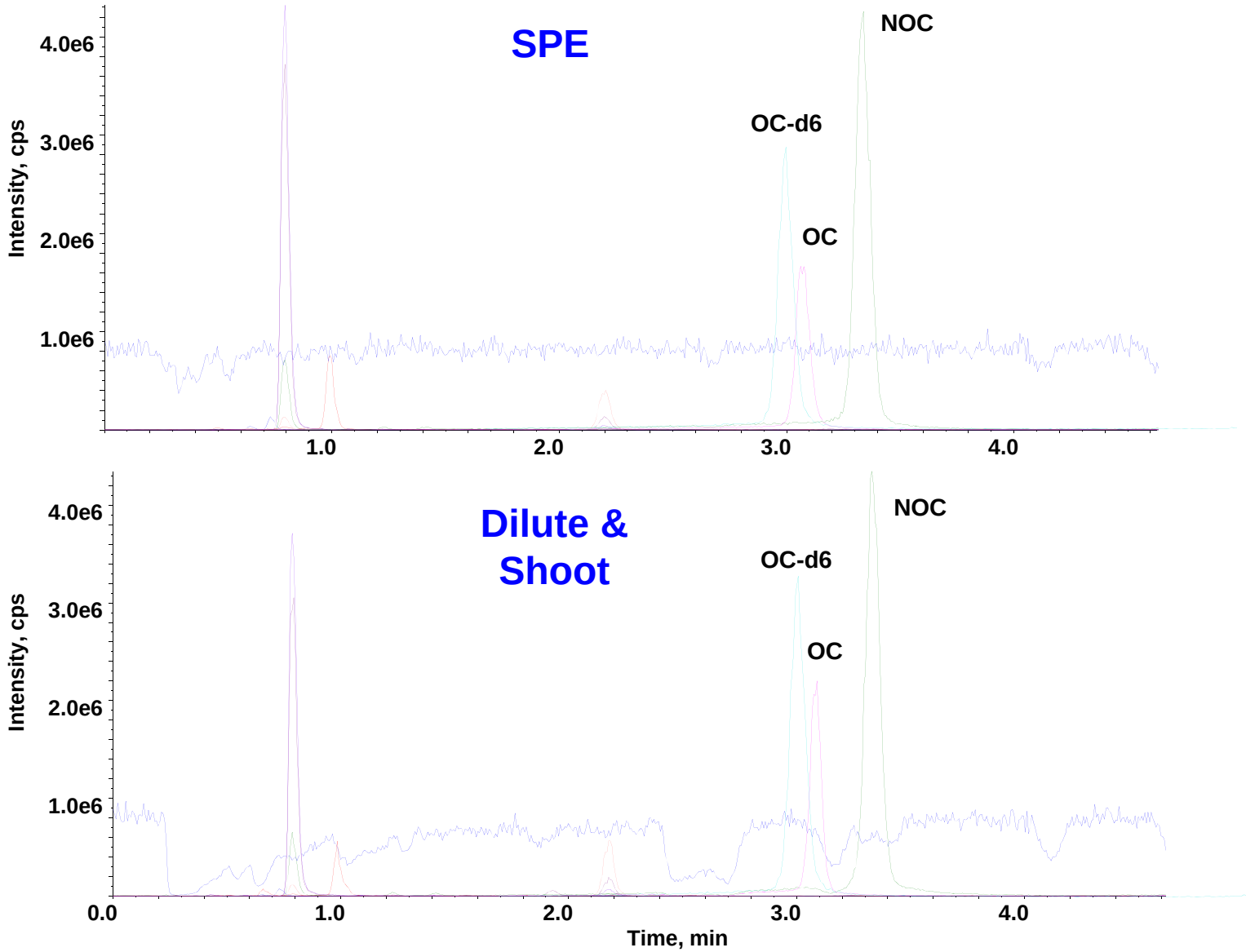
- Sample preparation method
  - Hydrolysis, and associated efficiency
  - Extraction method, and associated recoveries
- Analytical method and reporting
  - Cutoff and reportable range
  - Normalization of results
  - Inaccuracies due to ion suppression

Hydrolysis efficiency for morphine

	Percent (%) recovery of opioids using different hydrolysis methods		
Morphine Metabolite	**Chemical (acid)**	**Enzyme (*P. vulgata, 2 hrs*)**	**Enzyme (*H. pomatia, 16 hrs*)**
Morphine-3-glucuronide	100 ± 4	94 ± 2	50 ± 13
Morphine-6-glucuronide	98 ± 5	12 ± 1	0 ± 0
Patient urine	100 ± 0	64 ± 19	35 ± 20

Wang et al, *JAT* 30:570-5, 2006

Ion suppression comparison



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

Heltsley R et al, *JAT* 36:75-80, 2012

Challenges

Blood

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

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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