

Behavioral Health is Essential To Health

Prevention Works



Treatment is Effective



People Recover





Detection of Opioid Glucuronides in User Hair

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Drug Testing Advisory Board



Purpose

- 2004 proposed revision to mandatory guidelines for workplace drug testing to expand types of specimens that may be tested
 - Hair
 - Sweat
 - Oral fluid

Advantages of Hair Testing

- Less invasive than urinalysis
- Controlled or observed collection
- More difficult to substitute or adulterate
- Longer window of detection



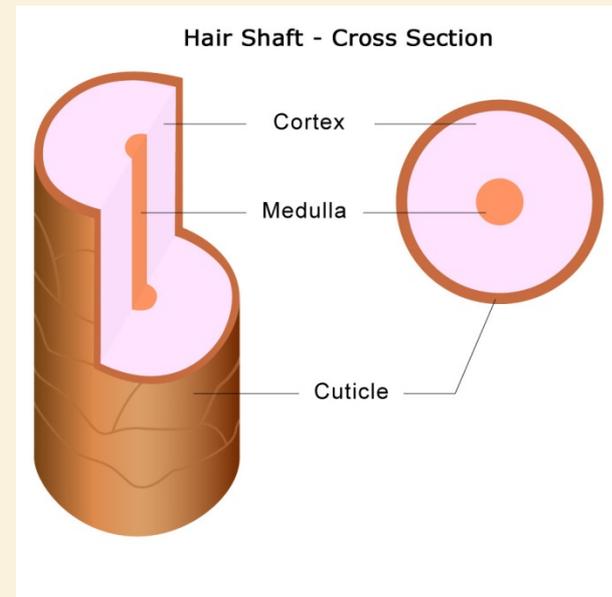
Use of hair as an alternative matrix is currently limited by issues that may affect the defensibility of results

External Contamination

- Issue of external contamination
 - Drug incorporated into hair from a mechanism other than drug use
- Currently, most testing in hair targets parent drugs
- Is a positive test in hair indicative of drug use, or could it be from external contamination?

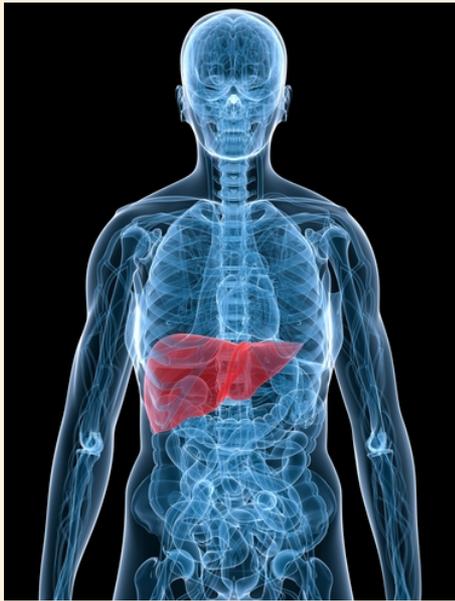
Addressing External Contamination

- Extensive wash procedures
- Metabolites in place of parent drugs
- Metabolite ratios
- Unique metabolites



Metabolism

- Chemical reactions that change drugs into compounds that are easier to eliminate.



Phase I

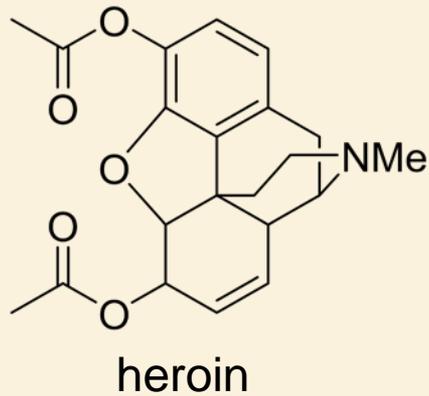
oxidation, reduction, hydrolysis

Phase II

(conjugation reactions)

glucuronidation, acetylation,
sulfation

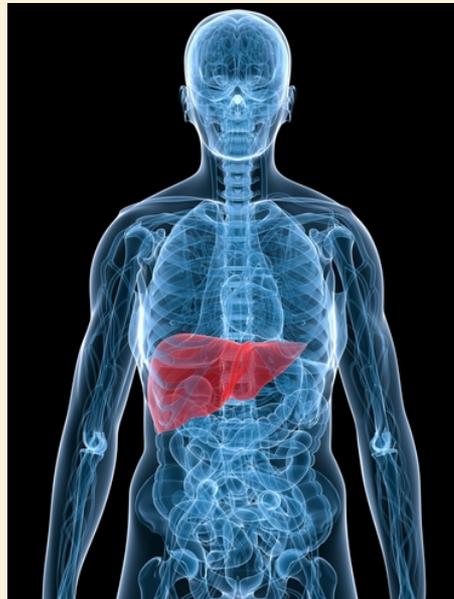
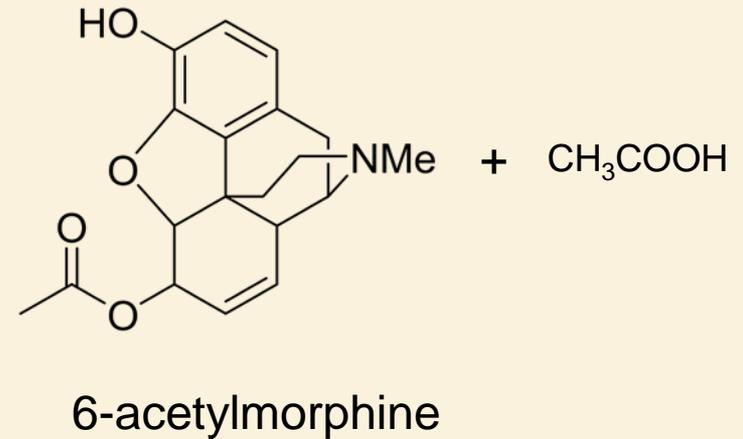
Phase I Metabolism



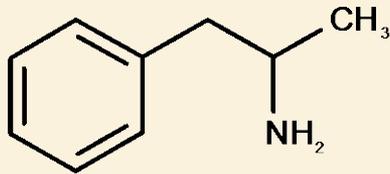
hydrolysis



H₂O

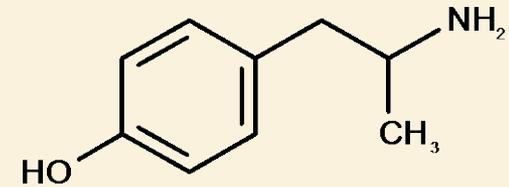


Phase I Metabolism

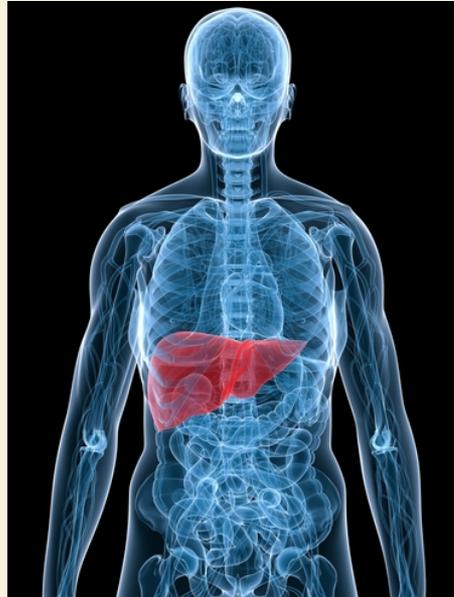


amphetamine

Oxidation

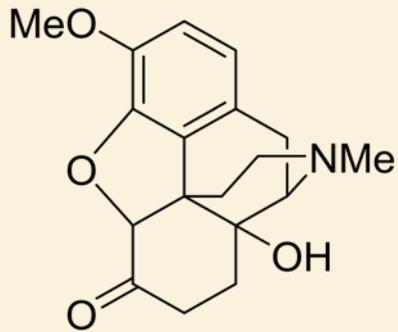


4-hydroxyamphetamine

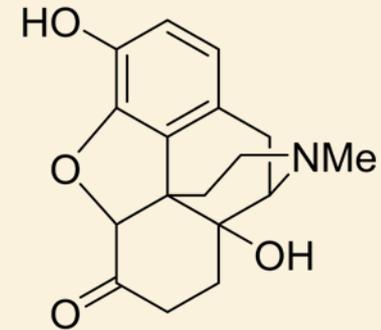


Phase I Metabolism

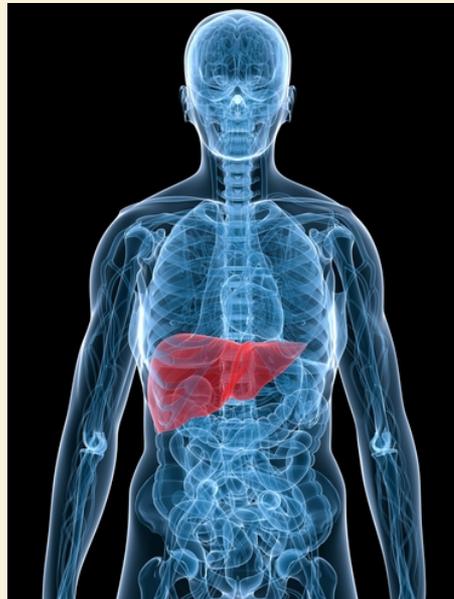
Oxidative dealkylation



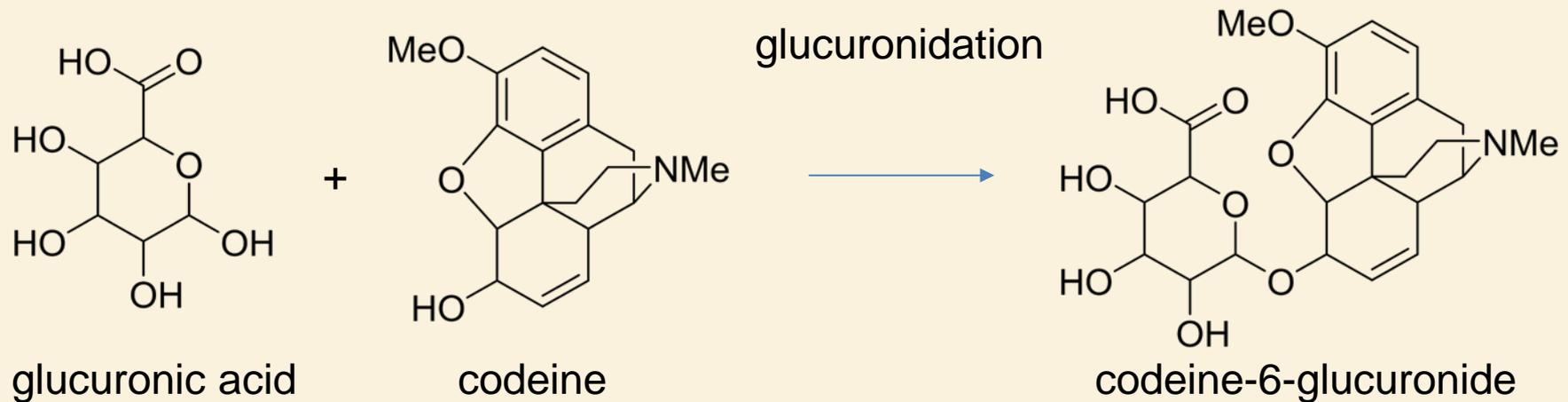
oxycodone



oxymorphone



Phase II Metabolism



- Not products of common degradation pathways
- Not manufacturing impurities
- Not formed by in vitro reactions on hair
- Not commercially available drugs

Background

- Research prompted by the need to identify metabolites for use in hair testing that are unambiguous markers of use, not present from external contamination
- Preliminary studies in July and August 2016 indicated that opioid glucuronide conjugates were present in hair of drug users
- RTI developed a validated method for quantification of several opioids and their glucuronides
- Analyzed 46 user hair samples (confirmed positive for opiates by Psychomedics) using the validated method

Extraction Method

- Hair washed using isopropanol and phosphate buffer wash published by Cairns et al.
- 25 mg of hair weighed out +/- 0.2 mg
- 500 uL of extraction solvent added
- Hair is heated at 100 °C for 1 hr (gently mixed after 30 min)
- Cooled to room temperature prior to SPE

Notes

- Hair is cut with scissors into < 1cm pieces
- Extraction solvent is “M3” reagent from Comedical
- 500 µL completely covers the hair
- After extraction hair retains its morphological characteristics

LC Method

Instrument: Agilent 1290 LC coupled to an Agilent 6490 triple quadrupole

Column: Agilent poroshell 120 SB C-18 (2.7 μm , 2.1 x 100 mm)

Flow Rate: 500 $\mu\text{L}/\text{min}$

Column Temperature: 50 $^{\circ}\text{C}$

Mobile phase A: 5mM ammonium formate with 0.1% formic acid

Mobile phase B: Methanol with 0.1% formic acid

Time (min)	% A	% B
Initial	95	5
0.5	95	5
5.0	25	75
5.1	25	75
6.1	10	90
6.2	95	5
8.2	95	5

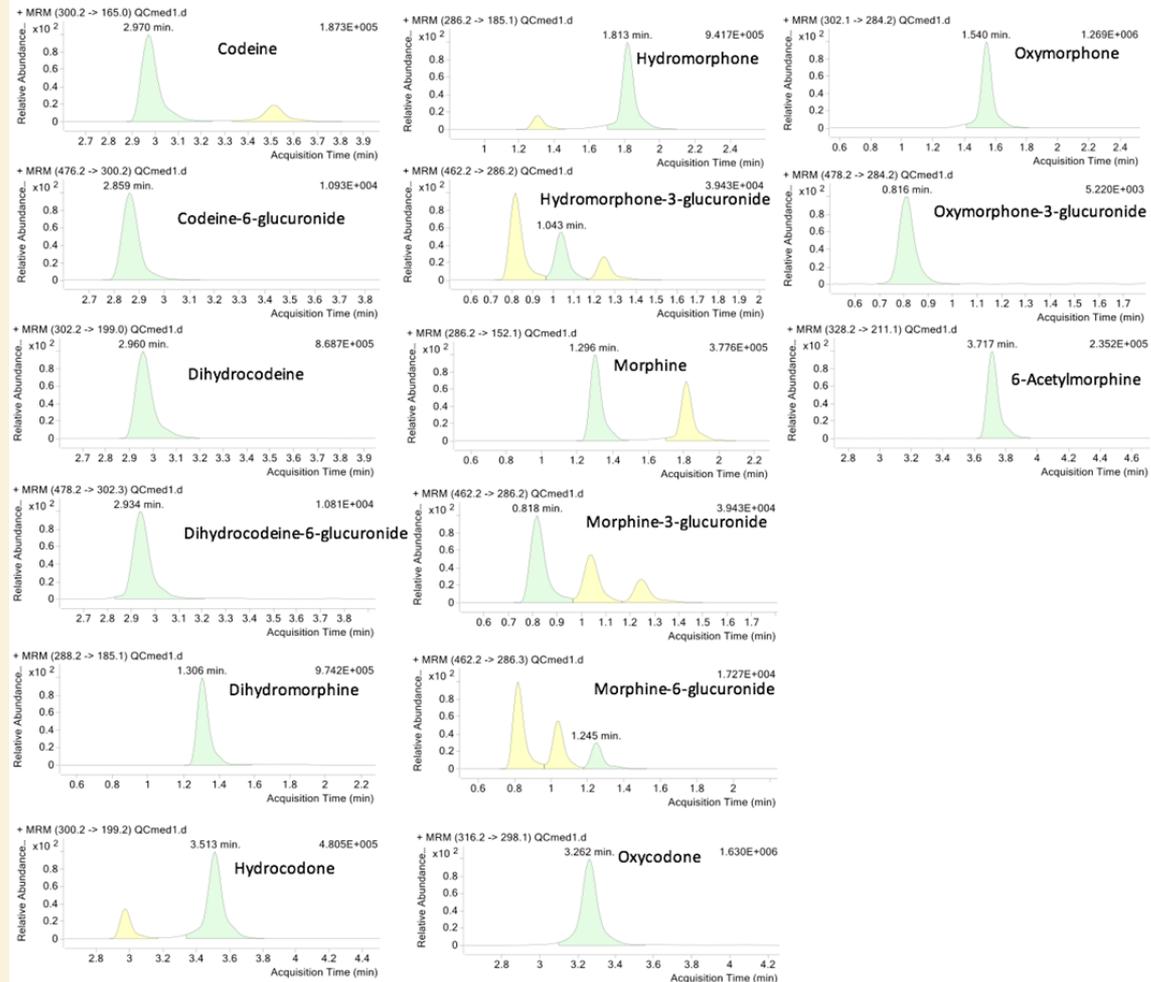
LC-MS/MS Parameters for Analytes

Analytes	Retention Time (min)	Precursor Ion (m/z)	Product Ion 1 (m/z)	Product Ion 2 (m/z)
Codeine	2.971	300.16	165.0	152.1
Codeine-6B-D-glucuronide	2.866	476.19	300.2	215.1
Dihydrocodeine	2.954	302.18	199.0	128.1
Dihydrocodeine-6B-D-glucuronide	2.943	478.21	302.3	199.2
Dihydromorphine	1.294	288.16	185.1	157.0
Dihydromorphine-3B-D-glucuronide	0.804	464.19	288.2	185.1
Hydrocodone	3.505	300.16	199.2	128.0
Hydromorphone	1.806	286.15	185.1	157.1
Hydromorphone-3B-D-glucuronide	1.035	462.18	286.2	185.1
Morphine	1.293	286.15	165.2	152.1
Morphine-3B-D-glucuronide	0.814	462.18	286.2	-
Morphine-6B-D-glucuronide	1.246	462.18	286.3	-
Oxycodone	3.266	316.16	298.1	241.1
Oxymorphone	1.537	302.14	284.2	227.0
Oxymorphone-3B-D-glucuronide	0.803	478.17	284.2	227.0
6-Acetylmorphine	3.715	328.16	211.1	164.9

LC-MS/MS Parameters for ISTDs

Internal Standards	Retention Time (min)	Precursor Ion (m/z)	Product Ion 1 (m/z)	Product Ion 2 (m/z)
Codeine-d6	2.93	306.2	165.0	-
Codeine-6B-D-Glucuronide-d3	2.85	479.2	61.1	-
Dihydrocodeine-d6	2.90	308.2	171.2	-
Hydrocodone-d6	3.47	306.2	202.3	-
Hydromorphone-d3	1.80	289.2	185.0	-
Morphine-d6	1.27	292.2	151.9	-
Morphine-3B-D-glucuronide-d3	0.81	465.2	289.2	-
Morphine-6B-D-glucuronide-d3	1.24	465.2	289.0	-
Oxycodone-d6	3.23	322.2	304.1	-
Oxymorphone-d3	1.53	305.2	287.1	-
Oxymorphone-3B-D-Glucuronide-d3	0.79	481.2	287.1	-
6-Acetylmorphine-d6	3.70	334.2	164.9	-

LC/MS/MS Transitions



Validation Results

	LOQ (pg/mg)	ULOL (pg/mg)	Estimated LOD (pg/mg)	Extraction Recovery (%)	Process Efficiency (%)
Codeine	40	1200	1	69.9	53.0
Codeine-6B-D-glucuronide	2	120	0.2	67.7	73.0
Dihydrocodeine	40	1200	1	71.9	53.9
Dihydrocodeine-6B-D-glucuronide	2	80	0.5	56.6	63.1
Dihydromorphine	40	1200	0.1	71.0	56.7
Dihydromorphine-3B-D-glucuronide				66.9	35.9
Hydrocodone	40	1200	10	66.9	35.9
Hydromorphone	40	1200	0.1	71.4	54.0
Hydromorphone-3B-D-glucuronide	2	120	0.2	61.5	28.4
Morphine	40	1200	0.2	75.1	54.8
Morphine-3B-D-glucuronide	2	120	0.1	68.1	30.9
Morphine-6B-D-glucuronide	2	120	0.5	65.6	51.0
Oxycodone	40	1200	0.5	72.1	57.1
Oxymorphone	40	1200	0.5	72.7	58.6
Oxymorphone-3B-D-glucuronide	2	120	0.5	67.6	35.5
6-Acetylmorphine	40	1200	1	64.9	28.5

Routine sample preparation uses 25 mg of hair. Validated a “dilution” procedure to use 10 mg of hair for samples with high concentrations. Increases the upper limit of quantification to 3000 pg/mg hair.

Interferences

- Potentially interfering compounds
 - Heroin, norcodeine, norhydrocodone, normorphine, noroxycodone and noroxymorphone
 - 6-AM was affected by heroin
 - No other compound produced interference in blank samples or caused quantifications to be out of tolerance (+/- 20%)
- Possibility of glucuronide conjugate interference as a result of sample preparation and extraction processes
 - No glucuronide conjugate signal detected in extracted blank hair samples from 10 non-drug users or blank hair fortified with the 8 analytes of interest
 - Morphine at 3,000 pg/mg spiked into blank hair and extracted caused an interference for morphine-6-glucuronide that could be separated by adjusting the LC gradient.

Results (46 hair samples)

	Number of Samples > LLOQ	Number of Samples > 200 pg/mg	Maximum Concentration (pg/mg)
Codeine	16	5	427.19
Codeine-6-glucuronide	7	0	7.22
Dihydrocodeine	2	0	43.04
Dihydrocodeine-6-glucuronide	0	0	< 2 (1.16)
Dihydromorphine	0	0	6.34
Dihydromorphine-3-glucuronide	0	0	n/a
Hydrocodone	26	9	981.81
Hydromorphone	9	3	843.46
Hydromorphone-3-glucuronide	2	0	4.97
Morphine	27	17	> 3000 (3113)
Morphine-3-glucuronide	10	0	9.41
Morphine-6-glucuronide	12	0	24.97
Oxycodone	28	21	2987.96
Oxymorphone	10	6	2183.15
Oxymorphone-3-glucuronide	6	0	8.93
6-Acetylmorphine	28	22	> 3000 (6846)

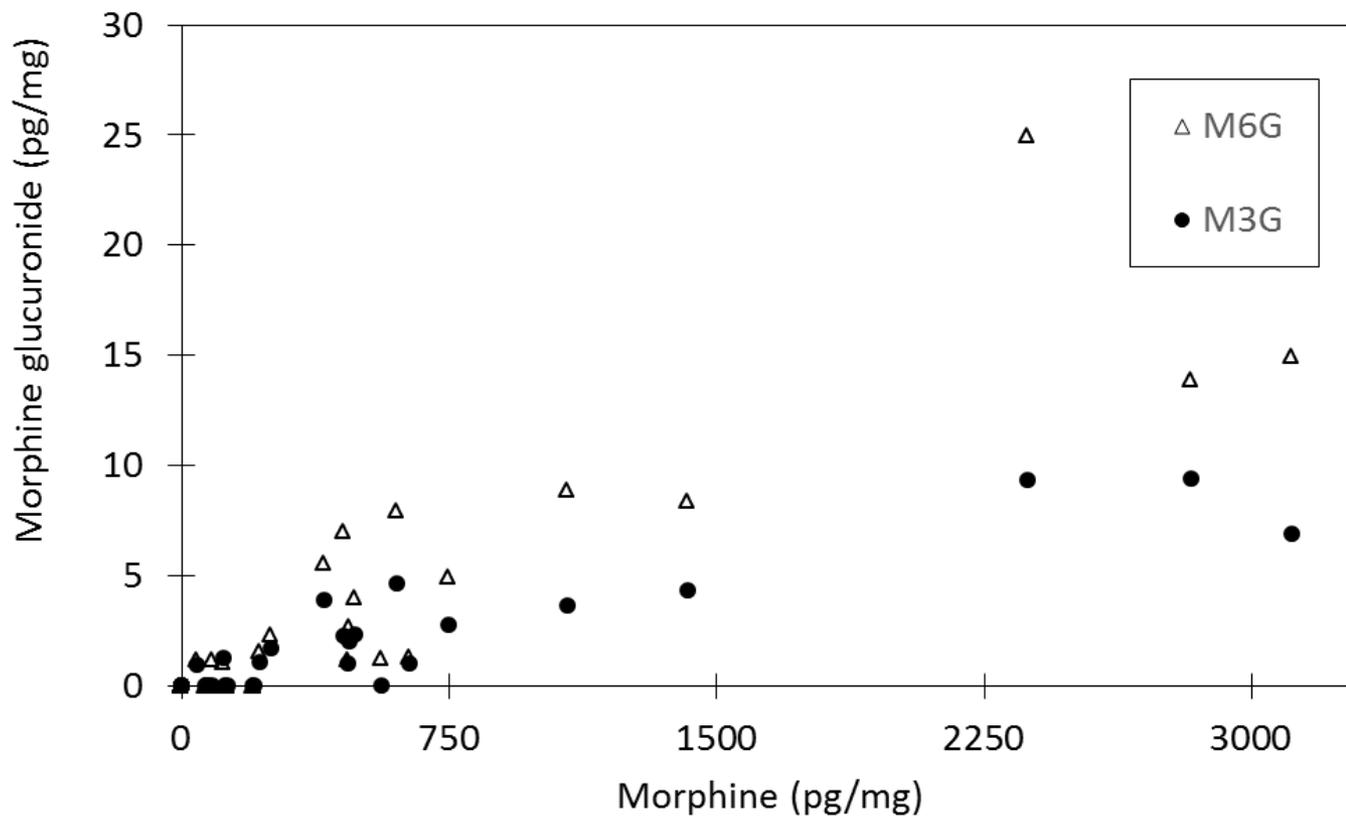
RTI-Psychemedics Comparison

	RTI		Psychemedics	
	Number of Samples > 200 pg/mg	Maximum Concentration (pg/mg)	Number of Samples > 200 pg/mg	Maximum Concentration (pg/mg)
Codeine	5	427	5	566
Hydrocodone	9	982	10	1004
Hydromorphone	3	843	3	847
Morphine	17	> 3000 (3113)	16	3370
Oxycodone	21	2988	21	3240
Oxymorphone	6	2183	6	1765
6-AM	22	> 3000 (6846)	22	6950

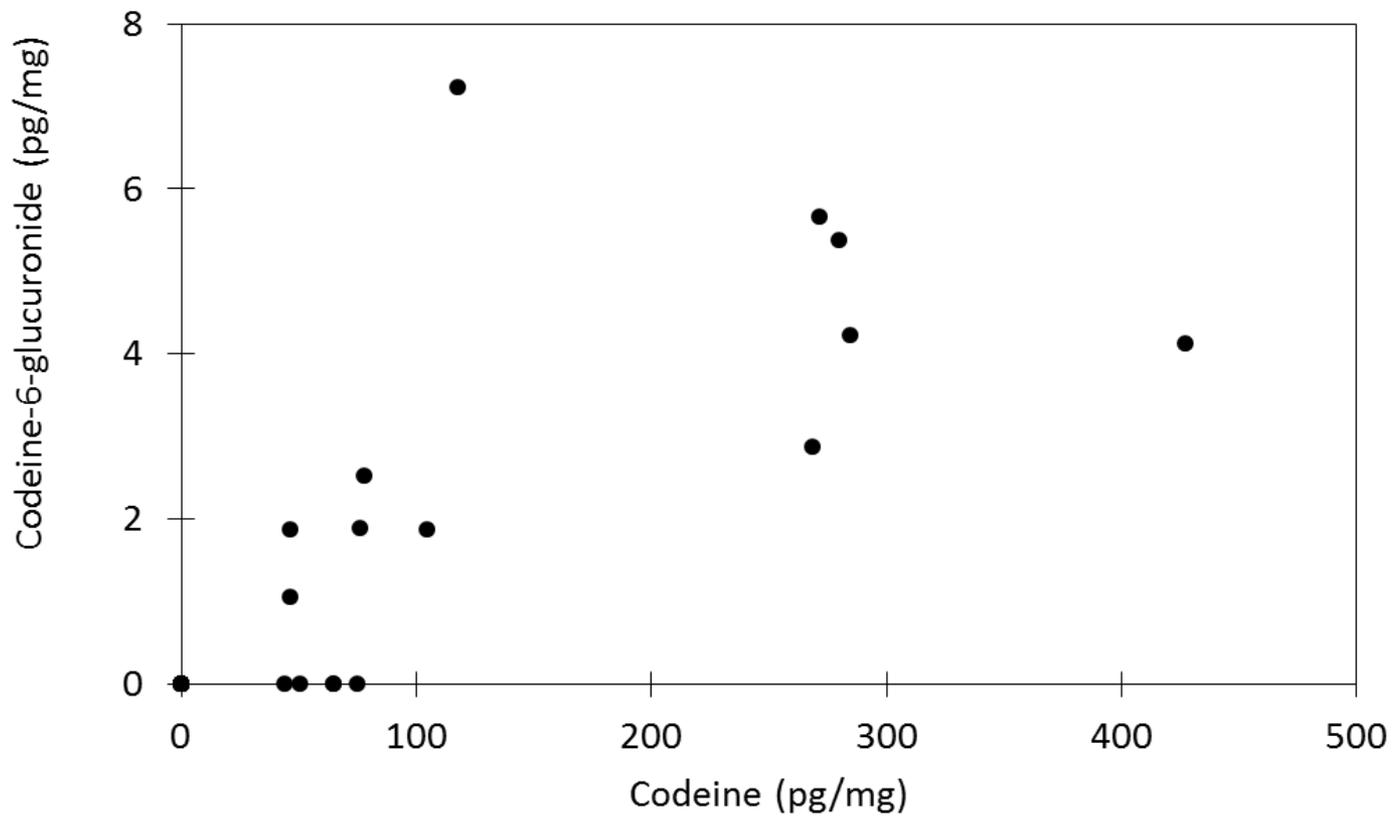
Results

	Parent concentration 200 pg/mg or more	All samples with parent and glucuronide conjugate both present
	Absolute Concentration Range (pg/mg)	Concentration Range Relative to Parent
Codeine-6-glucuronide	2.86 – 5.65	0.97 – 6.12%
Hydromorphone-3-glucuronide	2.37 – 4.97	0.59 – 1.30%
Oxymorphone-3-glucuronide	< 2 (0.98) – 8.93	0.16 – 2.13%
Morphine-3-glucuronide	< 2 (1.01) – 9.41	0.15 – 2.13%
Morphine-6-glucuronide	< 2 (1.20) – 24.97	0.20 – 2.73%

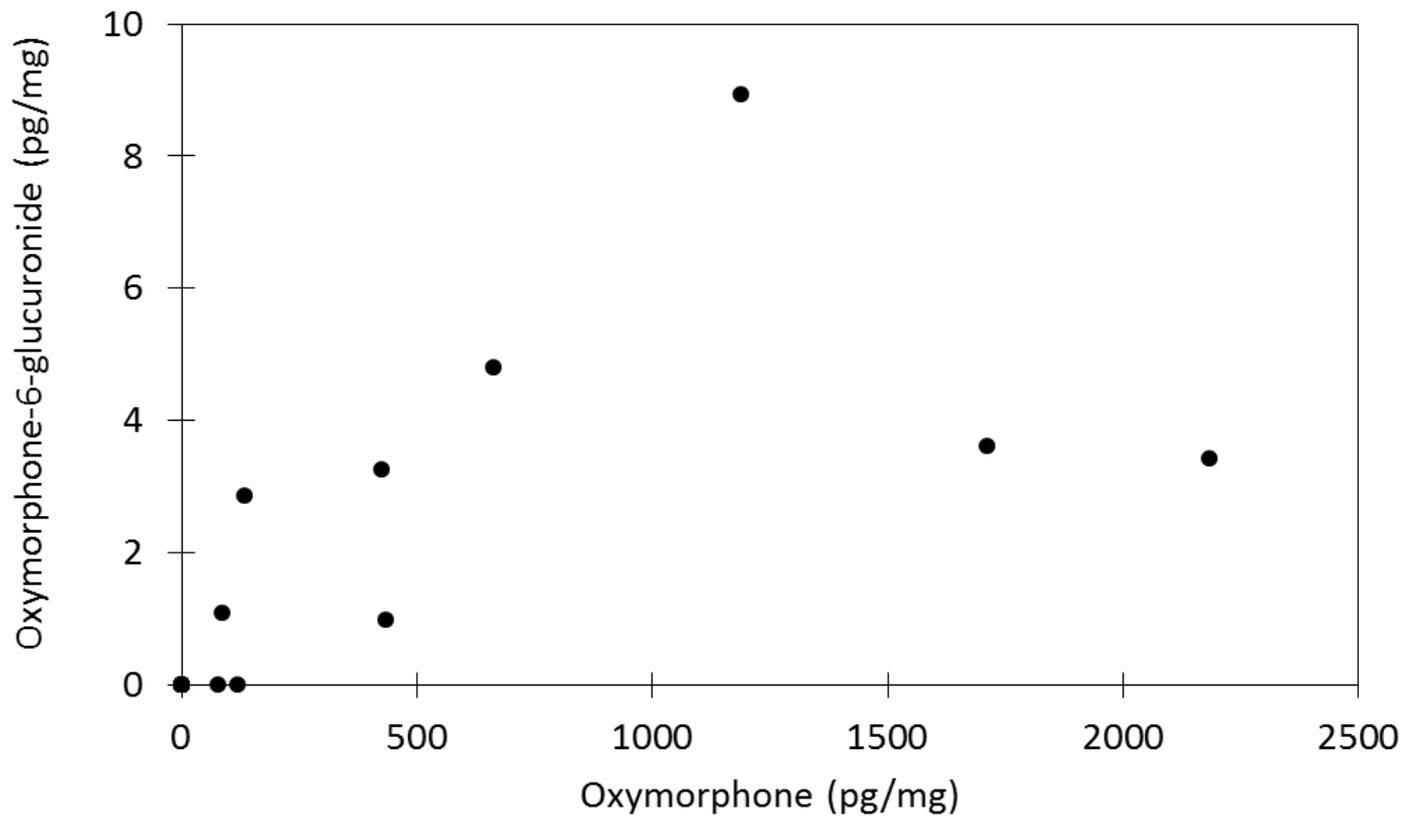
Morphine



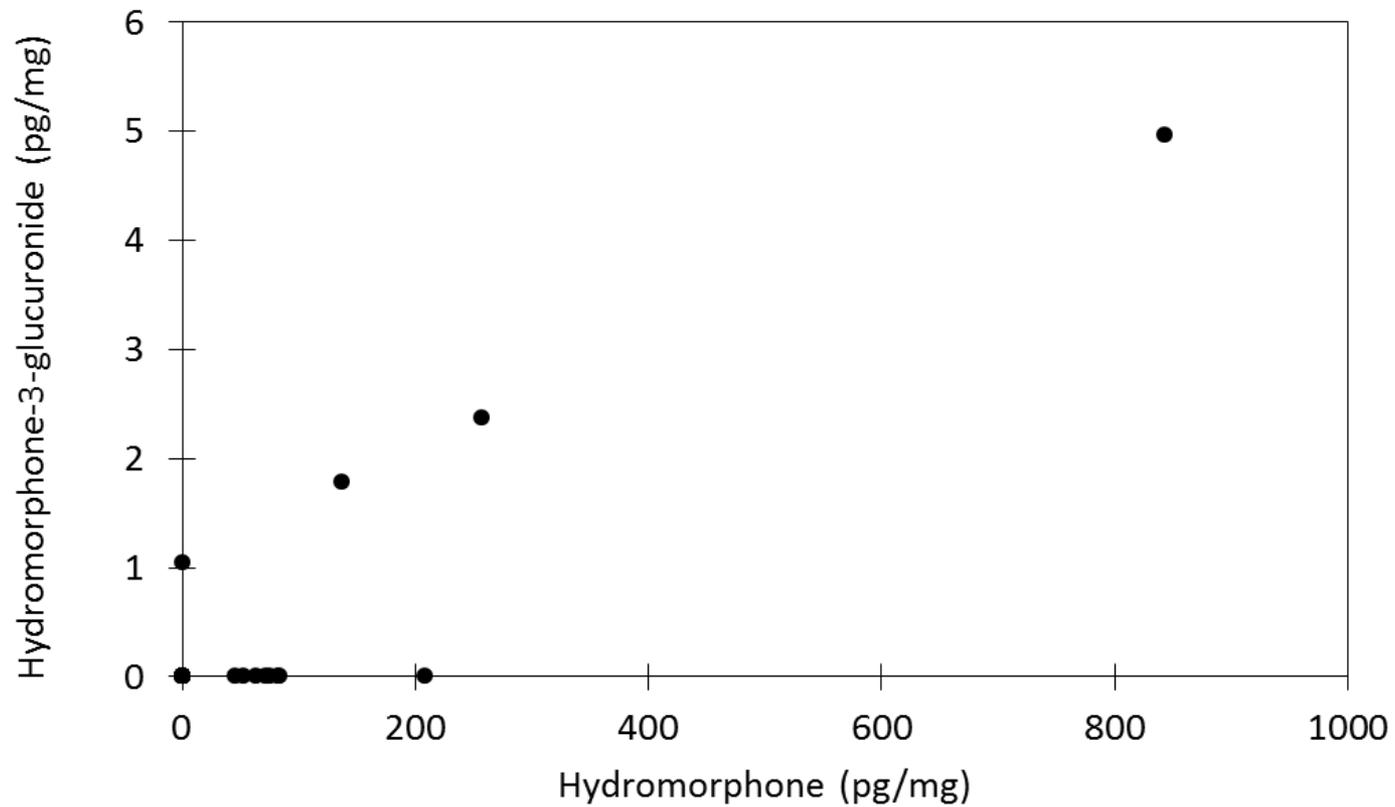
Codeine



Oxymorphone



Hydromorphone



Summary

- Codeine-6-glucuronide, Oxymorphone-3-glucuronide, Morphine-3-glucuronide, Morphine-6-glucuronide, Hydromorphone-3-glucuronide are present in drug user hair above LLOQ of 2 pg/mg and generally increase with parent concentration
- Glucuronide concentrations generally approximately 1 pg/mg or greater in samples with greater than 200 pg/mg parent compound present
- Maximum relative abundance of glucuronides < 3% of parents for all but codeine-6-glucuronide (6%)
- Room for improvement in extraction method to increase process efficiency and sensitivity
- Not enough data from hair samples containing dihydrocodeine and dihydromorphine
 - Dihydrocodeine-6-glucuronide, Dihydromorphine-3-glucuronide

Manuscript with full method details and results submitted to Journal of Analytical Toxicology
Detection and quantification of codeine-6-glucuronide, hydromorphone-3-glucuronide, oxycodone-3-glucuronide, morphine 3-glucuronide, and morphine-6-glucuronide in human hair from opioid users by LC-MS/MS.

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Questions?