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Phase II Metabolites of Drugs in Hair: A Potential Solution for Environmental Contamination

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Project Summary

Major Goals and Objectives

Although drugs and some metabolites can be measured reliably in hair, it can be difficult if not impossible to differentiate drugs deposited in hair due to actual use of the parent drug from drug that is present due to external contamination. External contamination may occur when an individual is in the company of people who use drugs, or from environmental exposure in contaminated environments. Wash procedures are commonly employed as a step to minimize the impact of external contamination; however, there are no standardized wash procedures and washing may incorporate drug into the internal matrix of the hair shaft. The use of metabolites as markers in addition to or instead of parent drugs has also been suggested as a way to differentiate drug use from contamination and this is reflected in current hair testing guidelines.

The objective of this project was to look for unique metabolites in hair that are indicators of consumption in addition to, or in place of, the parent drugs. The choice of metabolite is critical because some metabolites can be present as process impurities, meaning they may be present as a result of contamination by the parent compounds. Other metabolites may be degradation products formed because of exposure to hair care products subsequent to parent drug contamination. Also, it is possible that some parent drugs are converted to metabolites during analysis. In addition, some metabolites are commercially available drugs themselves.

Phase II conjugated metabolites are ideal markers of use because they are not products of common degradation pathways, as is the case for many phase I metabolites, and are not commercially available for the purposes of therapeutic use or abuse. We hypothesize that conjugated metabolites of drugs are present in the hair of people who use drugs as a result of metabolic activity and excretion into the hair. We conducted exploratory research to look for the presence of these metabolites in hair from people known to have used drugs using a variety of

sample preparation and liquid chromatography – mass spectrometry (LC-MS) detection techniques.

Data acquisition included targeted LC-MS/MS acquisition methods commonly employed in metabolite identification applications, as well as full scan high resolution non-targeted MS data acquisition.

Research Questions

- Can phase II metabolites (i.e., conjugated metabolites) serve as an alternative to current decontamination procedures for drug testing in hair?
    - Can phase II metabolites be inadvertently produced during sample analysis?
    - Are concentrations of phase II metabolites high enough for quantification?

Research Design, Methods, and Analytical Techniques

Hair specimens were selected from RTI's hair inventory including blank drug-free hair and hair from people known to have used drugs that had high concentrations of the drugs of interest. Hair specimens were extracted using techniques developed that retain opioid glucuronides.

Combined Drug Category Hair Extractions and SPE Clean-up

Two methods were developed to extract analytes of interest (opioids, amphetamines, cocaines) from hair, one using acidic methanol (overnight at room temperature) and one using M3 reagent from Comedical (Trento TN, Italy). Each of these was followed by an SPE clean-up prior to injection into the LC-MS system. For LC-MS/MS we used the extraction with the highest recovery for each drug category, e.g., acidic methanol for hydroxy cocaine and M3 for opioid glucuronides. Since QTOF is a nontargeted method, we used a single extraction method for all drug categories. The M3 extraction method was used for all hair specimens for QTOF analysis.

Sample Extraction: M3

1. To 25 mg pulverized hair add calibrator, QC, and/or ISTD
2. Add 500 µL of M3 Reagent to each sample
3. Vortex mix
4. Centrifuge at 4,000 rpm for 5 minutes
5. Place tubes on heating block (100°C) for 30 minutes
6. Remove, gently mix, then return to the heating block for an additional 30 minutes
7. Cool to room temperature
8. Add 500 µL of 1% formic acid in water to screw cap tubes and vortex mix
9. Centrifuge at 4,000 rpm for 5 minutes
10. Proceed with SPE cleanup

Sample Extraction: Acidic Methanol

1. To 25 mg pulverized hair add calibrator, QC, and/or ISTD
2. Add 500 µL of methanol with HCl (1%) to each sample
3. Vortex mix
4. Centrifuge at 4,000 rpm for 5 minutes
5. Place tubes on horizontal mixer (low speed) overnight
6. Add 500 µL of 1% formic acid in water to screw cap tubes and vortex mix
7. Centrifuge at 4,000 rpm for 5 minutes
8. Proceed with SPE cleanup

Solid Phase Extraction Cleanup: Biotage Evolute Express CX

1. Condition 1 mL methanol
2. Condition 1 mL 1% formic acid
3. Load sample
4. Wash with 1 mL 1% formic acid
5. Wash with 1mL 50/50 methanol-water (v/v)
6. Dry column (1 min at full vacuum or pressure)
7. Elute with 2 x 1.5mL dichloromethane/methanol/ammonium hydroxide 78/20/2 (v/v)
8. Prior to dry down add 100 µL of 1% HCl in methanol
9. Evaporate to dryness at 40 °C
10. Reconstitute samples in 100 µL 95:5 5 mM ammonium formate with .1% formic acid: methanol with .1% formic acid
11. Centrifuge with Millipore 0.22 micron spin filter at 4000 rpm for 4 min then transfer to LC vials

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Data Acquisition and Analysis

Specimens were analyzed using targeted MRM and SIM and semi-targeted precursor ion and neutral loss scanning on an Agilent 6490 triple quadrupole mass spectrometer coupled to an Agilent 1290 LC system using electrospray ionization in positive ion mode. Masshunter Qualitative Analysis version 10.0 was used for data analysis. The LC methods used 5 mM ammonium formate with 0.1% formic acid as mobile phase A and methanol with 0.1% formic acid as mobile phase B. Specific gradients for each drug category are shown in Table 1

Table 1 LC methods for targeted and semi-targeted data acquisition

COC/OXY	%B		MAMP	%B		Opioids	%B
Initial	5		Initial	5		Initial	5
1.00 min	5		1.00 min	5		0.5 min	5
7.00 min	55		5.00 min	25		5.00 min	25
7.50 min	95		6.00 min	95		5.10 min	90
9.00 min	95		7.50 min	95		8.10 min	90
All LC-MS/MS followed by 1.5-2 min post run at initial conditions						8.20 min	5

Targeted SIM and MRM transitions were developed for each parent compound and major metabolite (cocaine, benzoylecgonine, norcocaine, cocaethylene, hydroxycocaine, methamphetamine, amphetamine, hydroxymethamphetamine, hydroxyamphetamine, morphine, hydromorphone, codeine, hydrocodone, 6-acetylmorphine, oxycodone, oxymorphone, noroxycodone) based on the m/z of the parent and most prominent product ions found during MS optimization. Predicted m/z values were calculated for glucuronide, glutathione, S-glutathione, and sulfate conjugation. Example tables of SIM m/zs and MRM transitions for cocaine are shown in Table 2 and Table 3, respectively.

Table 2 Calculated SIM m/zs for cocaine

Description	Formula	$[M+H]^+$
Cocaine	C17H21NO4	304.15
Methylation	C18H23NO4	318.17
Hydroxylation	C17H21NO5	320.15

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Hydroxylation + methylation	C18H23NO5	334.17
2 x Hydroxylation	C17H21NO6	336.14
Acetylation	C19H23NO5	346.17
Glycine conjugation	C19H24N2O5	361.18
Sulfate conjugation	C17H21NO7S	384.11
Hydroxylation + sulfation	C17H21NO8S	400.11
Cysteine conjugation	C20H26N2O5S	407.16
Taurine conjugation	C19H26N2O6S	411.16
S-Cysteine conjugation	C20H26N2O6S	423.16
Decarboxylation + glucuronidation	C22H29NO9	452.19
N-acetylcysteine conjugation	C22H28N2O7S	465.17
Glucuronide conjugation	C23H29NO10	480.19
2 x O-sulfate conjugation	C17H21NO12S2	496.06
Hydroxylation + glucuronide conjugation	C23H29NO11	496.18
Glutathione conjugation	C27H36N4O9S	593.23
Desaturation + S-Glutathione conjugation	C27H34N4O10S	607.21
S-Glutathione conjugation	C27H36N4O10S	609.22
OH + S-Glutathione conjugation	C27H36N4O11S	625.22
2 x Glucuronide conjugation	C29H37NO16	656.22

Table 3 Calculated MRM transitions for cocaine

Description			m/z	
Precursor	**Product**		**Precursor**	**Product**
parent	product 1		304.10	182.20
parent	product 2		304.10	82.10
parent + gluc	parent		480.13	304.10
parent + gluc	product 1		480.13	182.20
parent + gluc	product 2		480.13	82.10
parent + gluc	product 1 + gluc		480.13	358.23
parent + gluc	product 2 + gluc		480.13	258.13
parent + glutathione	parent		593.17	304.10
parent + glutathione	product 1		593.17	182.20
parent + glutathione	product 2		593.17	82.10
parent + glutathione	product 1 + glutathione		593.17	471.27
parent + glutathione	product 2 + glutathione		593.17	371.17
parent + S-glutathione	parent		609.17	304.10
parent + S-glutathione	product 1		609.17	182.20

parent + S-glutathione	product 2		609.17	82.10
parent + S-glutathione	product 1 + glutathione		609.17	487.27
parent + S-glutathione	product 2 + glutathione		609.17	387.17
parent + sulfate	parent		384.06	304.10
parent + sulfate	product 1		384.06	182.20
parent + sulfate	product 2		384.06	82.10
parent + sulfate	product 1 + sulfate		384.06	262.16
parent + sulfate	product 2 + sulfate		384.06	162.06

Neutral losses to monitor were selected for each parent drug based on the m/z of the parent and most prominent product ions found during MS optimization, known metabolites, and potential conjugation reactions. An example table of neutral losses monitored for cocaine is shown in Table 4.

Table 4 Neutral losses monitored for cocaine

Biotransformation	Mass of Neutral Loss	Description of Neutral Loss
Glutathione conjugation	307.1	Glutathione
Glutathione conjugation	129.0	Pyroglutamic acid
Glutathione conjugation	146.1	Glutamine
GlcNAc conjugation	221.1	N-acetylglucosamine (GlcNAc)
GlcNAc conjugation	203.0	N-acetylglucosamine (GlcNAc)
Glycine conjugation	75.0	Glycine
Sulfate conjugation	80.0	SO3
Cysteine conjugation	121.0	Cysteine
Glucuronide conjugation	176.0	Anhydroglucuronic-acid
Glucuronide conjugation	194.0	Glucuronic-acid
N-Acetylcysteine conjugation	163.0	Acetylcysteine
Glucoside conjugation	162.0	Anhydroglucose
Cocaine/BZE/NC/CE fragmentation	121.9	parent - product ion 1
Cocaine fragmentation	222.0	parent - product ion 2
BZE/NC fragmentation	185.0	parent - product ion 2
Cocaethylene fragmentation	236.0	parent - product ion 2
Hydroxycocaine fragmentation	138.0	parent - product ion 1
Hydroxycocaine fragmentation	199.0	parent - product ion 2
Norcocaine fragmentation	154.0	parent - product ion 3

Product ions selected for precursor ion scanning were the m/z of the parent and most prominent product ions found during MS optimization, combinations of these product ions with possible conjugates, and the conjugate ions. An example of the m/z values selected for precursor ion scanning for cocaine is shown in Table 5.

Table 5 Precursor ions monitored for cocaine

Biotransformation	m/z of Product Ion	Description of Product Ion
Glucuronide conjugation	175.0	[Glu+H-2H]+
Glucuronide conjugation	177.0	[Glu+H]+
Glutathione conjugation	274.0	[GSH-S-2H+H]+
Glutathione conjugation	308.1	[GSH+H]+
None - Cocaine	304.1	parent
Cocaine fragmentation	182.2	product ion 1
Cocaine fragmentation	82.1	product ion 2
Glucuronide conjugation	358.2	product 1 + gluc
Glucuronide conjugation	258.1	product 2 + gluc
Glutathione conjugation	471.3	product 1 + glutathione
Glutathione conjugation	371.2	product 2 + glutathione
Glutathione conjugation	487.3	product 1 + S-glutathione
Glutathione conjugation	387.2	product 2 + S-glutathione
Sulfate conjugation	262.2	product 1 + sulfate
Sulfate conjugation	162.1	product 2 + sulfate

Specimens were also analyzed using non-targeted simultaneous high and low energy mass spectra acquisition using a Waters Xevo G2-XS QTOF coupled to a Waters Acuity LC system using electrospray ionization in positive ion mode. For the non-targeted data acquisitions all specimen data was acquired using the same LC method which was as follows: 5% B for 1 min followed by a linear gradient to 55% B from 1 to 7 min, hold at 55% B for 0.5 min, linear gradient to 95% B from 7.5 to 9 min, then hold at initial conditions until 10.5 min. Mobile phase

A was 5 mM ammonium formate with 0.1% formic acid and mobile phase B was methanol with 0.1% formic acid. The injection volume was 5 µL onto a poroshell SB-C18 2.1 mm x 100 mm x 2.7 µm held at 50 °C. The m/z range was 50 to 700. Source parameters for the QTOF were as follows: desolvation temperature at 600 °C, desolvation gas at 1000 L/h, sample cone voltage at 25 V, source temperature at 150 °C, cone gas at 20 L/h and capillary voltage at 0.80 kV.

QTOF data acquisition, processing and analysis were done using Waters Connect version 1.9.13. The data processing component list included over 300 expected components added from the scientific library. Twenty-nine of these entries were based on analysis of individual reference standards and included experimentally determined expected retention time, expected neutral mass, expected fragments, formula, and a .mol structure file. The components with reference standards and QTOF retention times are shown in Table 6. The other components are potential conjugates based on the SIM method example shown for cocaine in Table 2, applied to each parent compound and major metabolite (cocaine, benzoylecgonine, norcocaine, cocaethylene, hydroxycocaine, methamphetamine, amphetamine, hydroxymethamphetamine, hydroxyamphetamine, morphine, hydromorphone, codeine, hydrocodone, 6-acetylmorphine, oxycodone, oxymorphone, noroxycodone). For QTOF data processing, the target by retention time absolute retention time tolerance was set at 0.35 min. Target by mass parameters were match tolerance 10 ppm and fragment match tolerance 2 mDa.

Expected Applicability of the Research

Distinguishing between consumption versus environmental contamination as the cause for incorporation of drugs in hair has been a long-standing concern in the field of forensic toxicology. Without the ability to distinguish people who have used drugs from people who have

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not used drugs, the utility of hair drug testing for criminal investigations, child welfare, and at-risk employment with known drug environments or high-risk employment situations (e.g., police, crime scene investigators, commercial drivers) will continue to be suspect. Detection of conjugated metabolites in hair may directly affect the current policies and procedures for hair drug testing. It could alleviate concerns over the potential for external contamination and issues with interpretation of hair drug testing results.

The findings from this research project include potential markers of drug use in hair. Highly sensitive targeted MRM methods can be developed using these potential markers and used to analyze hair from both people who have used drugs and people who have not used drugs to determine their relevance in drug testing from known populations.

Participants and Other Collaborating Organizations

Work on this project was conducted at RTI International's headquarters location in Research Triangle Park, NC. Megan Grabenauer, Ph.D., was the PI of the project. She had oversight responsibility for the full project, developed the project design, performed data analysis, and prepared all reports. Katherine Bollinger, M.S., was the technical lead for this project. She coordinated all laboratory analyses, was responsible for data acquisition and assisted with data analysis. She also contributed to semi-annual and final reports. Nichole Bynum, M.S., provided additional laboratory support by performing specimen extractions, and assisting with data acquisition and analysis. Svante Vikingsson, Ph.D., assisted with data analysis.

Changes in Approach from Original Design

No substantial changes in approach from the original project design were necessary. The project timeline was delayed by one year due to impacts from the COVID-19 pandemic resulting

in staffing shortages and laboratory closures that interfered with our ability to acquire data. During the project period RTI International replaced the existing QTOF instrument with a new system necessitating familiarization and training with the new system and software to complete data processing.

Outcomes

Activities and Accomplishments

- IRB Review
  - Prior to beginning any work on this project a study protocol was submitted to and reviewed by RTI's institutional review board (IRB) and received a determination of not research with human subjects

- Extraction and analytical method development
  - Developed and optimized extraction methods for opioids, cocaines, and amphetamines from human hair
  - Verified that the optimized methods did not produce phase II metabolites
  - Developed and optimized liquid chromatography methods for each drug category

- Targeted and semi-targeted data acquisition
  - Created target lists for SIM, MRM, precursor ion, and neutral loss acquisition methods
  - Extracted and analyzed reference standards, controls, and hair specimens from people known to have used drugs using SIM, MRM, precursor ion, and neutral loss acquisition methods

- Targeted and semi-targeted data analysis
  - Reviewed all peak integrations and compiled combined peak lists for each acquisition method
  - Filtered integrated peaks for validity based on blank response, signal-to-noise, and retention time
  - Consolidated common transitions observed across multiple acquisition methods and visually compared to chromatograms
  - Prioritized observed transitions of interest and made tentative identification assignments

- QTOF data acquisition
  - Oversaw installation and underwent training on a new QTOF instrument
  - Extracted and analyzed reference standards, controls, and hair specimens from people known to have used drugs using a simultaneous high and low collision energy acquisition method

- QTOF data analysis

- Post-acquisition filtering criteria based on a variety of criteria including high, mid, and low confidence identification criteria
- Data reviewed by observed mass and mass error, retention time, comparison to blank, observation across multiple specimens with observed parent compounds, and filtering by response

- Delivery of project-related and technical reports to NIJ
  - Completed semi-annual and quarterly financial progress reports and submitted them through the JustGrants system

Results and Findings

Optimized hair extraction and SPE clean-up methods are listed in the Research Design, Methods, and Analytical Techniques section. These sample preparation methods were tested and found to not inadvertently produce phase II metabolites in response to the research question: Can phase II metabolites be inadvertently produced during sample analysis? Retention times for reference standards using the QTOF and MRM acquisition methods are listed in Table 6.

Table 6 QTOF and LC-MS/MS reference standard retention times.

Reference Standard	QTOF Retention Time	MRM Retention Time
Oxymorphone gluc	0.89	0.78
Morphine-3-gluc	0.90	0.78
Hydromorphone gluc	1.15	1.09
Morphine-6-gluc	1.32	1.18
Morphine	1.46	1.27
4OHAMP	1.75	2.07
Oxymorphone	1.77	1.52
4OHMAMP	1.91	2.25
Hydromorphone	2.08	1.92
Codeine gluc	2.82	3.72
Codeine	2.96	3.88
Oxycodone	3.06	4.23
Amphetamine	3.17	4.09
Hydrocodone	3.29	4.53
Methamphetamine	3.37	4.42
6-AM	3.41	4.77

p-OHBZE	3.41	3.50
p-OHCOC	3.62	3.71
m-OHBZE	3.64	3.70
m-OHCOC	3.87	3.98
BZE	4.29	4.41
Norfentanyl	4.54	NA
Cocaine	4.79	4.95
o-OHCOC	4.82	4.98
Norcocaine	5.12	5.27
Cocaethylene	5.51	5.68
Trazadone-d6	5.63	NA
Fentanyl	6.15	NA
EDDP	6.51	NA
Methadone	7.64	NA

Full result tables for possible conjugated metabolites resulting from LC-MS/MS and QTOF data analyses are included in the appendix. These tables include average retention time, observed m/z, and a tentative transformation identification if available. Selected key findings are presented here.

Morphine-3-glucuronide and morphine-6-glucuronide were reliably detected in hair specimens containing morphine. Codeine-6-glucuronide was reliably detected in hair specimens containing codeine. For codeine and morphine glucuronides fragmentations from glucuronide conjugate to parent drug had larger signal responses than transitions from glucuronide conjugate to known product ions of the parents. There is evidence for oxymorphone-3-glucuronide in specimens that had the highest concentrations of oxymorphone.

A peak with an m/z of 366 corresponding to a potential morphine sulfate metabolite was observed at 2.11 min in the QQQ data. MRM transitions for 366 -> 152 and 165, the dominant product ions for morphine, were both observed. There is literature evidence to suggest this might be morphine-3-sulfate.[1] Morphine-3-sulfate and morphine-6-sulfate have been reported to be

[1] Andersson (2012) Pharmacol Res Perspect, 2(6):e00071

minor urinary metabolites of morphine, and to fragment to m/z 165. While the morphine-3-sulfate was reported to have more than 100-fold lower concentrations than morphine-3-glucuronide in urine, they appear to produce peaks of similar abundance in hair.

Ortho, meta, and *para*-hydroxy cocaine as well as *meta*, and *para*-hydroxy benzoylecgonine were detected in hair specimens containing cocaine. Additional likely metabolites related to hydroxy cocaine and hydroxy benzoylecgonine were also observed, as shown in Figure 1.

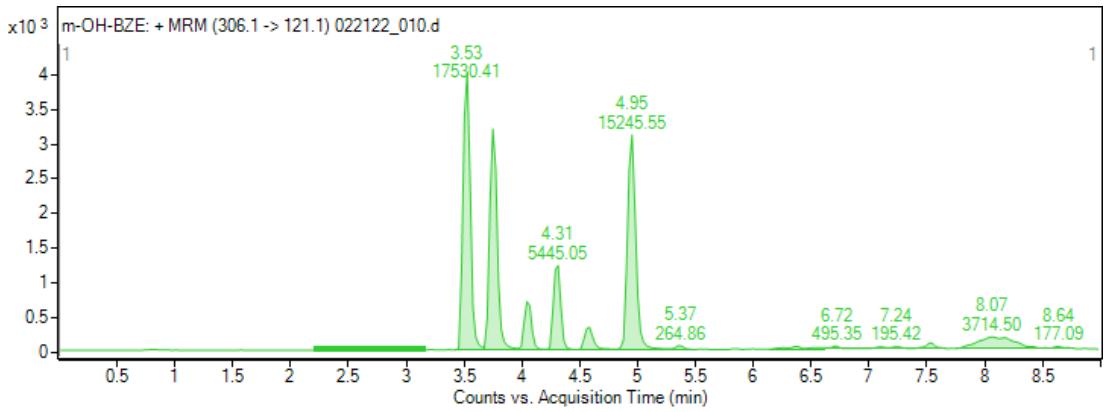

Figure 1 MRM chromatogram for 306.1 -> 121.1 m/z, a known transition for hydroxy-BZE. 3.53: p-OH-BZE, 3.75: p-OH-COC and m-OH-BZE, 4.04: m-OH-COC, 4.31: unknown, 4.58: unknown, 4.95: COC.

A potential cocaine related substance was observed eluting later than cocaine and cocaethylene, which is the latest eluting cocaine related reference standard used in this study. An extracted ion chromatogram for the $[M+H]^+$ ion of cocaine (304.1541 m/z) from the low energy QTOF data acquisition is shown in Figure 2. Cocaine (saturated) elutes at 4.72 min and this unknown substance elutes at 5.75 min. The low energy mass spectrum at 5.75 min shows a base peak of 304.1333 m/z followed by most abundant peaks at 182.1160 and 136.0749 m/z. The analytical evidence is consistent with norcocaethylene, a demethylated analog of cocaethylene.

Norcocaethylene has previously been reported as to fragment to m/z 182 and to elute after cocaine in a similar LC separation.[2]

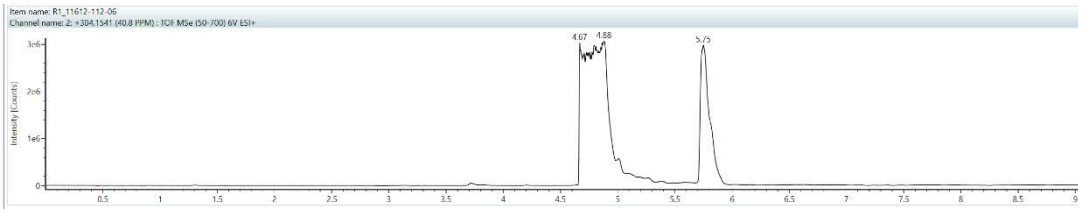

Figure 2 Low energy extracted ion chromatogram for the $[M+H]^+$ ion of cocaine, 304.1541 m/z

An extracted ion chromatogram for one of the dominant cocaine fragment ions (182.1160) is shown in Figure 3. The *ortho, meta*, and *para*-hydroxy cocaine metabolites can be seen at retention times of 4.87, 3.80, and 3.56 min, respectively. The chromatographic peak at 5.75 shows that the suspected norcocaethylene peak (see above) shares this common fragment ion with cocaine and other metabolites.

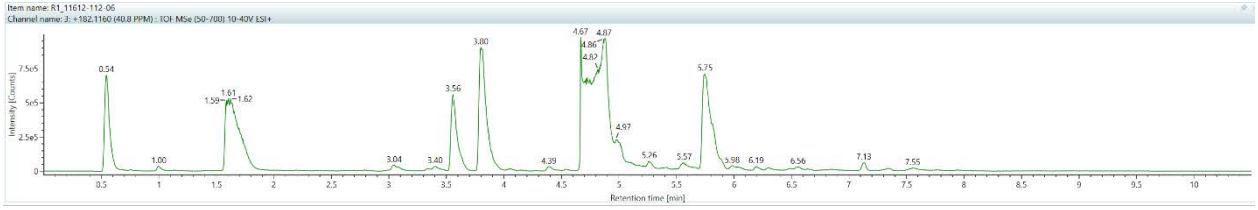

Figure 3 High energy extracted ion chromatogram for dominant cocaine fragment, 182.1160 m/z

Three distinct hydroxy-methamphetamine compounds were reliably detected in hair specimens containing methamphetamine. One of these matched the retention time of a 4-hydroxy-methamphetamine reference standard. There are several transitions related to amphetamines with precursor m/z of 162 at the retention time for a 4-hydroxy-amphetamine

[2] Chen et al (2017) J Pharm Biomed Anal, 134:243-251

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glucuronide reference standard, however no transitions with a precursor m/z greater than that of methamphetamine were observed at this retention time.

Limitations

Due to the exploratory nature of this project analyses were restricted to a small number of hair specimens from people known to have used drugs and may not be representative of the general population. There were limited conjugated metabolites available as reference standards to verify retention times and assist in method optimization and observed transition identification.

Artifacts

List of products

Grabenauer, M. "Identification of Phase II Opioid Metabolites in Human Hair." Presented at the NIJ R&D Symposium at American Academy of Forensic Sciences (AAFS) 2020 February 18, 2020, Virtual Symposium.

Data sets generated

This project produced peak integration results for reference standards and hair specimens from people known to have used drugs acquired under a variety of analytical conditions including both LC-MS/MS data acquisition and QTOF data acquisition. These peak lists from the MS data have been archived and study-level information was submitted to NACJD for this award (2019-DU-BX-0021).

Dissemination activities

Early results from this project were presented as an oral presentation at the NIJ R&D symposium at the 2020 AAFS Annual meeting. A manuscript intended for publication in a peer-reviewed journal is currently under development and datasets have been made publicly accessible. In addition, ongoing progress and results of this project have been shared informally with SAMHSA's drug testing advisory board and the National Laboratory Certification Program.

Appendix

QTOF Tables

Forty-three hair specimens from people known to have used drugs were analyzed by HRMS. Potential transformations or related fragments and their corresponding retention times, m/z, and the number of samples in which they were observed are shown in the following tables. All drug hair from people known to have used drugs analyzed by HRMS was independently analyzed by an external reference testing laboratory and the number of specimens positive for each target compound included in their testing panel is also included in the tables. Bolded entries indicate the identification is confirmed by a reference standard.

Cocaine

Observed RT	Predicted Formula	Observed m/z	Tentative Related Transformation or Fragmentation	Number of Specimens Observed	Number of Specimens Confirmed Positive by Reference Testing
0.55	C10H15NO2	182.1176	Cocaine related fragment	18	
1.61	C10H15NO2	182.1176	Cocaine related fragment	33	
2.80	C18H23NO4	318.1700	Cocaine + Methylation	15	
2.82	C18H21NO5	332.1494	BZE/NC + Acetylation; Codeine/Hydrocodone + 2 x Hydroxylation; Oxycodone + Hydroxylation; Oxymorphone/Noroxycodone + Hydroxylation + methylation	14	
3.05	C17H21NO6	336.1437	Cocaine + 2 x Hydroxylation; Hydroxycocaine + Hydroxylation	19	
3.37	**C16H19NO5**	**306.1331**	**para-Hydroxybenzoylecgonine**	**24**	**n/a**
3.41	C17H21NO6	336.1437	Cocaine + 2 x Hydroxylation; Hydroxycocaine + Hydroxylation	15	
3.57	**C17H21NO5**	**320.1487**	**para-Hydroxycocaine**	**26**	**26**

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3.59	**C16H19NO5**	**306.1333**	**meta-Hydroxybenzoylecgonine**	**24**	**n/a**
3.69	C17H21NO6	336.1437	Cocaine + 2 x Hydroxylation; Hydroxycocaine + Hydroxylation	32	
3.74	C16H19NO6	322.1282	BZE/NC + 2 x Hydroxylation	8	
3.81	**C17H21NO5**	**320.1489**	**meta-Hydroxycocaine**	**33**	**28**
3.83	C17H21NO6	336.1438	Cocaine + 2 x Hydroxylation; Hydroxycocaine + Hydroxylation	13	
3.83	C18H23NO6	350.1594	Cocaethylene + 2 x Hydroxylation; Hydroxycocaine + Hydroxylation + methylation	32	
4.13 ± 0.04	C16H19NO5	306.1333	BZE/NC + Hydroxylation	20	
4.13 ± 0.04	C17H21NO5	320.1479	BZE/NC + Hydroxylation + methylation; Cocaine + Hydroxylation	23	
4.13 ± 0.04	C18H23NO5	334.1647	Cocaethylene + Hydroxylation; Hydroxycocaine + Methylation; Cocaine + Hydroxylation + Methylation	20	
4.13 ± 0.04	C17H21NO6	336.1442	Cocaine + 2 x Hydroxylation; Hydroxycocaine + Hydroxylation	17	
4.31	**C16H19NO4**	**290.1383**	**Benzoylecgonine**	**41**	**31**

4.4	C18H23NO5	334.1647	Cocaethylene + Hydroxylation; Hydroxycocaine + Methylation; Cocaine + Hydroxylation + Methylation	11	
4.72	**C17H21NO4**	**304.1541**	**Cocaine**	**43**	**31**
4.85	**C17H21NO5**	**320.1489**	**ortho-Hydroxycocaine**	**25**	**26**
5.07	**C16H19NO4**	**290.1386**	**Norcocaine**	**30**	**21**
5.45	**C18H23NO4**	**318.1702**	**Cocaethylene**	**33**	**15**
5.75	C17H21NO4	304.1521	Norcocaethylene	4	

Opioids

Observed RT	**Predicted Formula**	**Observed m/z**	**Tentative Related Transformation or Fragmentation**	**Number of Specimens Observed**	**Number of Specimens Confirmed Positive by Reference Testing**
0.89	C17H19NO6	334.1281	Oxymorphone/Noroxycodone + 2 x Hydroxylation	13	
1.05	C17H19NO5	318.1337	Oxymorphone/Noroxycodone + Hydroxylation; Morphine/Hydromorphone + 2 x Hydroxylation	14	
1.26	C23H27NO9	462.176	**Morphine-6-glucuronide**	**2**	**n/a**
1.30	C17H19NO6	334.1285	Oxymorphone/Noroxycodone + 2 x Hydroxylation	13	
1.40	**C17H19NO3**	**286.1439**	**Morphine**	**20**	**21**
1.64	**C17H19O4**	**302.1381**	**Oxymorphone**	**23**	**8**
1.99	**C17H19NO3**	**286.1437**	**Hydromorphone**	**25**	**6**
2.13	C19H21NO5	344.1493	6-Acetylmorphine + Hydroxylation; Oxymorphone/Noroxycodone + Acetylation	19	
2.26	C18H21NO6	348.1437	Oxycodone + 2 x Hydroxylation	21	
2.51	C18H21NO3	300.1594	Morphine/Hydromorphone + Methylation	11	

2.63	C19H21NO6	360.1439	6-Acetylmorphine + 2 x Hydroxylation	26	
2.75	C24H29NO9	476.1901	**Codeine-6-glucuronide**	2	**n/a**
2.88	**C18H21NO3**	**300.1596**	**Codeine**	**23**	**12**
2.91	C18H21NO6	348.1443	Oxycodone + 2 x Hydroxylation	21	
3.08	**C18H21NO4**	**316.1543**	**Oxycodone**	**36**	**13**
3.23	**C18H21NO3**	**300.1593**	**Hydrocodone**	**28**	**11**
3.35	**C19H21NO4**	**328.1543**	**6-Acetylmorphine**	**32**	**20**
4.65	C20H23NO4	342.1701	Codeine/Hydrocodone + Acetylation; 6-Acetylmorphine + Methylation	21	
5.28	C19H23NO4	330.1698	Oxycodone + Methylation; BZE/NC + Acetylation	15	
5.43	C19H23NO4	330.1700	Oxycodone + Methylation; BZE/NC + Acetylation	20	
5.83	C19H23NO4	330.1726	Oxycodone + Methylation; BZE/NC + Acetylation	20	

Amphetamine and methamphetamine

Observed RT	Predicted Formula	Observed m/z	Tentative Related Transformation or Fragmentation	Number of Specimens Observed	Number of Specimens Confirmed Positive by Reference Testing
1.74	C12H17NO2	208.133	Hydroxymethamphetamine + Acetylation	2	
1.83	**C10H15NO**	**166.1224**	**4-hydroxymethamphetamine**	**14**	**n/a**
2.14	C11H15NO2	194.1161	Hydroxyamphetamine + Acetylation	2	
2.38	C9H13NO	152.1068	Amphetamine + Hydroxylation	2	
2.91	C10H15NO	166.1224	Amphetamine + Hydroxylation + Methylation	3	
3.11	**C9H13N**	**136.1117**	**Amphetamine**	**7**	**15**
3.30	**C10H15N**	**150.1276**	**Methamphetamine**	**19**	**14**

QQQ Tables

Cocaine

Avg RT	Type	Transition	Description of Transition	ID
1.60	NL	181.4 -> 52.3	Pyroglutamic acid	???
1.62	NL	181.3 -> 106.2	Glycine	???
1.62	NL	181.4 -> 59.5	coc to frag 182 BZE/NC to frag 168 cocaethylene to frag 196	???
1.62	NL	181.3 -> 43.3	ohcoc to frag 182	???
1.62	PreC	182.2 -> 105.1	BZE/NC frag 105	???
1.64	MRM	400.1 -> 182.1	hydoxycocaine-sulfate -> frag 182	???
2.85	NL	220.3 -> 44.3	Anhydroglucuronic-acid	???
2.85	NL	220.4 -> 91.3	Pyroglutamic acid	???
3.02	NL	202.9 -> 64.9	ohcoc to frag 182	???
3.03	NL	204.9 -> 41.9	Acetylcysteine	???
3.03	NL	206.0 -> 76.9	Pyroglutamic acid	???
3.03	PreC	205.1 -> 162.1	coc/cocaethylene frag 82 + sulfate	???
3.03	PreC	206.3 -> 121.1	ohcoc frag 121	???
3.03	PreC	205.1 -> 177	[Glu+H]+	???
3.03	NL	204.3 -> 42.3	anhydroglucose	???
3.03	NL	204.9 -> 58.8	Glutamine	???
3.04	NL	204.3 -> 129.2	Glycine	???
3.04	NL	204.9 -> 50.9	norcocaine to frag 136	???
3.04	PreC	206.3 -> 136	norcocaine frag 136	???
3.04	NL	202.9 -> 123.0	SO3	???
3.04	PreC	205.1 -> 105.1	BZE/NC frag 105	???
3.04	NL	408.4 -> 205.4	GlcNAc associated loss	???
3.51	NL	305.9 -> 120.9	BZE/NC to frag 105	P-OHBZE
3.51	NL	305.9 -> 167.9	ohcoc to frag 182	P-OHBZE
3.52	MRM	306.1 -> 168.1	hydroxyBZE -> frag 168	P-OHBZE
3.52	PreC	306.2 -> 168.2	BZE/NC frag 168	P-OHBZE
3.52	PreC	306.2 -> 121.1	ohcoc frag 121	P-OHBZE
3.52	MRM	306.1 -> 121.1	hydroxyBZE -> frag 121	P-OHBZE
3.71	PreC	320.3 -> 121.1	ohcoc frag 121	P-OHCOC REF
3.71	PreC	320.3 -> 182.2	coc/ohcoc frag 182	P-OHCOC REF
3.72	MRM	320.1 -> 121.1	hydoxycocaine -> frag 121	P-OHCOC REF
3.72	NL	319.8 -> 120.8	ohcoc to frag 121	P-OHCOC REF
3.72	MRM	320.1 -> 182.1	hydoxycocaine -> frag 182	P-OHCOC REF
3.72	PreC	321.1 -> 201.1	ohcoc frag 121 + sulfate	P-OHCOC C13
3.72	NL	319.8 -> 181.8	ohcoc to frag 182	P-OHCOC REF
3.72	PreC	320.3 -> 82.1	coc/cocaethylene frag 82	P-OHCOC REF
3.73	MRM	320.1 -> 200	hydoxycocaine -> frag 200	P-OHCOC REF

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3.74	NL	305.9 -> 120.9	BZE/NC to frag 105	M-OHBZE
3.74	PreC	306.2 -> 168.2	BZE/NC frag 168	M-OHBZE
3.75	MRM	306.1 -> 121.1	hydroxyBZE -> frag 121	M-OHBZE
3.75	MRM	306.1 -> 168.1	hydroxyBZE -> frag 168	M-OHBZE
3.97	PreC	320.3 -> 121.1	ohcoc frag 121	M-OHCOC REF
3.97	NL	319.8 -> 181.8	ohcoc to frag 182	M-OHCOC REF
3.98	MRM	320.1 -> 182.1	hydoxycocaine -> frag 182	M-OHCOC REF
3.98	NL	319.8 -> 120.8	ohcoc to frag 121	M-OHCOC REF
3.98	MRM	320.1 -> 121.1	hydoxycocaine -> frag 121	M-OHCOC REF
3.98	MRM	320.1 -> 200	hydoxycocaine -> frag 200	M-OHCOC REF
3.98	PreC	320.3 -> 82.1	coc/cocaethylene frag 82	M-OHCOC REF
3.99	PreC	320.3 -> 182.2	coc/ohcoc frag 182	M-OHCOC REF
4.04	MRM	306.1 -> 121.1	hydroxyBZE -> frag 121	???
4.04	MRM	306.1 -> 168.1	hydroxyBZE -> frag 168	???
4.26	PreC	334.3 -> 82.1	coc/cocaethylene frag 82	???
4.27	NL	333.7 -> 195.7	ohcoc to frag 182	???
4.27	PreC	334.3 -> 121.1	ohcoc frag 121	???
4.27	PreC	334.3 -> 196	cocaethylene frag 198	???
4.30	PreC	306.2 -> 136	norcocaine frag 136	???
4.30	MRM	306.1 -> 121.1	hydroxyBZE -> frag 121	???
4.31	MRM	306.1 -> 168.1	hydroxyBZE -> frag 168	???
4.51	PreC	203.1 -> 175	[Glu+H-2H]+	???
4.51	PreC	203.1 -> 121.1	ohcoc frag 121	???
4.51	PreC	203.1 -> 162.1	coc/cocaethylene frag 82 + sulfate	???
4.51	PreC	203.1 -> 177	[Glu+H]+	???
4.51	NL	202.3 -> 73.2	Pyroglutamic acid	???
4.58	MRM	306.1 -> 168.1	hydroxyBZE -> frag 168	???
4.58	MRM	306.1 -> 121.1	hydroxyBZE -> frag 121	???
4.59	PreC	320.3 -> 136	norcocaine frag 136	???
4.59	NL	333.7 -> 195.7	ohcoc to frag 182	???
4.59	PreC	334.3 -> 196	cocaethylene frag 198	???
4.59	MRM	609.2 -> 320.1	hydoxycocaine-glutathione -> hydoxycocaine	???
4.60	MRM	320.1 -> 200	hydoxycocaine -> frag 200	???
4.60	MRM	320.1 -> 182.1	hydoxycocaine -> frag 182	???
4.60	MRM	320.1 -> 121.1	hydoxycocaine -> frag 121	???
4.61	NL	319.7 -> 97.7	coc to frag 82	???
4.78	NL	218.2 -> 143.1	Glycine	???
4.78	PreC	219.1 -> 162.1	coc/cocaethylene frag 82 + sulfate	???
4.79	PreC	219.1 -> 201.1	ohcoc frag 121 + sulfate	???
4.79	PreC	219.1 -> 121.1	ohcoc frag 121	???
4.79	PreC	219.1 -> 175	[Glu+H-2H]+	???

4.80	NL	206.0 -> 76.9	Pyroglutamic acid	???
4.93	PreC	306.9 -> 185.1	BZE/NC frag 105 + sulfate	???
4.94	MRM	306.1 -> 121.1	hydroxyBZE -> frag 121	???
4.97	NL	319.8 -> 181.8	ohcoc to frag 182	O-OHCOC
4.98	PreC	321.1 -> 201.1	ohcoc frag 121 + sulfate	O-OHCOC
4.99	MRM	320.1 -> 182.1	hydoxycocaine -> frag 182	O-OHCOC
4.99	MRM	320.1 -> 121.1	hydoxycocaine -> frag 121	O-OHCOC
4.99	MRM	320.1 -> 200	hydoxycocaine -> frag 200	O-OHCOC
5.99	NL	303.7 -> 118.7	BZE/NC to frag 105	Norcocaethylene?
5.99	NL	303.7 -> 182.6	Cysteine	Norcocaethylene?
5.99	PreC	304.2 -> 136	norcocaine frag 136	Norcocaethylene?
6.00	NL	303.7 -> 181.8	coc to frag 182 BZE/NC to frag 168 cocaethylene to frag 196	Norcocaethylene?
6.00	NL	303.8 -> 67.8	cocaethylene to frag 82	Norcocaethylene?
6.00	PreC	304.2 -> 105.1	BZE/NC frag 105	???
6.01	MRM	304.1 -> 82.1	cocaine -> frag 82	Norcocaethylene?
6.01	PreC	304.2 -> 182.2	coc/ohcoc frag 182	Norcocaethylene?
6.01	PreC	318.2 -> 196	cocaethylene frag 198	Norcocaethylene?
6.01	NL	303.8 -> 104.8	ohcoc to frag 121	Norcocaethylene?
6.01	MRM	304.1 -> 182.2	cocaine -> frag 182	Norcocaethylene?
6.04	MRM	480.1 -> 182.2	cocaine-gluc -> frag 182	Coc-gluc?
6.23	MRM	480.1 -> 182.2	cocaine-gluc -> frag 182	???
6.25	MRM	480.1 -> 82.1	cocaine-gluc -> frag 82	???

Amphetamine and methamphetamine

Avg RT	Type	Transition	Description of Transition	ID
0.62	NL	149.9 -> 28.9	Cysteine	???
0.64	NL	112.2 -> 95.2	AMP to frag 119 hydroxyamp to frag 135	???
0.65	NL	117.3 -> 58.3	MAMP to frag 91 hydroxymamp to frag 107	???
0.68	NL	235.9 -> 28.9	hydroxymamp gluc to frag 135	???
0.72	PreC	150.0 -> 119.1	amp/mamp frag 119	???
0.83	PreC	151.9 -> 135.1	hydroxyamp frag 135	AMP-OH
0.84	MRM	152.1 -> 135.1	hydroxyamp -> frag 135	AMP-OH
0.84	NL	133.3 -> 74.3	MAMP to frag 91 hydroxymamp to frag 107	???
0.96	NL	138.2 -> 93.2	AMP to frag 91 hydroxyamp to frag 107	???
0.96	NL	138.2 -> 121.2	AMP to frag 119 hydroxyamp to frag 135	???
1.12	NL	162.3 -> 82.3	SO3	???
1.13	NL	162.3 -> 117.3	AMP to frag 91 hydroxyamp to frag 107	???
1.13	NL	161.3 -> 130.3	MAMP to frag 119, hydroxymamp to frag 135	???
1.14	NL	162.2 -> 103.2	MAMP to frag 91 hydroxymamp to frag 107	???

1.14	NL	162.6 -> 41.6	Cysteine	???
1.18	NL	112.2 -> 95.2	AMP to frag 119 hydroxyamp to frag 135	???
1.19	NL	262.4 -> 86.4	Anhydroglucuronic-acid	???
1.24	NL	230.1 -> 84.0	Glutamine	???
1.30	NL	173.7 -> 44.7	Pyroglutamic acid	???
1.34	PreC	182.0 -> 136.1	amphetamine	???
1.34	MRM	136.1 -> 119.1	AMP -> frag 119	???
1.43	NL	159.7 -> 30.7	Pyroglutamic acid	???
1.43	NL	159.8 -> 100.8	MAMP to frag 91 hydroxymamp to frag 107	???
1.48	NL	95.3 -> 78.3	AMP to frag 119 hydroxyamp to frag 135	???
1.56	PreC	150.2 -> 91.0	amp/mamp frag 91	???
1.56	NL	122.8 -> 42.9	SO3	???
1.59	NL	313.1 -> 110.1	GlcNAc associated loss	???
1.72	MRM	136.1 -> 119.1	AMP -> frag 119	???
1.73	PreC	150.2 -> 91.0	amp/mamp frag 91	???
1.73	PreC	136.0 -> 119.1	amp/mamp frag 119	???
1.76	NL	330.0 -> 135.9	Glucuronic-acid	???
1.77	NL	329.3 -> 136.3	hydroxyamp gluc to frag 135	???
1.83	MRM	152.1 -> 135.1	hydroxyamp -> frag 135	AMP-OH
1.93	NL	99.8 -> 54.8	AMP to frag 91 hydroxyamp to frag 107	???
1.99	PreC	166.1 -> 107.1	hydroxyamp frag 107	MAMP-OH
2.19	NL	121.3 -> 41.3	SO3	???
2.26	PreC	166.1 -> 107.1	hydroxyamp frag 107	4OH-MAMP REF
2.27	MRM	166.1 -> 135.1	hydroxyMAMP -> frag 135	4OH-MAMP REF
2.27	MRM	166.1 -> 107.1	hydroxyMAMP -> frag 107	4OH-MAMP REF
2.48	MRM	152.1 -> 107.1	hydroxyamp -> frag 107	???
2.77	MRM	166.1 -> 107.1	hydroxyMAMP -> frag 107	MAMP-OH
2.77	MRM	166.1 -> 135.1	hydroxyMAMP -> frag 135	MAMP-OH
2.78	PreC	166.1 -> 107.1	hydroxyamp frag 107	MAMP-OH
3.07	NL	327.4 -> 124.4	GlcNAc associated loss	???
3.25	PreC	328.5 -> 136.1	amphetamine	???
3.25	NL	329.0 -> 126.0	GlcNAc associated loss	???
3.33	NL	237.1 -> 44.1	hydroxyamp gluc to frag 135	???
3.34	PreC	150.2 -> 91.0	amp/mamp frag 91	???
3.36	MRM	166.1 -> 135.1	hydroxyMAMP -> frag 135	???
3.40	MRM	152.1 -> 107.1	hydroxyamp -> frag 107	???
3.40	MRM	152.1 -> 135.1	hydroxyamp -> frag 135	???
3.42	MRM	342.1 -> 283.1	hydroxyMAMP-gluc -> frag 107-gluc	???
3.79	NL	188.4 -> 157.4	MAMP to frag 119, hydroxymamp to frag 135	MAMP-OH Na+
3.81	MRM	166.1 -> 107.1	hydroxyMAMP -> frag 107	MAMP-OH
3.81	MRM	166.1 -> 135.1	hydroxyMAMP -> frag 135	MAMP-OH

3.81	PreC	166.1 -> 107.1	hydroxyamp frag 107	MAMP-OH
3.82	NL	204.2 -> 129.1	Glycine	???
3.85	PreC	205.3 -> 91.0	amp/mamp frag 91	???
3.85	PreC	205.2 -> 135.1	hydroxyamp frag 135	???
3.91	NL	193.9 -> 64.8	Pyroglutamic acid	???
4.02	NL	321.8 -> 246.7	Glycine	???
4.03	NL	321.3 -> 262.3	MAMP to frag 91 hydroxymamp to frag 107	???
4.51	MRM	328.1 -> 152.1	hydroxyamp-gluc -> hydroxyamp	???
4.71	NL	191.1 -> 160.1	MAMP to frag 119, hydroxymamp to frag 135	???
4.89	NL	156.6 -> 76.7	SO3	???
4.90	MRM	152.1 -> 135.1	hydroxyamp -> frag 135	???
5.16	NL	210.8 -> 81.7	Pyroglutamic acid	???
5.24	NL	234.3 -> 58.3	Anhydroglucuronic-acid	???
5.83	NL	211.8 -> 49.8	anhydroglucose	???
5.95	NL	289.3 -> 168.3	Cysteine	???
5.95	MRM	439.2 -> 150.1	MAMP-glutathione -> MAMP	???
5.96	NL	290.0 -> 83.0	hydroxymamp gluc to frag 135	???
5.96	PreC	290.1 -> 150.1	methamphetamine	???
5.97	MRM	150.1 -> 91.0	MAMP -> frag 91	???
6.05	MRM	136.1 -> 119.1	AMP -> frag 119	???
6.10	MRM	150.1 -> 91.0	MAMP -> frag 91	???
6.17	MRM	230.1 -> 91.0	MAMP-sulfate -> frag 91	???
6.18	NL	313.1 -> 110.1	GlcNAc associated loss	???
6.21	MRM	342.1 -> 283.1	hydroxyMAMP-gluc -> frag 107-gluc	???
6.23	NL	305.0 -> 70.0	hydroxymamp gluc to frag 107	???
6.24	NL	303.4 -> 82.4	hydroxyamp gluc to frag 107	???
6.24	NL	303.3 -> 182.2	Cysteine	???
6.24	NL	303.5 -> 82.4	N-acetylglucosamine (GlcNAc)	???
6.25	NL	303.9 -> 96.9	hydroxymamp gluc to frag 135	???
6.27	NL	211.4 -> 65.4	Glutamine	???
6.28	NL	301.9 -> 98.9	GlcNAc associated loss	???
6.31	NL	265.9 -> 71.9	Glucuronic-acid	???
6.32	MRM	230.1 -> 199.1	MAMP-sulfate -> frag 119-sulfate	???
6.32	MRM	232.1 -> 187.1	hydroxyamp-sulfate -> frag 107-sulfate	???
6.33	NL	149.7 -> 90.7	MAMP to frag 91 hydroxymamp to frag 107	???
6.34	MRM	136.1 -> 91.0	AMP -> frag 91	???
6.36	MRM	326.1 -> 150.1	MAMP-gluc -> MAMP	???
6.37	MRM	232.1 -> 107.1	hydroxyamp-sulfate -> frag 107	???
6.38	MRM	326.1 -> 267.0	MAMP-gluc -> frag 91-gluc	???
6.38	NL	349.1 -> 114.1	hydroxymamp gluc to frag 107	???
6.39	MRM	152.1 -> 135.1	hydroxyamp -> frag 135	???

6.40	NL	185.8 -> 56.8	Pyroglutamic acid	???
6.41	MRM	150.1 -> 91.0	MAMP -> frag 91	???
6.43	MRM	230.1 -> 91.0	MAMP-sulfate -> frag 91	???
6.43	MRM	328.1 -> 283.1	hydroxyamp-gluc -> frag 107-gluc	???
6.45	NL	206.3 -> 43.3	Acetylcysteine	???
6.45	NL	274.0 -> 39.0	hydroxymamp gluc to frag 107	???
6.47	NL	380.0 -> 72.9	Glutathione	???
6.48	NL	379.9 -> 172.9	hydroxymamp gluc to frag 135	???
6.49	NL	268.0 -> 73.9	Glucuronic-acid	???
6.49	NL	267.8 -> 91.8	Anhydroglucuronic-acid	???

Opioids

Avg RT	Type	Transition	Description of Transition	ID
0.59	PreC	320.2 -> 177.0	[Glu+H]+	???
0.67	NL	281.6 -> 133.5	Codeine to frag 152	???
0.73	MRM	302.2 -> 199.1	dihydrocodeine -> frag 199	???
0.74	MRM	302.1 -> 227.2	noroxycodone -> frag 227	???
0.74	MRM	302.2 -> 128.1	dihydrocodeine -> frag 128	???
0.77	MRM	302.1 -> 284.2	oxymorphone -> frag 284	???
0.82	MRM	462.2 -> 286.2	morphine + gluc -> morphine	MOR-3-GLUC REF
0.86	NL	397.0 -> 175.9	N-acetylglucosamine (GlcNAc)	???
0.86	PreC	483.8 -> 177.0	[Glu+H]+	???
0.87	NL	483.3 -> 176.2	Glutathione	???
0.93	NL	162.9 -> 42.0	Morphine to frag 165	???
0.94	NL	162.0 -> 82.1	SO3	???
0.94	NL	163.8 -> 83.9	SO3	???
1.02	NL	225.9 -> 125.0	hydromorphone to frag 185	???
1.03	MRM	302.2 -> 199.1	dihydrocodeine -> frag 199	???
1.03	MRM	302.2 -> 128.1	dihydrocodeine -> frag 128	???
1.04	MRM	302.1 -> 227.2	noroxycodone -> frag 227	???
1.07	NL	160.0 -> 59.1	hydromorphone to frag 185	???
1.10	NL	261.0 -> 85.1	6am to frag 152	???
1.11	MRM	302.1 -> 284.2	oxymorphone -> frag 284	???
1.15	NL	313.8 -> 137.9	6am to frag 152	???
1.17	MRM	300.2 -> 199.1	hydrocodone -> frag 199	???
1.17	NL	313.8 -> 192.9	Morphine to frag 165 (Cysteine)	???
1.19	MRM	462.2 -> 286.2	morphine + gluc -> morphine	MOR-6-CLUC REF
1.25	PreC	488.2 -> 308.0	[GSH+H]+	???
1.25	PreC	288.1 -> 175.0	[Glu+H-2H]+	isotope
1.26	NL	449.3 -> 286.4	6am to frag 165 (Acetylcysteine)	???
1.27	NL	308.3 -> 87.2	N-acetylglucosamine (GlcNAc)	MOR + Na?

1.28	MRM	288.2 -> 185.1	dihydromorphine -> frag 185	isotope
1.28	MRM	288.2 -> 157.1	dihydromorphine -> frag 157	isotope
1.46	NL	213.0 -> 83.9	Pyroglutamic acid & hydromorphone to frag 157	
1.47	NL	220.2 -> 91.1	Pyroglutamic acid & hydromorphone to frag 157	
1.47	MRM	302.2 -> 128.1	dihydrocodeine -> frag 128	???
1.47	MRM	302.1 -> 227.2	noroxycodone -> frag 227	OXM REF
1.47	MRM	302.1 -> 284.2	noroxycodone -> frag 284	OXM REF
1.48	MRM	302.2 -> 199.1	dihydrocodeine -> frag 199	???
1.54	MRM	302.1 -> 227.2	noroxycodone -> frag 227	OXM REF
1.55	PreC	286.3 -> 152.1	morphine/codeine frag 152	???
1.55	MRM	300.2 -> 128.1	hydrocodone -> frag 128	???
1.55	MRM	302.1 -> 284.2	noroxycodone -> frag 284	OXM REF
1.55	MRM	300.2 -> 152.1	codeine -> frag 152	???
1.55	MRM	300.2 -> 165.1	codeine -> frag 165	???
1.60	MRM	286.2 -> 152.1	morphine -> frag 152	???
1.61	MRM	286.2 -> 165.1	morphine -> frag 165	???
1.86	NL	285.8 -> 184.9	hydromorphone to frag 185	???
1.86	NL	285.8 -> 156.7	Pyroglutamic acid & hydromorphone to frag 157	HYM REF
1.86	PreC	286.3 -> 165.2	morphine/codeine frag 165	HYM REF
1.87	PreC	286.3 -> 152.1	morphine/codeine frag 152	HYM REF
1.91	MRM	286.2 -> 152.1	morphine -> frag 152	HYM REF
1.91	MRM	286.2 -> 165.1	morphine -> frag 165	HYM REF
1.92	MRM	288.2 -> 157.1	dihydromorphine -> frag 157	HYM C13
1.92	MRM	286.2 -> 157.1	hydromorphone -> frag 157	HYM REF
1.93	MRM	288.2 -> 185.1	dihydromorphine -> frag 185	HYM C13
1.93	MRM	286.2 -> 185.1	hydromorphone -> frag 185	HYM REF
2.00	NL	231.2 -> 85.1	Glutamine	???
2.05	NL	269.4 -> 121.3	Codeine to frag 152	???
2.05	NL	191.1 -> 43.0	Codeine to frag 152	???
2.15	MRM	366.1 -> 152.1	morphine + sulfate -> frag 152	MOR-SULF
2.15	MRM	366.1 -> 165.1	morphine + sulfate -> frag 165	MOR-SULF
2.15	MRM	366.1 -> 245.2	morphine + sulfate -> frag 165 + sulfate	MOR-SULF
2.24	MRM	316.2 -> 241.1	oxycodone -> frag 241	???
2.24	MRM	316.2 -> 298.3	oxycodone -> frag 298	???
2.52	MRM	288.2 -> 185.1	dihydromorphine -> frag 185	???
2.52	MRM	288.2 -> 157.1	dihydromorphine -> frag 157	???
2.55	NL	199.0 -> 52.9	Glutamine	???
2.64	NL	199.0 -> 52.9	Glutamine	???
2.84	MRM	302.1 -> 284.2	oxymorphone -> frag 284	???
2.86	MRM	302.2 -> 128.1	dihydrocodeine -> frag 128	???
2.87	MRM	302.1 -> 227.2	oxymorphone -> frag 227	???

2.93	MRM	316.2 -> 241.1	oxycodone -> frag 241	???
2.95	NL	327.7 -> 124.7	GlcNAc associated loss	???
3.07	MRM	300.2 -> 165.1	codeine -> frag 165	???
3.07	MRM	300.2 -> 152.1	codeine -> frag 152	???
3.07	MRM	300.2 -> 128.1	hydrocodone -> frag 128	???
3.08	MRM	300.2 -> 199.1	hydrocodone -> frag 199	???
3.21	NL	327.7 -> 124.7	GlcNAc associated loss	???
3.21	MRM	300.2 -> 152.1	codeine -> frag 152	???
3.21	MRM	300.2 -> 165.1	codeine -> frag 165	???
3.22	NL	327.7 -> 193.6	Morphine to frag 152	???
3.22	MRM	300.2 -> 128.1	hydrocodone -> frag 128	???
3.27	PreC	498.2 -> 470.3	morphine/codeine frag 152 + S-glutathione	???
3.63	NL	247.0 -> 117.9	Pyroglutamic acid & hydromorphone to frag 157	???
3.69	MRM	476.2 -> 300.2	codeine + gluc -> codeine	COD-6-GLUC REF
3.72	MRM	286.2 -> 152.1	morphine -> frag 152	???
3.72	MRM	286.2 -> 165.1	morphine -> frag 165	???
3.77	NL	204.2 -> 42.2	anhydroglucose	???
3.78	NL	205.9 -> 76.8	Pyroglutamic acid & hydromorphone to frag 157	???
3.79	NL	203.3 -> 123.4	SO3	???
3.79	NL	470.4 -> 267.4	GlcNAc associated loss	???
3.79	NL	205.1 -> 59.0	Glutamine	???
3.79	NL	205.1 -> 42.2	6am to frag 165	???
3.79	NL	204.2 -> 103.3	hydromorphone to frag 185	???
3.82	NL	299.6 -> 164.5	Codeine to frag 165	???
3.82	MRM	328.2 -> 152.1	6-acetylmorphine -> frag 152	???
3.82	PreC	300.3 -> 165.2	morphine/codeine frag 165	???
3.82	NL	299.6 -> 165.5	Morphine to frag 152	???
3.83	PreC	300.3 -> 152.1	morphine/codeine frag 152	???
3.84	MRM	302.1 -> 227.2	noroxycodone -> frag 227	DIHYDROCOD REF
3.84	MRM	302.2 -> 199.1	dihydrocodeine -> frag 199	DIHYDROCOD REF
3.85	MRM	302.2 -> 128.1	dihydrocodeine -> frag 128	DIHYDROCOD REF
4.08	MRM	328.2 -> 152.1	6am -> frag 152	???
4.09	MRM	328.2 -> 165.1	6am -> frag 165	???
4.12	NL	264.8 -> 43.7	N-acetylglucosamine (GlcNAc)	???
4.19	PreC	316.3 -> 175.0	[Glu+H-2H]+	OXYC REF
4.19	MRM	316.2 -> 241.1	oxycodone -> frag 241	OXYC REF
4.20	MRM	316.2 -> 298.3	oxycodone -> frag 298	OXYC REF
4.22	NL	206.4 -> 58.3	Codeine to frag 152	???
4.25	MRM	302.1 -> 227.2	noroxycodone -> frag 227	NOROXYC REF
4.25	MRM	302.1 -> 284.2	noroxycodone -> frag 284	NOROXYC REF
4.48	NL	234.2 -> 99.1	Codeine to frag 165	???

4.48	NL	299.6 -> 198.7	hydromorphone to frag 185	???
4.48	MRM	328.2 -> 152.1	6am -> frag 152	???
4.51	MRM	316.2 -> 241.1	oxycodone -> frag 241	???
4.51	MRM	286.2 -> 165.1	morphine -> frag 165	???
4.52	MRM	286.2 -> 152.1	morphine -> frag 152	???
4.54	MRM	286.2 -> 185.1	hydromorphone -> frag 185	???
4.54	PreC	575.7 -> 245.1	morphine/codeine frag 152 + sulfate	???
4.60	NL	341.4 -> 193.3	Codeine to frag 152	???
4.71	NL	327.7 -> 164.8	6am to frag 165	6AM REF
4.72	PreC	328.3 -> 177.0	[Glu+H]+	6AM REF
4.72	PreC	328.3 -> 232.1	morphine/codeine frag 165 + sulfate	6AM REF
4.72	NL	327.7 -> 165.7	anhydroglucose	6AM REF
4.72	NL	327.7 -> 193.6	Morphine to frag 152	6AM REF
4.72	NL	327.7 -> 179.6	Codeine to frag 152	6AM REF
4.72	MRM	286.2 -> 152.1	morphine -> frag 152	???
4.72	MRM	476.2 -> 328.2	codeine + gluc -> frag 152 + gluc	not 6AM
4.72	NL	327.7 -> 206.8	Morphine to frag 165 (Cysteine)	6AM REF
4.72	NL	327.7 -> 192.6	Codeine to frag 165	6AM REF
4.72	PreC	328.3 -> 165.2	morphine/codeine frag 165	6AM REF
4.72	PreC	328.3 -> 152.1	morphine/codeine frag 152	6AM REF
4.72	PreC	328.3 -> 286.1	morphine	6AM REF
4.73	NL	327.7 -> 198.6	Pyroglutamic acid & hydromorphone to frag 157	6AM REF
4.73	NL	327.7 -> 151.8	6am to frag 152	6AM REF
4.73	NL	327.7 -> 226.8	hydromorphone to frag 185	6AM REF
4.73	PreC	328.3 -> 175.0	[Glu+H-2H]+	6AM REF
4.73	NL	327.7 -> 133.6	Glucuronic-acid	6AM REF
4.73	NL	327.7 -> 181.6	Glutamine	6AM REF
4.74	MRM	286.2 -> 165.1	morphine -> frag 165	???
4.74	MRM	316.2 -> 241.1	oxycodone -> frag 241	???
4.74	NL	327.7 -> 106.6	N-acetylglucosamine (GlcNAc)	6AM REF
4.75	MRM	328.2 -> 165.1	6am -> frag 165	6AM REF
4.75	MRM	328.2 -> 152.1	6-acetylmorphine -> frag 152	6AM REF
4.76	MRM	300.2 -> 199.1	hydrocodone -> frag 199	???
4.76	MRM	302.2 -> 128.1	dihydrocodeine -> frag 128	???
4.76	MRM	302.2 -> 199.1	dihydrocodeine -> frag 199	???
4.77	MRM	302.1 -> 227.2	noroxycodone -> frag 227	???
5.12	MRM	328.2 -> 152.1	6-acetylmorphine -> frag 152	???
5.15	MRM	300.2 -> 128.1	hydrocodone -> frag 128	???
5.24	MRM	366.1 -> 165.1	morphine + sulfate -> frag 165	???
5.25	MRM	366.1 -> 152.1	morphine + sulfate -> frag 152	???
5.29	NL	234.2 -> 86.1	Codeine to frag 152	???

5.32	PreC	344.4 -> 152.1	morphine/codeine frag 152	???
5.66	MRM	328.2 -> 152.1	6am -> frag 152	???
5.93	MRM	328.2 -> 152.1	6am -> frag 152	???
6.42	MRM	575.2 -> 286.2	morphine + glutathione -> morphine	???
6.42	MRM	589.2 -> 300.2	codeine + glutathione -> codeine	???