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Development of a liquid chromatography–tandem mass spectrometry method to address the increased utilization of umbilical cord in the assessment of in utero drug exposure

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ABSTRACT

Objectives: The method described supports the detection of drugs and drug metabolites in the assessment of in utero drug exposure. The presented method employs liquid chromatography–tandem mass spectrometry (LC–MS/MS) as an alternative to a previously validated method using liquid chromatography-time-of-flight mass spectrometry (LC-TOF/MS). A reduction in required chromatographic time and consolidation from two injections to one injection per sample was desired to reduce turnaround time while maintaining the high specificity required.

Design and methods: Homogenized and extracted umbilical cord samples were analyzed using LC-TOF/MS and LC–MS/MS. The LC–MS/MS chromatographic method used a 3.5 min gradient with an injection-to-injection time of 5 min. Dynamic multiple reaction monitoring was utilized.

Results: A 55% reduction in total analytical time was achieved by incorporating positive and negative mode acquisition in a single injection with the LC–MS/MS (5 min cycle time) compared to the LC-TOF/MS method that required two total injections (one for positive mode, one for negative mode) and a combined ~11.5 min cycle time. 514 patient samples were analyzed by both methods over 20 days. Of the 260 LC-TOF/MS negative samples, 259 were LC–MS/MS negative. Inter-assay imprecision conducted over 20 days using the 50% and 150% QC samples yielded $_amp_$gt;97% qualitative acceptance and an average percent deviation from the target of 12% and 21%, respectively, using a single point calibration strategy.

Conclusions: In comparison to the existing LC-TOF/MS method, the LC–MS/MS method delivered the required specificity in a single injection with a 55% reduction in instrument time. Use of a single-point calibration standard and eight representative internal standards provided adequate accuracy for the quantitative assessment of quality control results with qualitative reporting of patient results.

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1. Introduction

The assessment of in utero drug exposure using umbilical cord tissue as an alternative to meconium provides advantages including ease of collection and larger specimen volume. We previously reported a method for the measurement of 57 drugs and metabolites using liquid chromatography-time-of-flight mass spectrometry (LC-TOF/MS) [1]. LC-TOF/MS uses chromatographic retention time, measured mass errors routinely less than 10 ppm, and isotope ratios to identify compounds. Because of the lack of fragmentation with this method, compounds with identical masses are indistinguishable unless chromatographically separated. In situations where turnaround time is of minimal concern, extended chromatographic methods easily allow for separation of these isobaric compounds. However, time to results is critical to the area of in utero drug exposure. The availability of the umbilical cord at birth potentially allows the incorporation of timely results into discharge planning. Because the high specificity of our previously developed LC-TOF/MS method was dependent on an extended chromatographic separation and two injections, we explored the use of tandem mass spectrometry (LC–MS/MS) with a single injection as an alternative technique to reduce in-laboratory turnaround time while maintaining the high specificity needed.

Abbreviations: LC-TOF/MS, liquid chromatography-time of flight/mass spectrometry; LC–MS/MS, liquid chromatography–tandem mass spectrometry; QC, quality control.

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2. Materials and methods

2.1. Patients

514 individual umbilical cord samples sent to ARUP Laboratories for routine testing by LC–MS/TOF were de-identified for analysis by LC–MS/MS in compliance with University of Utah Institutional Review Board (IRB #00007275) with internally assigned tracking numbers used for comparison studies.

2.2. Specimens

Umbilical cords were stored refrigerated (2–8 °C) until testing. Samples were extracted and then analyzed by LC-TOF/MS and residual extracts were subsequently analyzed by LC–MS/MS immediately or stored frozen at −80 °C until testing.

2.3. Chemicals, reagents, and solutions

J.T. Baker methanol, acetonitrile, isopropanol, formic acid and ammonium formate were purchased from VWR (Radnor, PA). Clinical Laboratory Reagent Water (CLRW) was from a Barnstead Nanopure Diamond System (Waltham, MA). Stock standards were purchased from Cerilliant (Round Rock, Texas). Calibrators and controls were prepared in methanol and added to drug free umbilical cord matrix generated in house from negative specimens.

Table 1
Included drugs and metabolites with associated internal standard, average retention time (RT) over 3 lots of columns, quantifier and qualifier transitions and interassay imprecision at for the 50% and 150% controls. Presented cutoffs are in ng/g. Retention times (RT) are in minutes.

Compound	Internal standard	Precursor → quant/qual	RT	Cutoff	CV 50%	CV 150%
2-Hydroxyethyl-Flurazepam	a-OH-alprazolam-d5	333.1 → 194.0/109.1	3.03	10	18.3%	17.5%
6-Acetylmorphine	6-Acetylmorphine-d6	328.2 → 211.0/165.0	1.55	4	23.1%	20.4%
7-Amino-clonazepam	Meperidine-d4	286.1 → 222.1/121.1	2.19	5	28.3%	30.8%
7-Amino-flunitrazepam	Meperidine-d4	284.1 → 227.1/135.1	2.37	5	26.0%	29.6%
a-OH-alprazolam	a-OH-alprazolam-d5	325.1 → 297.0/216.0	2.99	5	11.8%	11.0%
a-OH-midazolam	a-OH-alprazolam-d5	342.1 → 324.0/203.0	2.93	5	19.6%	17.1%
Alprazolam	a-OH-alprazolam-d5	309.1 → 281.0/205.0	3.11	5	16.8%	18.6%
Amphetamine	Methamphetamine-d5	136.1 → 119.1/91.1	1.42	8	30.1%	27.3%
Benzoylecgonine	Benzoylecgonine-d3	290.1 → 168.1/77.2	1.96	8	24.8%	24.7%
Buprenorphine	Meperidine-d4	468.3 → 83.2/55.2	2.24	2	32.1%	43.3%
Buprenorphine glucuronide	Meperidine-d4	644.4 → 468.2/55.2	2.60	8	58.8%	23.2%
Butalbital	Phenobarbital-d5	223.1 → 180.1/42.2	2.50	75	21.8%	47.5%
Clonazepam	a-OH-alprazolam-d5	316.1 → 134.0/109.0	2.58	5	44.9%	26.5%
Cocaethylene	Meperidine-d4	318.2 → 196.1/82.2	2.29	8	26.3%	28.2%
Cocaine	Meperidine-d4	304.2 → 182.1/82.2	2.09	8	28.1%	27.0%
Codeine	6-Acetylmorphine-d6	300.2 → 165.0/153.1	1.45	6	28.2%	18.0%
Desalkylflurazepam	a-OH-alprazolam-d5	289.1 → 226.0/140.0	3.06	10	18.4%	23.0%
Diazepam	Diazepam-d5	285.1 → 193.0/154.0	3.33	5	23.3%	24.5%
Dihydrocodeine	6-Acetylmorphine-d6	302.2 → 199.0/128.0	1.43	4	26.9%	28.6%
EDDP	Meperidine-d4	279.2 → 250.1/235.1	2.59	10	41.2%	26.3%
Fentanyl	Meperidine-d4	337.2 → 188.0/105.0	2.50	1	28.6%	16.6%
Flunitrazepam	a-OH-alprazolam-d5	314.1 → 268.0/239.0	3.07	5	19.7%	44.7%
Flurazepam	a-OH-alprazolam-d5	388.2 → 317.0/315.0	2.58	5	45.0%	33.1%
Hydrocodone	6-Acetylmorphine-d6	300.2 → 199.0/128.0	1.60	6	31.7%	26.6%
Hydromorphone	Morphine-d3	286.2 → 185.0/157.0	1.15	4	26.3%	25.3%
Lorazepam	a-OH-alprazolam-d5	321.0 → 302.9/275.0	2.98	5	25.7%	29.1%
MDA	Methamphetamine-d5	180.1 → 163.0/105.1	1.50	8	28.8%	33.0%
MDEA	Methamphetamine-d5	208.1 → 163.0/105.1	1.74	8	31.3%	43.3%
MDMA	Methamphetamine-d5	194.1 → 163.0/105.1	1.60	8	29.3%	21.9%
Meperidine	Meperidine-d4	248.2 → 220.1/174.1	2.10	2	21.9%	26.6%
Methadone	a-OH-alprazolam-d5	310.2 → 265.1/105.1	2.84	10	46.2%	20.5%
Methamphetamine	Methamphetamine-d5	150.1 → 119.1/NA	1.54	8	22.6%	25.4%
m-Hydroxy-benzoylecgonine	Benzoylecgonine-d3	306.1 → 168.1/121.0	1.73	8	20.4%	21.5%
Midazolam	a-OH-alprazolam-d5	326.1 → 291.1/223.0	2.77	5	28.5%	21.2%
Morphine	Morphine-d3	286.2 → 165.1/152.0	1.00	4	21.7%	27.7%
Naloxone	6-Acetylmorphine-d6	328.2 → 310.1/212.0	1.40	8	28.1%	25.2%
Naltrexone	6-Acetylmorphine-d6	342.2 → 270.0/55.2	1.54	8	26.1%	37.1%
N-Desmethyl-tramadol	6-Acetylmorphine-d6	250.2 → 232.1/44.3	2.01	2	37.2%	31.5%
Nitrazepam	a-OH-alprazolam-d5	282.1 → 236.0/180.0	2.97	5	23.0%	23.4%
Nordiazepam	Diazepam-d5	271.1 → 165.0/140.0	3.17	5	26.5%	24.5%
O-Desmethyl-tramadol	6-Acetylmorphine-d6	250.2 → 58.2/NA	1.57	2	29.6%	25.8%
Oxazepam	a-OH-alprazolam-d5	287.1 → 269.0/241.0	2.99	5	24.8%	28.9%
Oxycodone	6-Acetylmorphine-d6	316.2 → 298.1/241.0	1.56	4	30.5%	24.9%
Oxymorphone	Morphine-d3	302.1 → 284.1/227.0	1.07	4	27.5%	26.1%
PCP	Meperidine-d4	244.2 → 159.1/86.2	2.41	4	27.5%	23.6%
Phenobarbital	Phenobarbital-d5	231.1 → 85.0/42.2	2.33	75	23.9%	24.8%
Phentermine	Methamphetamine-d5	150.1 → 133.1/105.1	1.64	8	21.7%	31.6%
Propoxyphene	a-OH-alprazolam-d5	340.2 → 266.1/58.2	2.76	10	45.9%	51.2%
Secobarbital	Phenobarbital-d5	237.1 → 194.1/42.2	2.80	75	19.0%	18.9%
Tapentadol	Benzoylecgonine-d3	222.2 → 107.1/77.2	1.99	2	37.4%	28.4%
Temazepam	Diazepam-d5	301.1 → 283.0/255.0	3.16	5	25.7%	27.9%
Tramadol	Benzoylecgonine-d3	264.2 → 58.2/NA	1.97	2	32.8%	23.2%
Triazolam	a-OH-alprazolam-d5	343.1 → 308.1/239.0	3.07	5	18.1%	15.9%
Zolpidem	Meperidine-d4	308.2 → 235.0/92.1	2.29	10	28.4%	33.9%

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