Drug of abuse quantitation in hair using LC-MS/MS

Xiaolei Xie, Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA 95134, USA

ABSTRACT

Purpose: To demonstrate a simple and fast pre-treatment method, in combination with the Thermo Scientific™ TSQ Quantis™ mass spectrometer (MS), for quantitative detection of 16 drugs in hair, which can meet the high efficiency and high sensitivity requirements in forensic toxicology laboratories.

Methods: 0.2 g hair was cut and grounded after being washed twice by 5 mL water and 5 mL acetone. 0.1 g±0.001g grounded hair was weighed. The analytes were extracted by sonication in 1 mL mixture of methanol and acetonitrile. After centrifugation, the supernatant was diluted with water and injected into LC-MS/MS. The Phenyl Hexyl column was used to separate compounds with a 15.5 min LC gradient. Triple quadruple mass spectrometer was used for quantification under SRM mode. Data was analyzed by quantification software.

Results: We evaluated two different grounding methods and several different extraction solvents. The whole extraction process took less than 30 minutes. The method had baseline chromatographic separation for all compounds and achieved desirable limit of quantification. Our method demonstrated the lower limit of quantification ranged from 2 ng/g to 10 ng/g with good linearity up to 2 mg/g for the monitored compounds. We also evaluated method reproducibility with the RSD% from 1.4-7.6%.

INTRODUCTION

In forensic toxicology laboratories, rapid and accurate results are very beneficial to advance case progress and are quite in line with the needs of the first-line police. Although urine and blood are routine matrixes for drug detection and analysis, it is difficult to use them as a source of long-term drug use information. The drug components in these two matrixes are easy to degrade due to metabolic rate. In contrast, hair is easier to be obtained and stored. In addition, the inherent growth rate and structure of hair can ensure the long-term stability of drug residues, making hair as testing matrix for history of long-term drug use. According to the growth rate of the hair, it is also possible to evaluate the first/long-term drug use, and roughly estimate the time of the last drug use. This work investigated two different grounding methods, several different extraction solvents, and finally developed an easy and quick LC-MS/MS method to quantify 16 drugs of abuse in hair.

MATERIALS AND METHODS

Sample Preparation

Hair samples were washed twice in oscillation with appropriate amount of water and acetone in sequence. 0.1 g was accurately weighted, and chopped into a 2 mL centrifuge tube. After adding two porcelain beads, it was grinded twice at 20 Hz for 3 min. 1 mL of 0.2% formic acid in methanol and acetonitrile (50:50, v:v) was added. After sonicating for 5 min, The samples were centrifuged at 8,000 rpm for 5 min. 0.5 mL supernatants were added to 0.5 mL water, and filtered through 0.22 µm polyethersulfone Microfiltration membrane.

Liquid Chromatography

5 uL was injected onto a 2.1 x 100 mm, 2.6 µm Thermo Scientific Accucore™ Phenyl Hexyl Column, which was thermostatted at 30° C. Compound separation was accomplished with the Thermo Scientific Vanquish™ UHPLC system using a binary reverse-phase gradient as shown in Table 1. Mobile phases were (A): 0.1% formic acid, 2 mM aqueous ammonium formate; mobile phase (B): 0.1% formic acid, 2 mM ammonium formate, 1% water, 50/50 methanol acetonitrile; LC effluent was diverted to waste until after the column void to prevent salts from fouling the ion source.

Table 1: LC Gradient

Time (min)	Flow (ml/min)	A (%)	B (%)
0	0.3	99	1
1.0	0.3	99	1
10.0	0.3	1	99
11.5	0.3	1	99
11.6	0.3	99	1
15.5	0.3	99	1

Mass Spectrometry

Electrospray ion source (ESI); ionization mode: Positive ion mode; monitoring mode: Select Reaction Monitoring (SRM); Spray Voltage: 3000 V; sheath gas pressure: 40 Arb; auxiliary gas pressure: 15 Arb; evaporation temperature: 325 C; ion transfer tube temperature: 325 C; collision gas pressure: 1.5 mTorr

RESULTS

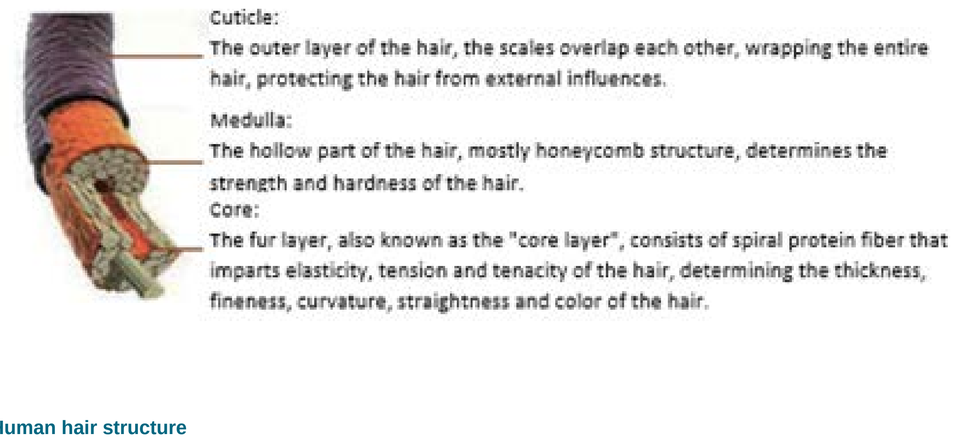

Figure 1: Human hair structure

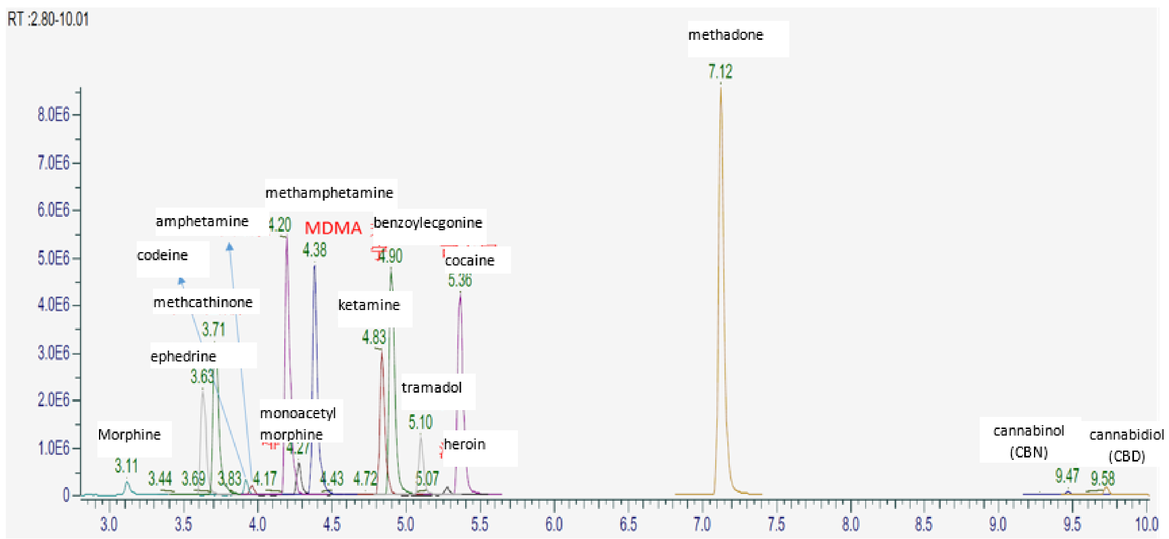

Figure 2: Extracted ion currents diagram for these 16 drug compounds

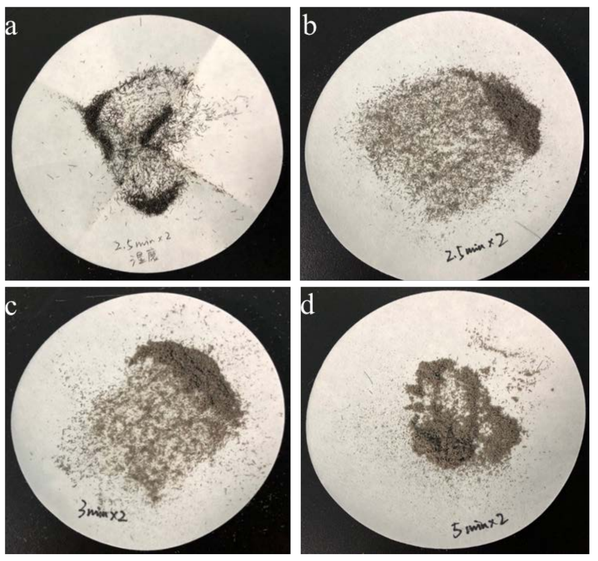

Figure 3: Comparison of effects from different grinding methods and grinding periods. Under the wet grinding (grinding the hair soaked in methanol) 2.5 minX2 (a) condition, the hair was not pulverized due to liquid buffering. When the hair was ground by dry grinding(b, c and d), the hair debris became more and more delicate as the grinding period extended. When the grinding period reached 5 minX2 (d), almost all the hair was ground to very fine powder.

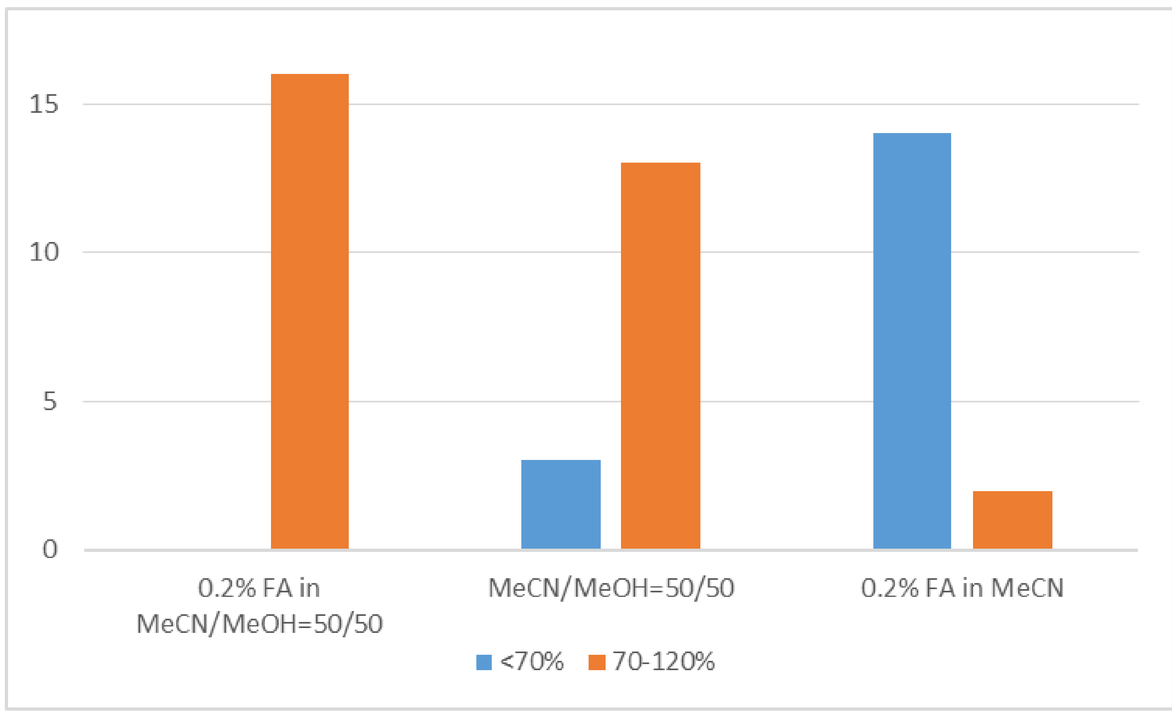

Figure 4: Distribution of recovery rates of each compound under conditions with different extractants.

Table 3. Method parameters of 16 compounds

Compound	Linear range (ng/mL)	Linear relationship	Linear coefficient (R2)	Limit of quantitation (ng/mL)
Morphine	0.1-100	Y=3663.65*X-	0.9973	0.1
Ephedrine	0.1-100	Y=46751.4*X-5428.79	0.9949	0.1
Methcathinone	0.1-100	Y=40553.8*X-4608.73	0.9966	0.1
Codeine	0.5-100	Y=3565.79*X-291.492	0.9942	0.5
Amphetamine	0.1-100	Y=1687.31*X-38.7655	0.9951	0.1
Methamphetamine	0.1-100	Y=90866.9*X-13210.9	0.9868	0.1
Monoacetylmorphine (acetylmorphine)	0.1-100	Y=9060.99*X-642.172	0.9954	0.1
3,4 methylenedioxymethamphetamine (MDMA)	0.1-100	Y=83167.4*X-9591.63	0.9947	0.1
Ketamine	0.1-100	Y=53583.6*X-4777.3	0.9962	0.1
Benzoylecgonine	0.1-100	Y=97687.7*X-7255.8	0.9979	0.1
Tramadol	0.1-100	Y=25974.2*X-3900.5	0.9863	0.1
Heroin	0.5-100	Y=1838.6*X-768.219	0.9861	0.5
Cocaine	0.1-100	Y=93418.3*X-3771.29	0.9950	0.1
Methadone	0.1-100	Y=165220*X-18489.7	0.9951	0.1
Cannabidiol (CBD)	0.1-100	Y=1138.37*X-15.9651	0.9989	0.1
Cannabinol (CBN)	0.1-100	Y=2535.61*X-116.177	0.9991	0.1

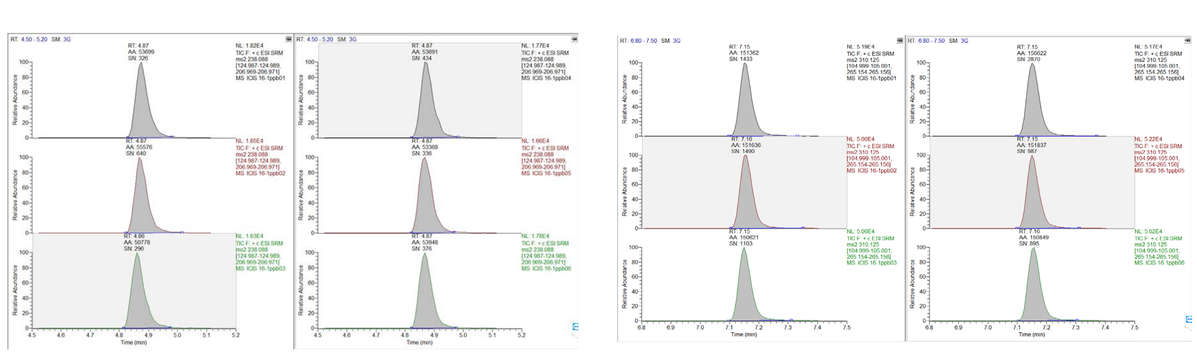

Figure 5: Extracted ion diagrams of ketamine (a) and methadone (b) 1 ng/mL in continuous 6 injections

CONCLUSIONS

We demonstrate an easy and quick LC-MS/MS method to reliably quantify 16 drugs of abuse in hair. We evaluated our method using several positive samples. The lowest positive result for methamphetamine was 66 ng/g in hair.

TRADEMARKS/LICENSING

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PO73212-EN 0819S