RayKol

Knowledge Optimized Laboratory

Determination of 12 common drugs and metabolites in hair

Introduction

Through the analysis of hair samples, it can trace back the drug use for months, and make probable estimation for the duration and level of drug use. Nevertheless, hair samples also have some disadvantage, such as low drug content, high requirements for detection sensitivity.

Based on the above considerations, the method of extracting 12 kinds of drugs and metabolites by LC-MS/MS was established. In this method, methanol was used as the extraction solvent and extracted with pressurized fluid extraction, the extracted solvent was evaporated with vacuum evaporator, reconstituted with methanol to 0.5mL and analyzed by LC-MS/MS. Using the method suggested in this paper, the average recovery rate of 12 kinds of targeted compounds was 85.92% to 120.88% with an RSD value less than 10%. At the same time, this method used 4 hair samples from different personnels, and the results were consistent with the results of the traditional frozen grinding ultrasonic extraction method.

Instruments	RayKol HPFE series High-throughput Pressurized Fluid Extraction System
	RayKol MPE Automated Vacuum Evaporation System
	Agilent HPLC/Tandem Mass Spectrometer
Reagents	Methanol, acetonitrile, acetone: chromatographic pure; diatomite (SiO_2, 61790-53-2): Excellent grade pure; water made from Milli-Q ultra-pure water system
	12 kinds of drugs and metabolites standards supported from a forensic lab

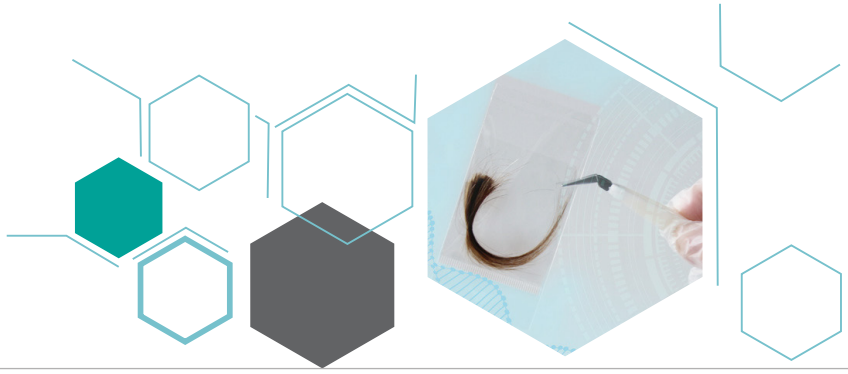
Sample Preparation

Washing the hair samples

The purpose of hair washing is to remove environmental pollutants, dirt and grease on the hair surface, which might cause false positive results, and hair as court evidence of drug identification, only considering the intake of the body into the hairs. Therefore, the washing of hair samples is fundamental step before further pretreatment. But at present, there is no unified operating standard for hair washing. In this experiment, the hair samples were washed via oscillation with water and acetone for 1min, then dried and cut into about 1mm segments.

Experimental method

01. Take a set of clean 11mL extraction cells, put these cells upside down on the table, and place a piece of lower filter paper, then the upper metal filter and screw, then close and tighten with a hexagonal wrench;
02. Place the extraction cells back on the table, and load 2 to 3cm height quartz sand, then load pre-treated 20mg hair samples into the cell via filling funnel;
03. Load the quartz sand again, so that the height of the samples in the cell is 0.5 to 1cm away from the cell mouth, then fully wipe clean with a brush and place the loaded cells into the chamber of HPFE.



HPFE Extraction procedure: with methanol as the extraction solvent, static extraction at 10.34MPa and 90°C for 6min, 2 cycles of extraction, set the purge time as 90s and rinse time as 60s.

RayKol HPFE series High-throughput Pressurized Fluid Extraction System

After extraction, the extract was collected in vials then evaporated with MPE Automated Vacuum Evaporation System to less than 0.5mL. Then reconstitute to 0.5mL with methanol, and transfer to GC vials for later LC-MS/MS analysis.

RayKol MPE Automated Vacuum Evaporation System

Conditions for analysis

Chromatography conditions

The column was SHIMADZU shim-pack GIST-HP C18 column (2.1×150mm), with column temperature of 40°C, flow rate: 0.4mL/min, injection volume: 1μL. Mobile phase A: 0.1% aqueous formic acid; mobile phase B: acetonitrile; elution method: gradient elution.

Mass spectrometry conditions

Electrospray ion source (ESI): positive ion mode; Multiple reaction monitoring mode (MRM); Atomization gas flow: 3.0mL/min; Dryer flow: 10.0mL/min; Heating flow: 10.0mL/min; Interface temperature: 300°C; Devastation temperature: 526°C; DL temperature: 200°C; Heating block temperature 200°C; CID gas: 17kPa. With concentration of 5ng/mL, 12 kinds of drugs and metabolites under MRM mode would have a good separation degree.

Method Validation

The accuracy and precision of the method established in this paper are measured by spiked standard recovery and relative standard deviation (RSD). Twelve drug standards were added at a concentration of 5 ng / mL for pretreatment manipulation. The experimental results show that the average recovery of 12 compounds was between 70.82% and 115.80%, with RSD <10%.

Table-1 Recovery rate and RSD of the 12 drugs and metabolites

Target compound	Avg .(%)	RSD (%)
6-monoacetylmorphine	110.64	5.88
Morphine	73.07	5.95
Codeine	105.67	5.80
Cocaine	81.37	7.24
Benzoylecgonine	105.67	5.80
Methylamphetamine	103.54	3.14
Amphetamine	82.40	1.91
Ketamine	115.80	3.72
Norketamine	98.70	4.55
MDA	111.04	7.26
MDEA	88.17	3.70
MDMA	95.56	3.59

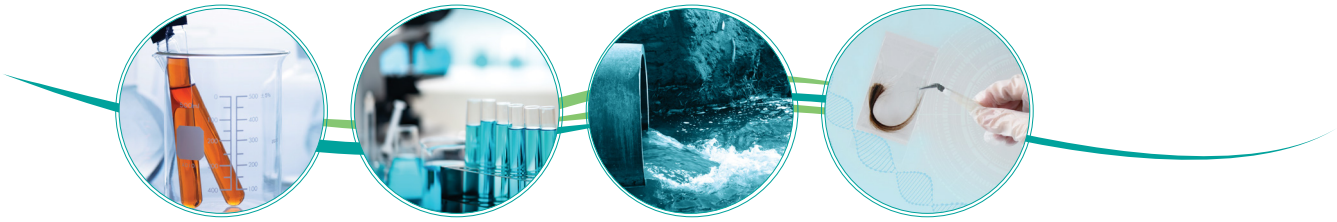
To compare the results from the PFE methods and freeze-grinding ultrasonic extraction, 4 samples of hair were divided into two groups following the two experimental procedures. For the samples using ultrasonic extraction, they were grounded in freeze grinder, then place with 1mL methanol in the ultrasonic ice bath for 2 hours for extraction, to make a parallel comparison, result data shown in Table-2.

Table-2 Comparison of extraction test results between pressurized fluid extraction and frozen grinding method

	No.1 to 4 hair sample	6-monoacetyl morphine	Morphine	Codeine	Cocaine	Benzoyle cgonine	Methylampheta mine	Ampheta mine	Keta mine	Norketa mine	MDA	MDEA	MDMA
PFE concentration ng/mg	No.1	–	–	–	–	–	8.46	0.69	–	–	–	–	–
		–	–	–	–	–	8.31	0.64	–	–	–	–	–
	No.2	5.23	1.39	2.34	–	–	–	–	–	–	–	–	–
		4.55	1.13	2.06	–	–	–	–	–	–	–	–	–
	No.3	3.63	1.43	0.78	–	–	26.57	0.63	–	–	–	–	–
		3.73	1.34	0.64			25.73	0.67					
	No.4	2.31	0.67	0.57	–	–	–	–	–	–	–	–	–
		2.22	0.60	0.39	–	–	–	–	–	–	–	–	–
Freeze grinding ultrasonic extraction concentration ng/mg	No.1	–	–	–	–	–	10.09	0.99	–	–	–	–	–
		–	–	–	–	–	8.82	0.90	–	–	–	–	–
	No.2	1.68	0.67	3.21	–	–	–	–	–	–	–	–	–
		3.40	1.85	5.47	–	–	–	–	–	–	–	–	–
	No.3	1.08	0.88	1.38	–	–	23.32	0.76	–	–	–	–	–
		3.49	3.11	4.81	–	–	36.35	2.51	–	–	–	–	–
	No.4	0.14	0.08	0.09	–	–	–	–	–	–	–	–	–
		0.19	0.15	0.11	–	–	–	–	–	–	–	–	–

By comparing results from two extraction methods for 4 groups of hair samples, it demonstrated that the pressurized fluid extraction and the conventional freeze grinding ultrasonic extraction were consistent in composition detection, however the pressurized fluid extraction method was more stable in concentration determination.

Since drugs and their metabolites are embedded in the keratin of the hair, the conventional method is to release the drug from the hair by digestion and then extract it with a solvent. At present, the detection of drugs in hair is alkaline hydrolysis, acid hydrolysis, enzyme hydrolysis and methanol ultrasonic extraction. The content of impurities in the process of digestion by alkali method and acid method is high, the cost of enzymatic digestion is high, and the ultrasonic extraction of methanol needs to be frozen grinding. Therefore, this paper combined the advantages of pressurized fluid extraction, methanol was used to extract 12 drugs and metabolites in hair. Since the pressurized fluid extraction with methanol can greatly reduce the hydrolysis of the substance to be measured upon release. This experiment processes one sample for about 30 minutes, and can process 6 samples at a time, which has the advantages of simple, efficient operation and reduced manual operation.



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