RayKol

Knowledge Optimized Laboratory

Determination of Methamphetamine in the Urine

Intruduction

This solution utilized automatic solid phase extraction with liquid chromatography tandem mass spectrometry to determine the concentration of methamphetamine in urine, the extract was purified by MCX cation exchange SPE column by RayKol Fotector Plus Automated Solid Phase Extraction System, eluted with 5% ammoniated methanol, eluate was evaporated by RayKol Auto EVA 80 Automated Evaporation System, then reconstituted before LC-MS/MS analysis and quantified by external standard method. At the spike level of 0.2ng/mL, the recovery rate of methamphetamine was between 78.9% and 88.6%, and the RSD value was less than 10.0%, which met the requirements of analysis and identification, indicating that this method can be used as an effective solution for methamphetamine detection in urine.

Instruments	RayKol Fotector Plus Automated Solid Phase Extraction System
	Auto EVA 80 Automated Evaporation System
	Agilent 1290 A + 6470 HPLC / mass spectrometer
Consumables	MCX cation exchange SPE column (RayCure, 60mg/3mL, part no.: RC-204-72855)
Reagents	Acetonitrile, formic acid, methanol as standards for chromatography Ammonium acetate, concentrated hydrochloric acid, ammonia as standards for analysis Ultrapure water made by Milli-Q system
	Methamphetamine standard product came from a public security user

Sample Preparation

Take 2mL urine sample, adjust pH to be less than 2 with dilute hydrochloric acid solution, centrifuge at 8000r/min for 5min, then obtain the supernatant for solid phase extraction.

Solid-phase extraction procedure

Conditioning

Use 3mL methanol and 5mL water respectively for SPE column conditioning, speed as 2mL/min.

Sample loading

Load the sample at the flow rate as 2mL/min.

Rinsing

Rinse sample tube with 2mL water then load into SPE column, then rinse column with 3mL methanol.

Elution

Air push the SPE column, then elute with 4mL 5% ammoniated methanol at rate 1mL/min for collection. See detailed SPE method Figure-1.

No	Step	Source	Output	Flow rate(mL/min)	Volume(mL)	Time(min)
1	Rinse Sample Path	Methanol				2.8
2	Condition	Methanol	Solvent	2	3	1.9
3	Condition	Water	Solvent	2	3	1.9
4	Load Sample		Solvent	2	2.5	1.7
5	Rinse Sample Tubes	Water	Solvent	60	2	1.7
6	Rinse	Methanol	Solvent	2	3	1.9
7	Air Push		Solvent	80	30	2.2
8	Rinse Syringe	5% ammonia...		60	3	0.7
9	Elute	5% ammonia...	Collect	1	4	4.7
10	Air Push		Collect	1	2	2.4
11	Air Push		Collect	20	10	1.3
12	End					

Figure-1

Evaporation

Evaporate the eluate to nearly dry under 40°C using Auto EVA 80 Automated Evaporation System, then add 1mL water, vortex mixed then filter before LC-MS/MS.

Solid-phase extraction conditions

SPE Equipment	Fotector Plus Automated Solid Phase Extraction System
SPE column	HLB Solid Phase Extraction Column (RayCure, 60mg/3mL)
Rinsing	Phosphate buffer (pH =6.0)
Elution	Dichloromethane

RayKol Fotector Plus
Automated Solid Phase Extraction System

Analysis conditions for LC-MS/MS

Column	ACQUITY UPLC BEH C18 (100mm ×2.1mm×1.7μm)
Flow rate	0.30mL/min
Mobile phase	A:5mmol/L Ammonium acetate solution (containing 0.1% formic acid), B: Acetonitrile
Column temperature	35°C
Sampling volume	2μL
Detector	Agilent 6470
Ion mode	ESI+
Purge	8L/min
Nitrogen temperature	330°C
Capillary voltage	3500
Atomizing pressure	45psi
Gradient elution	0.0-1.0min, 5-5% mobile phase B; 1.0-2.0min, 5-15% mobile phase B; 2.0-8.0min, 15-30% mobile phase B 8.0-8.5min, 30-95% mobile phase B; 8.5-11.0min, 95-95% mobile phase B 11.0-12.0min, 95-5% mobile phase B; 12.0-14.0min, 5-5% mobile phase B

Method Validation

The accuracy and precision of this method was demonstrated by spiked standard recovery rate and relative standard deviation (RSD). Blank urine samples were spiked with 0.2ng/mL methamphetamine in pretreatment. The experimental results showed that the recovery rate of methamphetamine was between 78.9% and 88.6%, and the RSD was less than 10.0%, which met the requirements of analysis and detection. The specific data are shown in Table-1.

Table-1 0.2 ng/mLRecovery rate andRSD(%)(n=3)

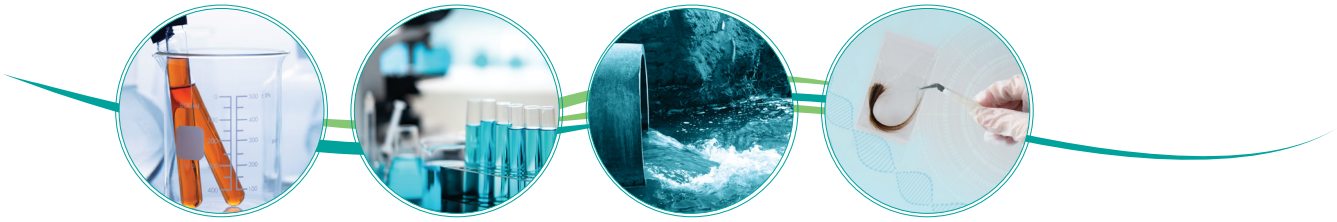
Target compounds	Recovery rate (%)			Avg .(%)	RSD (%)
	Spiked-1	Spiked-2	Spiked-3		
Methamphetamine	88.6	81.4	78.9	83.0	6.0

Result and discussion

Methamphetamine is a weak basic compound, the cation exchange solid phase extraction column could have better effects in purification and enrichment. Before going through the SPE column, the extract should be made acidic to better retain the target compound on the column, and finally the alkaline ammonified methanol solution is used for elution.

Conclusion

RayKol Fotector Plus Automated Solid Phase Extraction System is used to purify 6 samples simultaneously, and automatically process 60 samples with high flux in unattended operation, which greatly reduces the lab workload and improves the work efficiency. In addition, it can avoid the detection deviation caused by operational errors, and minimize the impact of the change of technician on the test results. RayKol Auto EVA 80 Automated Evaporation System can process 80 samples simultaneously for nitrogen blowing, with good parallelism; and the nitrogen blowing needle automatically follow the liquid level, no need to manually adjust the nitrogen blowing needle, to result in small gas consumption and less time.



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