



Determination of 9 kinds of Sedative-Hypnotic Drugs in Blood and Urine

Introduction

After centrifuging the forensic sample, dilute the sample with buffer, purified by RayKol Fotector Plus Automated Solid Phase Extraction System with HLB SPE column, eluted with dichloromethane. Eluate was evaporated by RayKol Auto EVA 80 Automated Evaporation System prior to the detection by GC/MS. The experimental results showed that the recovery of 9 kinds of targeted compounds was between 73% and 109%, and RSD <10%, indicating that this method can be used for the determination of these 9 kinds of sedative-hypnotics in blood and urine.

| | |
|-------------|--|
| Instruments | RayKol Fotector Plus Automated Solid Phase Extraction System |
| | Auto EVA 80 Automated Evaporation System |
| | Agilent 7890A/5975C GC-MS |
| Consumables | HLB SPE column (RayCure, 60mg/3mL, part no.: RC-204-36473) |
| | GC Column (Agilent HP-5MS, 50mX250µmX0.25µm) |
| Reagents | Dichloromethane, methanol, ultrapure water, sodium dihydrogen phosphate, |
| | disodium hydrogen phosphate, phosphate buffer (pH =6.0) |

Sample Preparation

Take 2mL aqueous sample, add 8mL phosphate buffer (pH =6.0), centrifuge at 5000r/min for 5min, take the supernatant to the glass tube for SPE purification.

If the blood has been decomposed, firstly centrifuge at 10000r/min for 5min, and then take the supernatant and add the phosphate buffer for SPE purification. If the blood has been added to the anticoagulant, add 2 to 8mL phosphate buffer then centrifuge before SPE purification.

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

Solid-phase extraction procedure

Conditioning

Use 5mL dichloromethane, 5mL methanol and 5mL phosphate buffer (pH =6.0) respectively for SPE column conditioning, speed as 2mL/min.

Sample loading

Load 10mL sample solution with flow rate 2mL/min, then load 3mL phosphate buffer (pH =6.0). Rinse the sample tube, then nitrogen purge the column under 25psi nitrogen for 5min.

Elution

Elute with 5mL dichloromethane at flow rate 1mL/min. Kindly see detailed SPE method as Figure-1.

| No | Step | Source | Output | Flow rate(mL/min) | Volume(mL) | Time(min) |
|----|--------------------|---------------|---------|-------------------|------------|-----------|
| 1 | Rinse Sample Path | Methanol | | | | 2.0 |
| 2 | Condition | Dichlorome... | Solvent | 2 | 5 | 3.3 |
| 3 | Condition | Methanol | Solvent | 2 | 5 | 2.9 |
| 4 | Condition | Phosphate ... | Solvent | 2 | 5 | 2.9 |
| 5 | Load Sample | | Waste | 2 | 10 | 5.8 |
| 6 | Rinse Sample Tubes | Phosphate ... | Waste | 60 | 3 | 2.3 |
| 7 | Air Push | | Solvent | 60 | 10 | 1 |
| 8 | Dry | | | | | 1 |
| 9 | Rinse Syringe | Water | | 60 | 3 | 0.4 |
| 10 | Rinse Syringe | Methanol | | 60 | 3 | 0.4 |
| 11 | Elute | Dichlorome... | Collect | 1 | 5 | 5.8 |
| 12 | Air Push | | Collect | 1 | 2 | 2.4 |
| 13 | Air Push | | Collect | 20 | 5 | 0.8 |
| 14 | End | | | | | |

Figure-1 SPE method for the analysis of sedative-hypnotics in blood and urine sample using Fotector Plus Automated SPE system

Evaporation

The eluent was concentrated to less than 0.5mL at 40°C with RayKol Auto EVA 80 Automated Evaporation System and reduced to 0.5 mL with dichloromethane.



RayKol Auto EVA 80 Automated Evaporation System

Conditions for GC-MS

GC-MS detection conditions

Inlet temperature 290°C, no diversion, inlet volume 1.0L; carrier gas is high-purity helium, constant flow mode, flow rate 1.0mL/min; electron bombardment ionization source (EI), ion source temperature 230°C, interface (transmission line) temperature 280°C, mass analyzer temperature 150°C, full scan mode (Scan), electron multiplier detection voltage 1576V.

GC heating procedure

Initial temperature 50°C, hold for 1min; heat up to 150°C at 20°C/min; heat up to 300°C at 10°C/min, then keep for 5min.

Method Validation

At the spiked level of 2mg/L, the average recovery was between 73% and 109% and RSD(n =4)<10%, meeting the quality control requirements. The detailed results are shown in Table-1.

Table-1 Recovery rate and RSD Value (n=4) for the results

| Target compound | Blood | | Urine | |
|---------------------|----------|---------|----------|---------|
| | Avg. (%) | RSD (%) | Avg. (%) | RSD (%) |
| Barbital | 89.5 | 2.9 | 75.3 | 5.4 |
| Amobarbital | 88.4 | 5.5 | 115 | 5.3 |
| Secobarbital sodium | 84.0 | 5.1 | 112.3 | 5.2 |
| Phenobarbital | 76.4 | 6.9 | 108.5 | 6.7 |
| Promethazine | 99.4 | 3.2 | 73.8 | 0.8 |
| Diazepam | 94.3 | 2.8 | 102.6 | 1.9 |
| Chlorpromazine | 84.1 | 3.4 | 99.4 | 1.3 |
| Trifluoperazine | 92.8 | 6.7 | 97.5 | 0.2 |
| Clozapine | 107.9 | 4.2 | 91.5 | 0.3 |

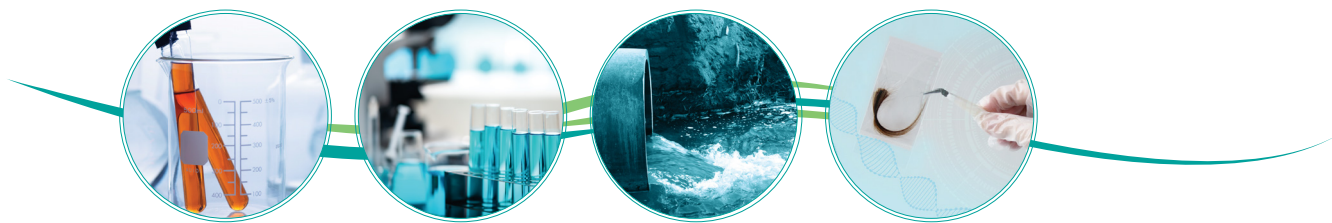
Result and discussion

Before the elution step, the solid phase extraction column should be blown dry as much as possible, otherwise it would affect the eluting effect and subsequent nitrogen blowing.

After method validation, this method has been demonstrated to be also applicable for determining other drugs and residual pesticides in aqueous forensic samples, including analysis of diacetylmorphine, 6-monoacetyl morphine, papaverine, terbaine, codeine, ketamine; analysis of dichlorvos, methomyl, chlorpyrifos, parathion, methyl parathion, loquat, methamidophos, chlorfenapyr, cypermethrin, bome-thrin, cypermethrin, fenpropathrin, bifenthrin.

Conclusion

This solution effectively solves the purification and evaporation in the analysis of sedative-hypnotic drugs in blood and urine. Fotector Plus Automated Solid Phase Extraction System with high-precision injection pump and precise flow rate control allow laboratory personnel to modify the issues in inaccurate sample volume, loading speed, and elution speed; also, multiple channels are operated simultaneously to improve workflow in labs. The purified eluate can be evaporated using the Auto EVA 80 Automated Evaporation System, completely solving the problem of large sample volume and longtime consumption. The combination of the two can greatly improve the lab efficiency.



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