Automation of Solid Phase Extraction (SPE) Multi-method for GC/MS Analysis of Drugs of Abuse in Human Serum

Application Note CL0212

Solid phase extraction is an important method to prepare samples for GC/MS by removing unwanted compounds and retaining the analyte of interest. This application describes the automation of a manual SPE multi-method performed with the GX-271 ASPEC™ System and TRILUTION LH software. Fifteen different drugs of abuse and metabolites, including compounds indicative of cannabis, meth, ecstasy, opiates, and cocaine, were extracted from human serum samples using an automated SPE method. The average recovery of all analytes was approximately 80%. This method shows excellent recoveries of both acidic and basic eluents in a single run, while achieving good reproducibility.

Introduction

Psychotropic drugs (cannabis, amphetamines, opiates, cocaine) are illegal, yet widely used in Germany. The quantitative detection of these substances in blood is essential to determine drug use in cases of driving under the influence, which is forbidden by law. The use of solid phase extraction (SPE) for the preparation of clinical samples is an established method for the analysis of drugs of abuse. An automated SPE method was used to process human serum samples for the analysis of several standard drugs and their metabolites (Table 1).

TRILUTION LH software and the GX-271 ASPEC system were employed to perform the liquid handling and SPE method (Figure 1). The samples were derivatized after extraction and analyzed by gas chromatography/mass spectrometry. The automated method was transferred and validated from a previously used manual method. This application demonstrates the extraordinary flexibility of the GX-271 ASPEC and TRILUTION LH software since the desired SPE products can be eluted in fractionated mode.

Figure 1. The Gilson GX-271 ASPEC™ System accommodates 1, 3, or 6 ml SPE cartridge and automates solid phase extraction of samples.

Gilson, Inc.
Middleton, WI 53562 USA
Telephone: 800-445-7661

Materials & Methods

SPE Materials

Gilson GX-271 ASPEC™ System (Figure 1)
TRILUTION® LH v3.0 Software

SPE Cartridges:

JT Baker® BAKERBOND C18 500 mg / 3 mL

SPE Solutions:

Methanol
Water
Acetic Acid
Dichloromethane: Acetone (1:1)
Dichloromethane: Isopropanol: Ammonia (40:20:2)
Phosphate buffer
Human serum (6-8 replicates per analysis)

SPE Method

1. Condition:
   i. 6 mL Methanol
   ii. 2 mL Water
2. Load:
   i. +6.5 mL Sample (1 mL Serum mixed with 6 mL Phosphate buffer)
3. Wash :
   i. 4 mL Water
   ii. 4 mL Water: Methanol (80:20)
   iii. 1 mL Acetic Acid 0.1%
4. Dry 10 minutes with Nitrogen gas
5. Elution 1 (acid):
   i. 3 mL Dichloromethane: Acetone (1:1)
6. Elution 2 (basic):
   i. 3 mL Dichloromethane: Isopropanol: Ammonia (40:20:2)

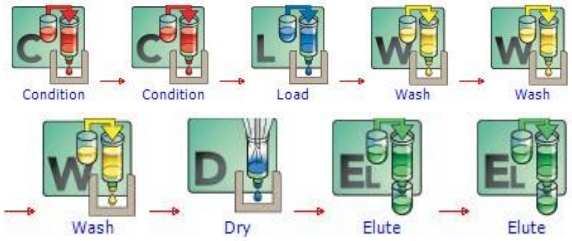

Figure 2. TRILUTION LH SPE Method

Results and Discussion

Fifteen different drugs of abuse and metabolites (Table 1) were extracted from human serum samples using an automated SPE method (Figure 2). A minimum of 6 replicates were performed for each analyte of interest, at 2 different concentrations in serum. The average recovery of all analytes was approximately 80% with Methylecgonine having the lowest recovery at 57 +/-5.5 %. The highest recovery was observed for MDMA (108 +/ 2 %), also known as ecstasy (Table 2). A high level of reproducibility was observed for the majority of the analytes of interest, with an average of 4.9% RSD. The highest %RSD came from Methylecgonine.

Analytes of Interest		
THC	Morphine	MDE
THC-OH	Methadone	MDMA
THC-COOH	Benzoylegonine	MDA
6-Monacetyl-morphine	Methylecgonine	Methamphetamine
Codeine	Cocaine	Amphetamine

Table 1. Analytes of interest to be extracted from human serum

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Analyte	Conc ng/mL	Recovery % (SD)	Conc ng/mL	Recovery % (SD)	Ave Rec %
THC	1	**63.3** (5.3)	5	**61.8** (4.3)	**62.6**
THC-OH	1	**81.0** (3.8)	5	**74.5** (6.5)	**77.8**
THC-COOH	10	**83.0** (2.0)	50	**73.6** (3.2)	**78.3**
6-Monacetylmorphine	2	**81.3** (6.5)	10	**74.7** (3.8)	**78.0**
Codeine	10	**92.0** (3.6)	50	**74.2** (2.8)	**83.1**
Morphine	10	**106.4** (8.6)	50	**73.6** (2.1)	**90.0**
Methadone	20	**76.4** (6.3)	100	**69.3** (3.2)	**72.9**
Benzoylecgonine	50	**79.9** (1.8)	250	**69.3** (3.0)	**74.6**
Methylecgonine	2	**70.2** (17.4)	10	**57.0** (5.5)	**63.6**
Cocaine	2	**62.4** (13.5)	10	**72.9** (4.3)	**67.7**
MDE	20	**94.0** (1.1)	100	**77.8** (2.4)	**85.9**
MDMA	20	**108.4** (2.0)	100	**89.9** (2.9)	**99.2**

Table 2. Recovery of Drugs of Abuse from Human Serum Using Automated SPE with TRILUTION® LH and the Gilson GX-271 ASPEC™.

Clinical analysis of drugs of abuse in human serum is essential for the determination of drug use. Solid phase extraction is an important method to prepare samples for GC/MS by removing unwanted compounds and retaining the analyte of interest. This application describes the automation of a manual SPE multi-method performed with the GX-271 ASPEC™. This method shows excellent recoveries of both acidic and basic eluents in a single run, while achieving good reproducibility. Further method optimization could be used to obtain greater recoveries for Methylecgonine, as it showed the lowest recovery and the highest %RSD.

Acknowledgements

The application was developed and supported by: Institut für Rechtsmedizin, Mainz Germany, A. Schlägel, Dr. Zörntlein, Dr. Röhrich, Dr. Becker, Prof. Dr. Dr. Urban.

Summary or Conclusions

- The use of solid phase extraction (SPE) for the preparation of clinical samples is an established method for the analysis of drugs of abuse.
- The GX-271 ASPEC system and TRILUTION LH software were used to automate an SPE method that processed human serum samples for the analysis of fifteen different drugs of abuse and metabolites.
- This method shows excellent recoveries of both acidic and basic eluents in a single run, while achieving good reproducibility.
- This application demonstrates the extraordinary flexibility of the GX-271 ASPEC and TRILUTION LH software since the desired SPE products can be eluted in fractionated mode.