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

Application Note CL0212

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Keywords

GX-271 ASPEC™, TRILUTION® LH Liquid Handling Software, Solid Phase Extraction (SPE), psychotropic drugs, Gas Chromatography Mass Spectrometry (GC/MS)

Introduction

Psychotropic drugs (cannabis, amphetamines, opiates, cocaine) are illegal; however, 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. Gilson GX-271 ASPEC, Insert: SPE Cartridges and Eluted Samples on the GX-271 ASPEC™.

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Materials & Methods

Materials

- Gilson GX-271 ASPEC™ (Figure 1)
- TRILUTION® LH
- 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)

Table 1. Analytes of interest to be Extracted from human Serum.

Analytes of Interest		
THC	Morphine	MDE
THC-OH	Methadone	MDMA
THC-COOH	Benzoylecgonine	MDA
6-Monacetylmorphine	Methylecgonine	Methamphetamine
Codeine	Cocaine	Amphetamine

SPE Method (Figure 2)

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

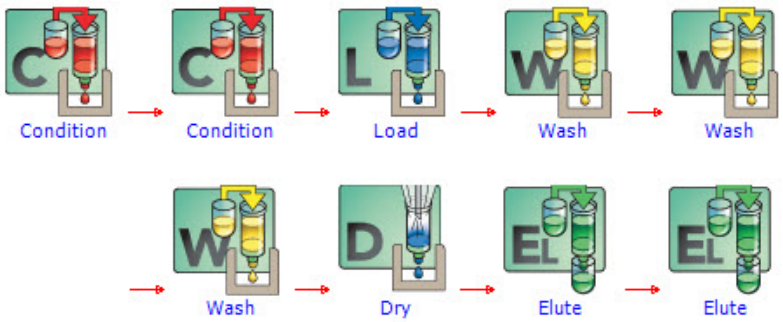

Figure 2. TRILUTION LH SPE Method

The samples were dried after elution and derivatized by PFP for GC/MS analysis. Following derivatization, samples were dried and reconstituted in 50 µL ethyl acetate. Samples (1 µL) were injected onto GC/MS for analysis.

Results

Fifteen different drugs of abuse and metabolites were extracted from human serum samples using an automated SPE method. 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.

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

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**
MDA	20	**90.6** (6.8)	100	**77.9** (2.6)	**84.3**
Methamphetamine	20	**106.7** (3.4)	100	**87.3** (2.2)	**97.0**
Amphetamine	20	**91.3** (2.4)	100	**69.6** (8.5)	**80.5**

Summary

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.

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