Identification and Quantification of Illegal Drugs and Benzodiazepines in Human Plasma by LC/MS-MS Analysis after Solid Phase Extraction (SPE)

Keywords: Amphetamines, Benzodizepines, Blood, Cocaine, Forensics, GX-271 ASPEC, Human plasma, LC/MS-MS, Opiates, Plasma, Solid phase extraction (SPE)

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Introduction

The identification and quantification of drugs of abuse in blood (plasma, serum, whole blood) has become very common in the forensic medicine laboratory. A variety of methods for the extraction and analysis of these drugs have been described in the literature (Sadeg and Dumontet, 2001; Moeller and Kraemer, 2002), with particular focus on the use of GC/MS or LC/MS for analysis.

Liquid/liquid extraction (LLE) has been traditionally used at a basic pH (pH = 9) for the extraction of amphetamines, cocaine and its metabolites and opiates from blood. This is sometimes followed by an additional clean-up step. LLE can be time-consuming and difficult to automate compared to solid phase extraction (SPE). Poor reproducibility can also be a factor when using a manual method such as LLE.

This study describes an automated SPE protocol using a Gilson GX-271 ASPEC™ system (Figure 1) for the simultaneous extraction of amphetamines, cocaine and its metabolites (benzoylecgonine, ecgonine methyl ester) and opiates as well as a variety of benzodiazepines prior to analysis by LC/MS-MS.

Figure 1. Gilson GX-271 ASPEC System with 406 Dual Syringe Pump (Part no. 2614008)

Experimental Conditions

Materials

All solvents were distilled in glass suitable for GC, HPLC, pesticide residues analysis and spectrophotometry. HPLC solvents were obtained from J.T. Baker (UK). All reagents were ACS grade quality or better. All samples (0.5 mL of plasma, serum or hemolyzed blood after centrifugation) were spiked with internal standards (LGC Standards, Molsheim, France).

The following internal standards were used:

- 100 ng benzoylecgonine-d3, internal standard used for cocaine metabolites and opiate quantification
- 100 ng amphetamine-d5, internal standard used for amphetamine quantification
- 50 ng clonazepam-d4, internal standard for benzodiazepine quantification

The Gilson GX-271 ASPEC System was configured as follows:

Description	Part numbers
GX-271 ASPEC w/ Dual 406 Syringe Pump	2614008
1mL and 10mL Syringes	2502343 and 2502345
406 Dual Adaption Kit for ASPEC and Two 10 mL Plumbing Packages	2644708 and 2644701
221x1.5x1.1 BV Tapered Probe and Guide Assembly for 1.5 mm Probes	27067374 and 26046228
Rinse Stations	(2) 26034551 and (1) 26034555
GX 2-channel rinse pump and 2.0 mm ID Pharmed Tubing Kit	261452 and 26032221
Locator Tray for five 20-Series Racks	26041033
DEC Accessory Kit for 1 mL SPE Cartridges	2604701
Rack Code 343 for 80 13x100 mm Tubes	260440025
Safety Shield Assembly, GX27X	2604706
TRILUTION LH Software Package	21063020, 210630R20 and ORACLE10GXE

Solid Phase Extraction (SPE) Protocol

The SPE procedure used 1 mL Waters Oasis™ HLB (30mg) Cartridges. The cartridges were sealed using Gilson 1 mL Sealing Caps.

The SPE protocol is entirely automated using the Gilson GX-271 ASPEC system. The SPE steps are summarized with the schematic provided in the GX-271 ASPEC control software, TRILUTION LH (Figure 2).

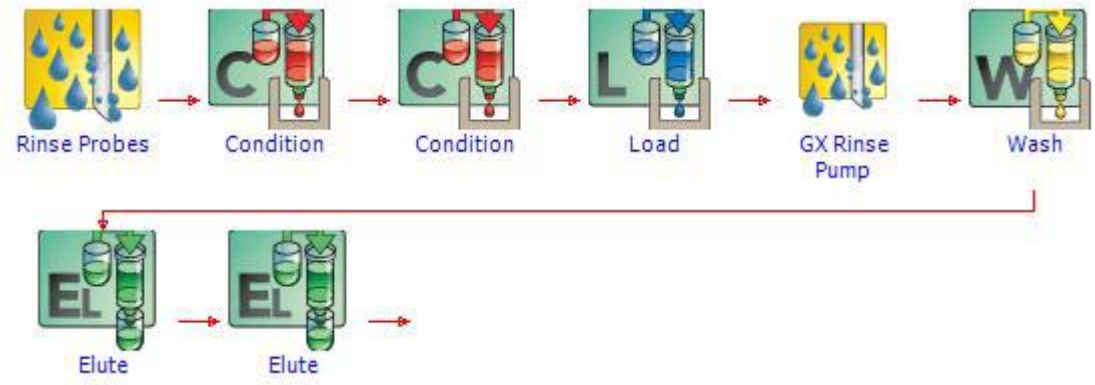

Figure 2. TRILUTION LH SPE Tasks for Extraction of Drugs

The details of each step are as follows:

- Initialization Step : Gilson Mobile SPE Racks are moved above the waste rack (Figure 3)
- Rinse probe with methanol
- Condition SPE Cartridge with 1 mL of methanol followed by 1 mL of deionized water at a flow rate of 3 mL/min
- Load sample at a flow rate of 1 mL/min followed by rinse
- Wash cartridge with 2 mL deionized water at a flow rate of 6 mL/min
- Move the Gilson Mobile SPE Rack over the collection tubes
- Elute the analytes of interest with 2 X 0.5 mL of methanol at a flow rate of 1.5 mL/min
- Eluate is now ready for injection on the LC/MS-MS system
- Inject 10 uL in the LC/MS-MS system at 0.2 mL/min

Figure 3. Gilson Mobile Rack

LC/MS-MS Analysis

HPLC Analysis was performed on a Waters Alliance 2695 (temperature at 30°C) with a Waters Xterra C18 column. Separation was accomplished using a binary gradient of water and acetonitrile both containing 0.5% TFA. The detection system was a Waters QuatroMicro triple quadropole mass spectrometer.

Ten microliters of SPE eluate was injected into the LC/MS-MS system at a flow rate of 0.2 mL/min

Results

Table 2. Recovery Values Obtained from Spiked Plasma Samples (n =3)

Analytes		Recoveries with plasma spiked at 50 ng/mL	Recoveries with plasma spiked at 150 ng/mL and 200 ng/ml for benzodiazepines
Amphetamines	Amphetamine	100	100
	Methamphetamine	96	98
	MDA	72	79
	MDMA	88	96
	MDEA	80	75
	MBDB	100	95
	Ephedrine	86	90
Cocaine and metabolites	Benzoylecgonine	84	75
	Ecgonine methyl ester	100	87
	Cocaine	74	79

Table 2. (continued)

Opiates	Morphine	90	84
	6monoacétylmorphine	100	92
	Codeine	88	80
	Pholcodine	84	80
	Ethylmorphine	100	100
Benzodiazepines	Diazepam	95	83
	Nordiazepam	87	75
	Hydroxynordiazepam	96	84
	Chlordiazepoxide	100	100
	Oxazepam	82	84
	Temazepam	78	79
	Clonazepam	95	83
	7aminoclonazepam	70	68

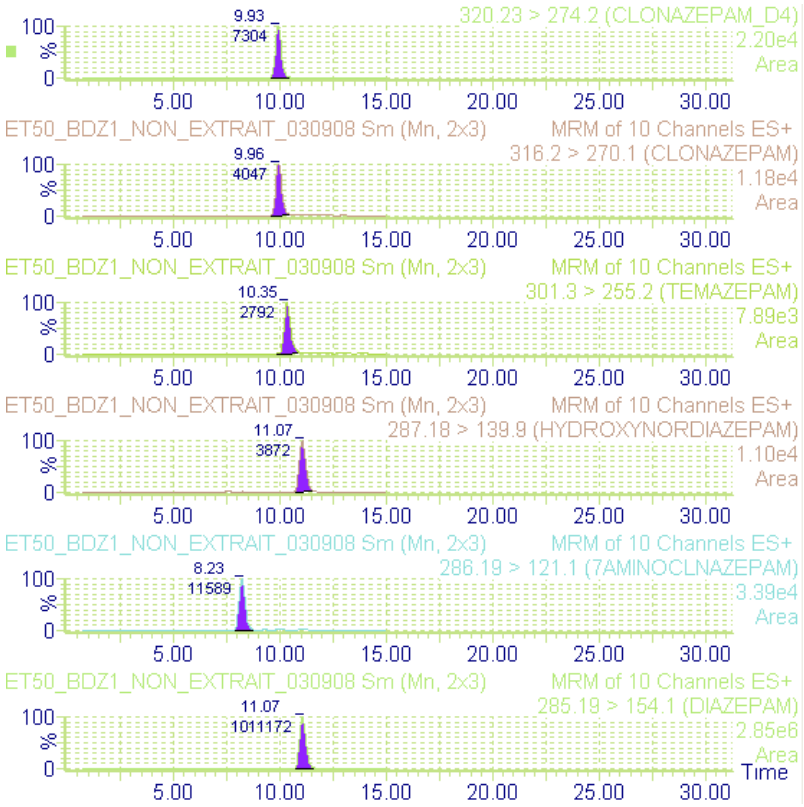

Figure 4. Assay of a plasma extract targeting benzodiazepines

Conclusion

The high quality of the automated SPE clean-up method allowed for the direct injection of extracts into the LC/MS-MS system. Recovery of all analytes was excellent. Automation of the SPE process increased recovery ranges 10 to 20% compared to results obtained using the manual liquid/liquid extraction method. This automated method has now been fully validated in our laboratory. Automation of the extraction process has the additional benefit of allowing scientists to spend more time developing new methods for the analysis of illegal drugs, therapeutic drugs and other compounds of interest in the forensic laboratory.

References

Sadeg, N and Dumontet, M. (2001). Intérêt de l'extraction en phase solide en toxicologie: exemple d'extraction de 15 substances toxiques et médicamenteuses par 7 colonnes SPE différentes par un protocole unique. (Interest of SPE in the toxicology field: example of extraction of 15 toxics or drugs by 7 different SPE columns using a simple extraction procedure). **Ann. Toxicol. Anal. 13** (1):35-40.

Moeller, S. and Kraemer, T. (2002). Drugs of abuse monitoring in blood for control of driving under the influence of drugs. **Therapeutic Drug Monitoring 24**: 210-221.