Identification and Quantification of Drugs of Abuse and Benzodiazepines by LC/MS-MS Analysis after Solid Phase Extraction (SPE)

Application Note CL1202

The Gilson GX-271 ASPEC™ system was used for automated solid phase extraction of illegal drugs and benzodiazepines from human plasma. Automation of the SPE process increased recovery 10 to 20% compared to the manual liquid/liquid extraction method. The high quality of the automated SPE clean-up method allowed for the direct injection of extracts into the LC/MS-MS system. The automated system increases walk-away time, 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.

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.

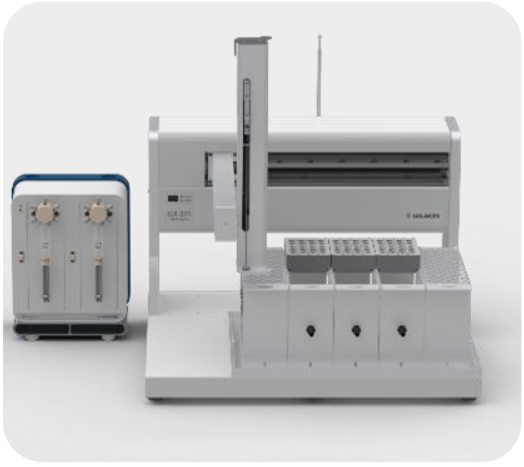

Figure 1. Gilson GX-271 ASPEC System shown with VERITY 4260 Dual Syringe Pump (Part no. 2614008)

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Materials & Methods

SPE Materials

Gilson GX-271 ASPEC System

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-D_3, internal standard used for cocaine metabolites and opiate quantification

100 ng amphetamine-D_5, internal standard used for amphetamine quantification

50 ng clonazepam-D_4, internal standard for benzodiazepine quantification

SPE Method

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).

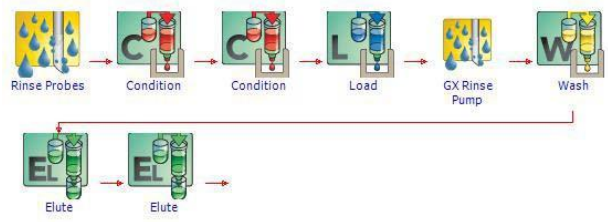

Figure 2. TRILUTION LH SPE Tasks for Extraction of Drugs

The details of each step are as follows:

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

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 quadrupole mass spectrometer.

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

Results and Discussion

Recovery of all analytes was excellent and the high quality of the automated SPE clean-up method allowed for the direct injection of extracts into the LC/MS-MS system. Recoveries are reported in Table 1 and Figure 3. Automation of the SPE process increased recovery ranges 10 to 20% compared to results obtained using the manual liquid/liquid extraction method.

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
Opiates	Morphine	90	84
	6-monoacétylmorphine	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
	7-aminoclonazepam	70	68

Table 1. Recoveries of the indicated compounds using the Gilson GX-271 ASPEC system.

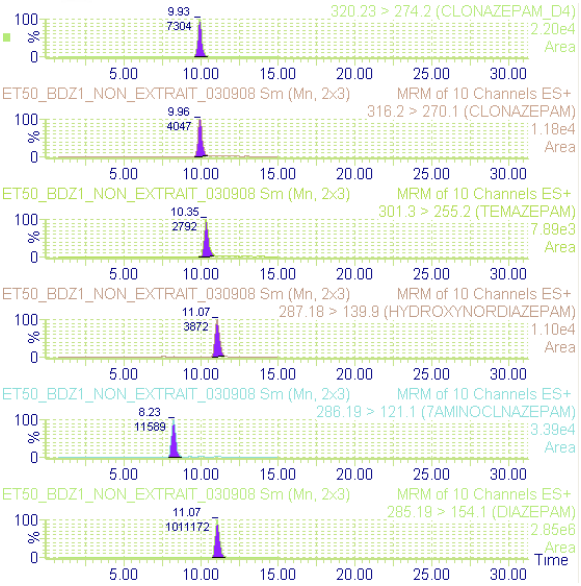

Figure 3. Assay of a plasma extract targeting benzodiazepines.

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

1. Sadeg, N and Dumontet, M. 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 toxic and medicinal substances by 7 different SPE columns using a simple extraction procedure). *Ann. Toxicol. Anal.* **13**, 35-40 (2001).
2. Moeller, S. and Kraemer, T. Drugs of abuse monitoring in blood for control of driving under the influence of drugs. *Therapeut. Drug Monitor.* **24**, 210-221 (2002).

Acknowledgements

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Summary or Conclusions

- The Gilson GX-271 ASPEC system automates sample cleanup using 1, 3, or 6 ml SPE cartridges.
- Recovery of all analytes was excellent: the automated method reported here resulted in 10-20% improvement in recovery of drugs of abuse and benzodiazepines from human serum compared to the manual method.
- The automated SPE clean-up method yields extracts of such high quality that they are compatible with on-line injections into HPLC or LC/MS systems using GX Direct Injection Module.