

Automation of Solid-Phase Extraction in Forensic Toxicology

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Abstract

Aim: In this study, the performance of different devices for the automation of a solid-phase extraction procedure for brain samples has been evaluated.

Methods: Homogenized brain tissue from a pig was spiked with a mixture of analytes. A universal SPE procedure was performed on different devices for automation and the analytes were quantified by GC-MS-SIM after silylation.

Results: The RapidTrace did not perform well with the brain samples, whereas ASPEC XL and MultiPurposeSampler showed reproducible results with acceptable recovery rates.

Discussion: For complex matrices (such as brain samples) the device to automate SPE should be carefully chosen. Moreover, during method development, special attention has to be paid to washing, to flow control, to the possibility that the cartridge could dry out, and to avoid systematic errors.

Conclusion: ASPEC XL and MultiPurposeSampler can be used for the automation of a SPE procedure even with complex matrices (such as brain samples) in postmortem forensic toxicology.

1. Introduction

In forensic toxicology, sample preparation is a crucial pre-requisite for the successful application of high-tech analytical instruments for qualitative and quantitative substance determination.

Because of its high extraction efficiency and its possibility for miniaturization and automation, solid-phase extraction (SPE) is rapidly gaining importance in analytical toxicology.

Automation of SPE is often driven by the need for laboratory accreditation; systematic errors, costs, and time spent per case are reduced; and finally technician safety is improved.

For automation of SPE, different devices can be used – each with its own advantages and disadvantages. Therefore the performance of different devices for the automation of SPE of brain samples was evaluated in this study.

2. Material and Methods

2.1. Sample pre-treatment [1]

0.5 g of brain tissue from a pig was homogenized with an IKA ULTRA-TURRAX Tube Drive (IKA, Staufen, Germany) and spiked with a mixture of analytes (amphetamine, benzoylecgonine, cocaine, codeine, diazepam, doxepine, ibuprofen, methadon, metoprolol, morphine, paracetamol, phenobarbitone, salicylic acid, THC, THCA) in concentrations between

50 and 5000ng/g. The sample was diluted with 5mL of phosphate buffer (0.05 M, pH 7.4), and after centrifugation (500 x g, 20 min) the supernatant was used for SPE.

2.2. Solid-phase extraction

For automated solid-phase extraction, three different devices were tested: RapidTrace (Zymark, Biotage AB, Uppsala, Sweden), ASPEC XL (Gilson, Middleton, USA), and Multi-PurposeSampler MPS (Gerstel, Mülheim, Germany).

EVOLUTE CX (50mg, 3mL, Biotage AB, Uppsala, Sweden) cartridges were used.

The sorbent was conditioned with a buffer solution. Then the sample was loaded at 0.5mL/min. After washing with demineralized water and pH-adjustment with 0.1N acetic acid, elution was performed with ethyl acetate/isopropanol (3:1) to obtain the acidic/neutral extract. Basic analytes were then eluted separately with ethyl acetate/isopropanol/triethylamine (75:25:3). The eluates were evaporated with a vacuum-concentrator Alpha RVC (Christ, Osterode, Germany) and silylated before analysis.

2.3. GC-MS detection

GC-MS quantitative analyses of the extracts were performed using a HP 6890 gas chromatograph with HP 6890 auto-sampler, connected to a HP 5973net mass spectrometer (Agilent Technologies Inc., Palo Alto, CA, USA). In the gas chromatograph, an MDN-5S-column (15 m x 0.25 mm i.d., 0.25 µm film thickness, Supelco, Bellefonte, PA, USA) was installed with a constant flow of Helium (Messer, Austria) at 1 mL/min. The following temperature program was used, with a total running time of 20 min: the initial column temperature was set to 100°C for 2 min, increasing to 200°C by 25°C/min and stopped at 290 °C after a ramp of 15 °C/min. Splitless injections were performed with the injection-port temperature set to 280°C. The transfer line of the instrument was adjusted to 300°C. The instrument was operated in SIM-mode.

3. Results and Discussion

When developing an automated SPE procedure, appropriate pre-treatment of body fluids and tissue samples, as well as a proper choice of the extraction device are essential.

In the field of postmortem toxicology, brain samples can provide information about the effect of drugs at their place of action which is why brain samples were homogenized for this study [2], and a mixture of analytes - recommended by the Scientific Committee for Extraction of the GTFCh - was spiked. Then the sample was diluted with a phosphate buffer solution using a physiological pH of 7.4 - protein precipitation could be avoided in this way, and the viscosity of the solution was minimized. After centrifugation, the supernatant could be used for automated SPE without clogging of the tightly packed extraction cartridges.

The control software of all three devices allowed the adjustment of the speed of sample application, and the ASPEC even allowed for feedback on the flow via an integrated pressure sensor.

To produce the necessary overpressure for SPE, the RapidTrace seals each extraction cartridge with the same plunger: This increases the risk of cross-contamination. Moreover, the sample is pumped through a HPLC-valve, which was blocked very quickly by the brain samples that are rich in proteins. Therefore, the RapidTrace could not be used for developing a robust and reliable SPE procedure using brain samples.

The ASPEC as well as the MPS showed a higher amount of flexibility in respect to maximal amount of samples, cartridge sizes, collection tubes, and speed of extraction. Both devices could handle the brain samples without problems. The control software of each instrument could successfully prevent cross-contamination; the ASPEC includes also error handling (e.g. after clogging of a cartridge).

To prevent analyte loss, the ASPEC was slightly modified: A 5mL-HPLC-loop was installed between the needle and the transportation loop, and the volume for individual steps was set to never exceed 5mL. Because the ASPEC does not clearly separate samples which are rich in proteins from solvents for washing and elution, there is an immanent risk of unpredictable protein precipitation within the system, which makes extensive washing steps necessary. Generally, the ASPEC performed well with the brain samples and the recovery rates were similar to the MPS (see below).

For the MPS, a new form of SPE - based on Disposal Pipette Extraction (DPX Labs, LLC) - was applied. DPX is a technique where a sorbent is loosely filled in a pipette tip and the sample is mixed with this sorbent within the tip in a batch mode. Based on the automation of the DPX-procedure on the MPS, a SPE procedure was developed by replacing the DPX-tips with SPE-cartridges.

Under these conditions, the protein-rich sample is aspirated into the cartridge from the opening on the bottom of the cartridge and the wash solution and solvents are applied from the opening on top of the sealed cartridge (see Figure 1).



Fig. 1. SPE-cartridge after aspiration of sample from the opening on the bottom.

The advantage of this procedure is that the sample is filtered via the lower frit before coming into contact with the sorbent. In this way, problems such as clogging of the tightly packed extraction cartridges can be ruled out. Moreover, because of the clear separation between sample and solvent, there is no more possibility of cross-contamination. The only problem that could not yet be solved is the drying of the cartridge before elution (for this study the cartridges had to be centrifuged prior to the elution step).

As with the ASPEC, the MPS was robust and flexible. Moreover, the blocking of cartridges and the risk of cross-contamination could be excluded, and multiple extractions with one cartridge would be theoretically possible (see Table 1 for results).

Tab. 1. Results for five brain samples extracted with a MultiPurposeSampler MPS (Gerstel); test-analytes, mean recovery, and standard deviation (SD); nd= not detectable.

Analyte	Mean Recovery	SD	Mean Recovery	SD
	(%, n=5) Acidic/neutral extract		(%, n=5) Basic extract	
amphetamine	-	-	46	3.9
benzoylecgonine	-	-	56	6.0
cocaine	-	-	83	8.2
codeine	-	-	78	8.4
diazepam	44	2.1	26	6.8
doxepine	-	-	59	6.9
ibuprofen	66	15.9	-	-
methadone	-	-	62	7.7
metoprolol	-	-	89	5.1
morphine	-	-	76	9.3
phenobarbitone	73	11.8	-	-
THC	nd	nd	-	-
THCA	23	6.7	-	-

4. Conclusion

Solid-phase extraction procedures can be automated with different devices. Whereas the RapidTrace is limited to sample matrices, which have a low protein content (such as urine or serum), the ASPEC and the MPS turned out to be more robust and could also handle very complex postmortem matrices such as brain tissue. When automating a SPE procedure attention should be paid to avoiding cross-contamination and systematic errors. On the other hand, the automation of the SPE procedure improves sample quality in respect to extraction yield, reproducibility and laboratory productivity [3]. The high reliability and consistency of results is essential in order to compare them to reference values (as well as for creating such reference values for complex matrices).

5. References

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