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Determination of Drugs in Brain Samples using Disposal Pipette Extraction

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Abstract

Aim: The aim of this study was to evaluate a new technique for the manual extraction of different drugs from postmortem brain samples.

Methods: Homogenized brain tissue from a pig was spiked with a mixture of analytes.
For sample pre-treatment, protein precipitation with acetonitrile was compared to direct dilution with phosphate buffer.
Disposal pipette extraction tips were loaded by hand with a syringe device. The extraction method was optimized and the drugs were detected by GC-MS-SIM after silylation.

Results: Most of the drugs analysed showed reproducible recovery rates. Recoveries were dependent on sample pre-treatment. It could be shown that avoiding protein precipitation resulted in more reliable results.

Discussion: Analytes with different physico-chemical properties were extracted from a complex biological matrix by manual Disposable Pipette Extraction. The homogenous mixture of the sorbent with the sample solution was essential for reliable results.
Conclusion: The developed method can be used for the manual extraction of complex postmortem brain samples in cases where no (automated) extraction devices for SPE are available.

1. Introduction

The analysis of drugs of abuse in biological specimens requires complex techniques in order to eliminate interferences of endogenous compounds and to obtain reproducible and efficient results.
In some toxicological laboratories, tissue extractions are not performed on a routine basis, and the acquisition of an (automated) extraction device for solid-phase extraction (SPE) is therefore not useful.
Disposal Pipette Extraction (DPX), which is a novel SPE method using a loosely packed sorbent in a disposal pipette tip, can be performed manually using a syringe device for loading the pipette tip.

2. Material and Methods

Brain tissue from a pig - homogenized with an IKA ULTRA-TURRAX Tube Drive, Staufen, Germany; see Figure 1 - was spiked with a mixture of analytes (amphetamine, benzoylecgonine, cocaine, codeine, diazepam, doxepine, ibuprofen, methadone, metoprolol, morphine, phenobarbitone, THC, THCA).

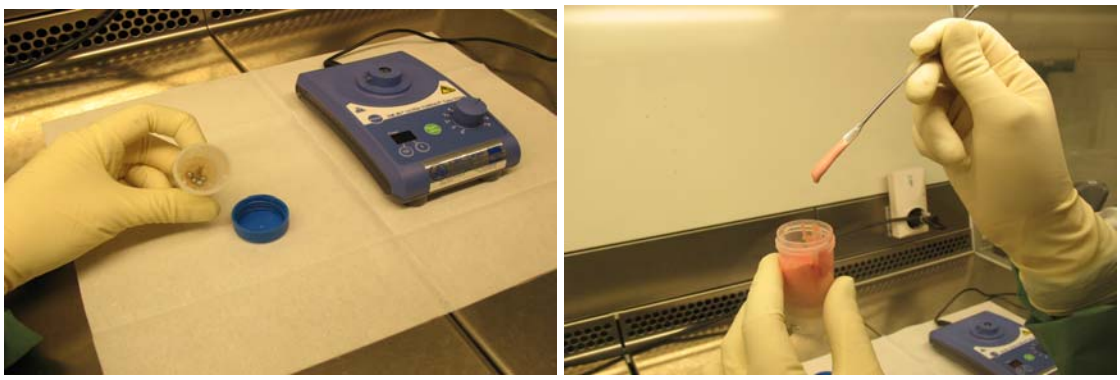

Fig. 1. Homogenization of brain samples.

During sample pre-treatment, protein precipitation with acetonitrile was compared to direct dilution with a phosphate buffer:

Figure 2a: 400 µL ACN was added to 0.1 g of homogenized tissue and the supernatant was diluted with a phosphate buffer (0.05M, pH 7.4) up to a volume of 2 mL;
Figure 2b: 0.1 g of homogenized tissue was diluted with 2 mL of phosphate buffer;
Samples were further treated in an ultrasonic bath and centrifuged (500 x g, 20 min.). The supernatant was used for further extraction.

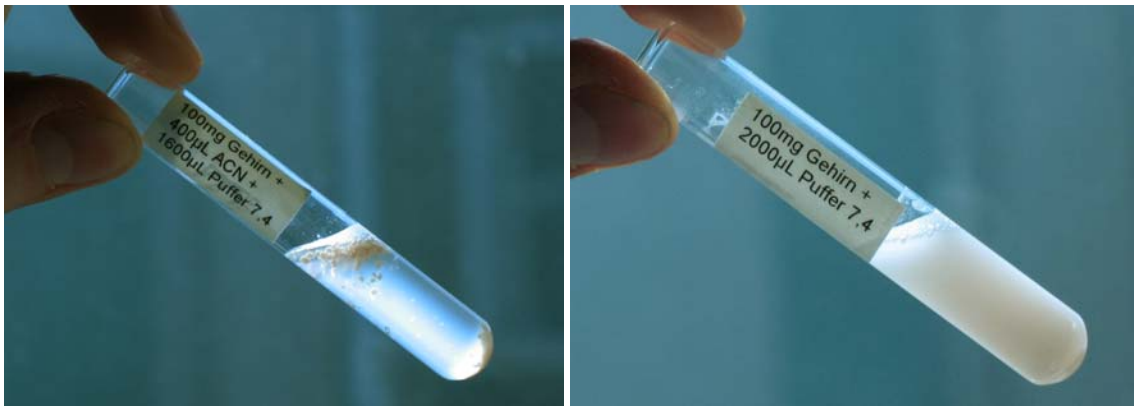

Fig. 2a and 2b. Pre-treated brain tissue sample with (Fig. 2a) and without (Fig. 2b) protein precipitation.

Disposable Pipettes (CX-1, 1 mL tips, DPX Labs, LLC) were used to extract drugs from the prepared supernatant (see Figure 3a).

The sorbent was conditioned with ACN followed by a phosphate buffer (0.05 M, pH 7.4). Using an attached syringe device, the tips were loaded with the sample solution and mixed thoroughly by hand until a homogenous mixture was reached with the sorbent (see Figure 3b). The solution was allowed to stay in the tip for about 2 minutes before it was discharged. After washing with water and pH-adjustment with hydrochloric acid (1 M), the tips were eluted, first with ethyl acetate/isopropanol (3:1) for acidic/neutral drugs, followed by ethyl acetate/isopropanol/triethylamine (75:25:3) for basic drugs.

The elution reagents were also allowed to equilibrate with the sorbent for 2 min. before the solution was subsequently dispensed directly into GC vials.

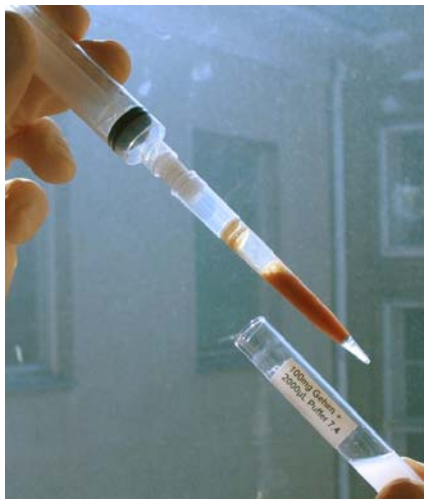

Fig. 3a and 3b. Dry DPX pipette tip with loose sorbent and syringe device (Fig. 3a.). Pipette tip loaded and homogenized with buffer solution (Fig. 3b).

To evaporate the solvents, 10µL HCl (0.01 M in methanol) was added to the basic extracts and a vacuum-concentrator Alpha RVC (Christ, Osterode, Germany) was used.

Detection and quantification: GC-MS-SIM (HP 6890 gas chromatograph with HP 6890 auto-sampler connected to a HP 5973 MSD) after silylation.

3. Results and Discussion

Six homogenized brain samples from a pig were spiked with analytes. Extraction was done manually using DPX pipette tips with a syringe (see Methods).

For method development, parameters like protein precipitation during sample pre-treatment, pH of the sample, wettability of the sorbent, homogenous distribution and equilibration time in the pipette tip were investigated.

Most of the drugs analysed showed reproducible recovery rates (see Table 1). It could be shown that recoveries were dependent on sample pre-treatment where avoiding protein precipitation resulted in more reliable results.

Moreover, the homogenous mixture of the sorbent with the sample solution turned out to be essential and could be improved by conditioning the sorbent with ACN.

4. Conclusion

Analytes with different physico-chemical properties could be extracted from a complex biological matrix (postmortem brain samples) by manual Disposable Pipette Extraction.

The developed method can be applied in laboratories where tissue extractions are not performed on a routine basis and therefore no device for (automated) extraction of solid-phase cartridges is available.

Tab. 1. Results for three brain samples without and with protein precipitation; test-analytes, mean recovery, and standard deviation (SD); nd= not detectable.

Analyte	**Mean recovery (%, n=3) without precipitation**	**SD**	**Mean recovery (%, n=3) with precipitation**	**SD**
amphetamine	16	0.9	9	0.9
benzoylecgonine	53	3.7	5	0.3
cocaine	84	2.1	59	1.5
codeine	70	5.5	37	2.4
diazepam	73	6.8	52	2.6
doxepine	69	7.5	62	1.6
ibuprofen	66	4.0	nd	-
methadone	82	7.6	64	3.4
metoprolol	35	6.7	20	0.3
morphine	62	6.2	25	3.9
phenobarbitone	43	7.1	nd	-
THC	34	5.8	nd	-
THCA	10	1.7	5	0.2

Experiments to fully automate the method with a MultiPurposeSampler (MPS, Gerstel, Germany) revealed difficulties regarding wettability of the sorbent. For the homogenous distribution of the sorbent and sample solution, a high amount of mixing energy was necessary which could not be achieved by the automation device but which could be easily controlled when extraction was performed manually.

5. References

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