SCREENING FOR THE GENERAL UNKNOWN IN TISSUE SAMPLES BY POLYSTYRENE RESINS

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ABSTRACT: Screening for a broad range of different compounds in complex biological matrices needs a non-selective clean-up procedure. To meet this challenge, a semi-automatic method, appropriate to handle 5 ml of post-mortem blood or 1 g of tissue sample, using the polystyrene resins OASIS™ (Waters) or ISOLUTE™ 101 (IST) was developed. This procedure enables the isolation of lipophilic organic compounds from post-mortem tissue samples and is suitable for the toxicological screening of general unknown poisons in target organs.

KEY WORDS: Drug screening; Post-mortem material; Polystyrene resin.

Problems of Forensic Sciences, vol. XLII, 2000, 169–172
Received 9 September 1999; accepted 16 May 2000

INTRODUCTION

One of the main objectives of forensic toxicology is the identification of general unknown poisons in biological materials.

For urine and serum samples, liquid/liquid extraction has proven convenient for this challenge. But these specimens are not always available, and liquid/liquid extraction is difficult to adapt for the clean-up of other samples, like post-mortem blood or tissue.

Today, solid phase extraction with modified silica is frequently applied for the extraction of a great number of different organic compounds from biological samples. For a general screening, the sorption characteristics of these sorbents are often too selective; moreover, their capacity for the clean-up of tissue samples is limited.

Therefore, a non-selective clean-up procedure using a general applicable sorbent for the isolation of a broad range of different compounds from complex biological matrices is needed.

RESULTS AND DISCUSSION

Macroporous organic polymers like Amberlite® XAD, which is produced by Rohm and Haas Company, have been used for the extraction of drugs from tissue samples since 1975 [2]. Quite recently new sorbents, such as Oasis™ from Waters Corporation and Isolute™ 101 from IST Company, were introduced on the market.

Both Amberlite® XAD and Isolute™ 101 are polystyrene resins.

Oasis™ is a copolymer of N-vinylpyrrolidon and styrene and therefore enables both hydrophilic and lipophilic retention characteristics.

Using these two sorbents, a semi-automatic procedure for the screening of the "general unknown" in complex biological matrices was developed.

The use of polystyrene resins has several advantages:

1. These sorbents are non-selective and therefore generally applicable for the isolation of a broad range of different compounds.
2. Because of the non-polar characteristics of polystyrene resins, no secondary interactions are observed, and therefore no "endcapping" as with bonded silica is needed.
3. Steep isotherms show fast adsorption and desorption characteristics, depending only on the particle size of the sorbent.
4. High capacity (specific surface areas from 500 to 800 m^2/g) and stability in a wide range of pH-values (from 1 to 14) are observed.
5. Finally, a defined relative pore volume between 5 and 10 nm enables a molecular sieve effect, leading to isolation and enrichment of low molecular lipophilic compounds from complex biological matrices.

In previous procedures, based on protein precipitation according to Jean Servais Stas, denaturation of proteins caused adsorption and occlusion of low molecular compounds, thus leading to an irreversible and varying loss of the analytes.

Alternatively, colloidal solutions, obtained by homogenization of postmortem samples of blood, liver and brain in buffer with a pH-value of 7.4 can avoid any precipitation of the proteins.

Comparing both clean-up procedures, it could be clearly seen that precipitation in methanol delivered extracts with much more concomitant substances, and that the isolation by polymeric sorbents extracted fewer matrix components, interfering with chromatography or mass spectrometry.

These observations are based on a process of micellar chromatography, which is slower compared to the rate of adsorption onto modified silica gel sorbents. Therefore, the adsorption rate for a volume of 50 ml colloidal solution is crucial and has to be controlled in the range of 1 ml per minute.

To improve the reproducibility, main parts of the extraction procedure were performed automatically on an Aspec XL from Gilson Company. This system consisted of two syringe pumps with a pressure sensor, a sample processor unit, and the racks and accessories to handle samples and solvents.

The Aspec XL was controlled by a personal computer with XTray-software from Abimed.

500 mg-columns of Oasis™ and Isolute™ 101 were applied to develop the extraction procedure on Aspec XL.

After homogenization and centrifugation of 5 ml post-mortem blood or 1 g of tissue sample in 50 ml buffer with a pH-value of 7.4, the adsorption step was performed at 1

ml/min. The elution with ethylacetate/isopropanol (3 + 1) was followed by evaporation of the solvent and separation of the acidic and basic fraction (Figure 1).

For both sorbents, the mean recovery of the reference substances phenobarbital, codeine and morphine on three successive work-ups was determined between 72 to 100 percent, using post-mortem blood, liver and brain. The concentration of the three drugs was selected according to toxicologically relevant concentrations [1].

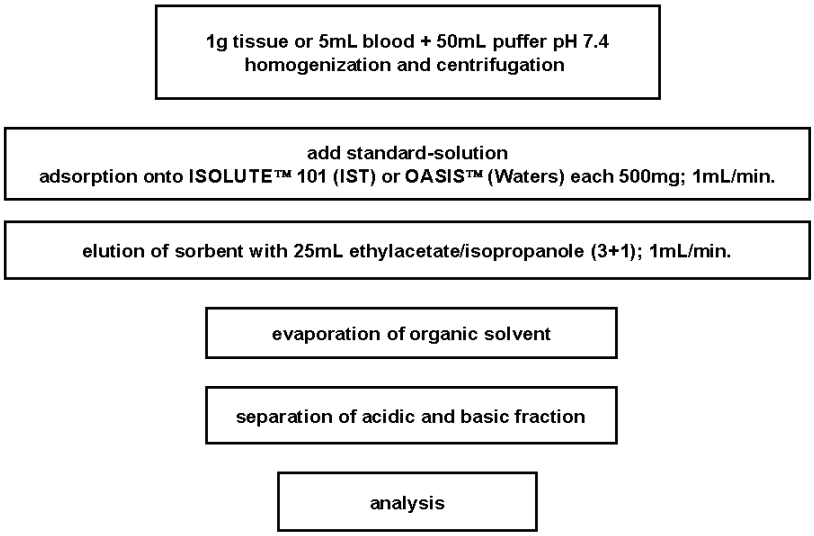

Fig. 1. Extraction procedure for post-mortem samples of blood and tissue.

The reproducibility of the whole process including sample preparation, sorption steps, liquid/liquid-extraction and gas chromatography mass spectrometry showed day-to-day relative standard deviations between 1 and a maximum of 17 percent.

CONCLUSIONS

A semi-automatic screening procedure for 5 ml of post-mortem blood or 1 g of tissue sample using the polystyrene resins Oasis™ (Waters) and Isolute™ 101 (IST) was developed.

This method enables the toxicological screening of general unknown poisons in post-mortem tissue samples.

Recovery of reference substances and day-to-day reproducibility meets the demands for a practical application of this procedure.

References:

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