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Semi-automated solid-phase extraction procedure for drug screening in biological fluids using the ASPEC system in combination with Clean Screen DAU columns

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ABSTRACT

The use of a semi-automated solid-phase extraction system (ASPEC) for the screening of drugs in plasma and urine on a single mixed-mode column (Clean Screen DAU) is described. The processes of column preconditioning, sample application, column wash, pH adjustment and elution of the drugs were accomplished by the ASPEC. After off-line evaporation, the residues were injected into a wide-bore capillary gas chromatograph. The recoveries of the tested drugs were in the range of 73–96%, with relative standard deviations less than 5% at a concentration level of 2 μg/ml.

INTRODUCTION

Solid-phase extraction (SPE), as a convenient and powerful tool for sample clean-up, is widely used in the analysis of drugs in biological samples. In recent years, SPE has not only been used for a single drug or a series of analogous compounds [1–3], but also for the screening of a wide range of drugs [4–7]. The advantages of SPE over traditional liquid–liquid extraction have been well documented in many publications (*e.g.* refs. 8 and 9). One of the major advantages is that the extraction procedure can be automated. Several semi- or fully automated SPE methods have been reported [10–12]. However, these methods have been developed for the extraction of individual drugs, or a series of analogues. It is evident that those automatic methods are less suitable for drug screening, since they have been optimized towards a given substance or a given class.

In this study, the use of a Gilson automatic extraction system ASPEC for screening purposes was investigated. The extraction procedure used was based on our earlier investigations into the extraction of acidic, neutral and basic drugs from plasma, whole blood and urine on Bond Elut Certify columns [6,7]; satisfactory recoveries were obtained, as well as clean extracts. In order to match the SPE rack of the ASPEC system, 1-ml size Clean Screen DAU SPE columns were used. These columns contain both hydrophobic and cation-exchange functional groups, which are claimed to be especially suitable for retaining drugs with different physicochemical properties [13].

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EXPERIMENTAL

Materials

Methamphetamine hydrochloride, hexobarbital, pentobarbital, methylphenobarbital, mepivacaine hydrochloride, methadone hydrochloride, amitriptyline hydrochloride, codeine, ketazolam, dipipanone hydrochloride and prazepam were obtained from commercial suppliers and were of pharmacopoeial quality. Individual stock solutions (1 mg/ml) were prepared by dissolving the appropriate amount of drug in methanol–ethyl acetate (1:1). These stock solutions were stored in glass tubes at 4°C. The chromatographic standard solution was prepared by diluting the stock solution of prazepam with ethyl acetate to 20 μg/ml. The phosphate buffer (0.1 *M*, pH 6.0), the acetic acid solution (0.01 *M*, pH 3.3), acetone–chloroform (1:1) and 2% ammoniated ethyl acetate were prepared as described in our previous paper [6].

All reagents were of analytical grade (Merck, Darmstadt, Germany). Clean Screen DAU columns (130 mg of sorbent mass, 1 ml of column volume) were kindly supplied by Worldwide Monitoring (Horsham, PA, USA). This sorbent contains *n*-alkyl (*ca.* C_8) functional groups and benzenesulphonic acid functional groups, as shown in Fig. 1 [13].

Instrumentation

The extraction was performed with an ASPEC (automatic sample preparation with extraction columns) system (Gilson Medical Electronics, Villiers le Bel, France). As shown in Fig. 2, the ASPEC system consists of three components, a Model 401 dilutor, a sample processor and a set of racks and accessories to handle 1-ml SPE columns and solvents. The system can receive up to 108 samples if 1-ml SPE columns are used.

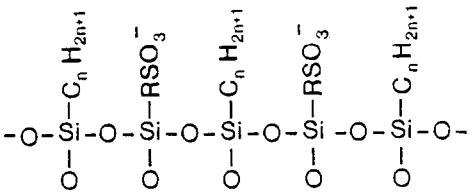

Fig. 1. Hydrophobic and cation-exchange functional groups in copolymeric bonded phase Clean Screen DAU columns.

A Hewlett-Packard 5880 A gas chromatograph (Avondale, PA, USA) equipped with a Hewlett-Packard 7671 A automatic sampler, a 5880 A GC terminal and a flame ionization detector was used for the analysis. An HP-1 fused-silica wide-bore capillary column (30 m × 0.53 mm I.D., film thickness 0.88 μm, Hewlett-Packard) was installed in the chromatograph. Injector and detector temperatures were maintained at 275 and 300°C, respectively. The oven temperature was held at 80°C for 2 min then increased at 20°C/min to 215°C, followed by an increase of 5°C/min to 285°C; it was held at this final temperature for 2 min. The injection port was set in the splitless mode, and the helium carrier gas flow-rate was 10 ml/min.

Specimen pretreatment

Citrated calf plasma (1.2 ml) or human urine (1.2 ml) spiked with appropriate drugs (2 μg/ml) was diluted with phosphate buffer (3.6 ml) in a glass sample tube. The mixture was vortex-mixed to ensure homogeneity. The concentration of 2 μg/ml was chosen for analytical convenience as well as to represent toxicologically relevant levels for most of drugs.

Extraction

Clean Screen DAU columns were sealed with polypropylene caps and installed on the SPE rack (Fig. 2). The solvents and the buffered samples were placed in the solvent positions and sample rack, respectively. Glass collection tubes (55 × 12 mm) were placed in the collection rack. The extraction was then performed by ASPEC in a sequential mode as follows:

(1) The column was preconditioned with 1 ml of methanol and subsquently with 1 ml of phosphate buffer (pH 6.0) at a flow-rate of 1.0 ml/min.

(2) The buffered sample (4 ml) was dispensed onto the column and pushed through the column by positive pressure at a flow-rate of 1 ml/min. 800-μl volume of air was applied to the column to push the sample through completely.

(3) The water-soluble endogenous compounds

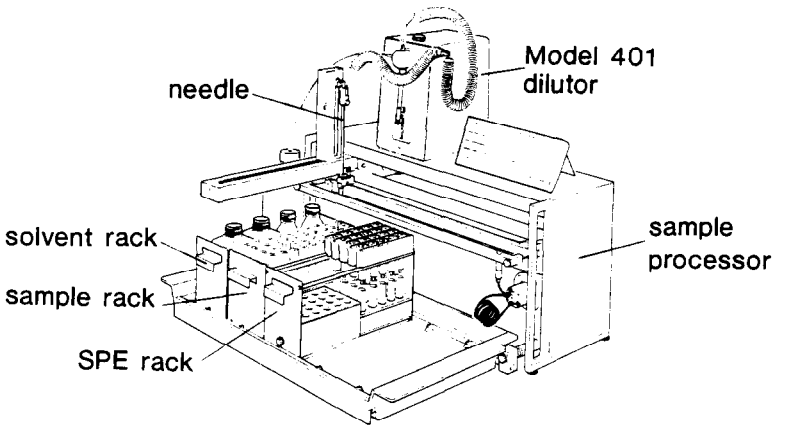

Fig. 2. The ASPEC system, which consists of three main components: a Model 401 dilutor, a sample processor and a set of racks and accessories to handle 1-ml SPE columns and solvents.

were washed from the column by 1 ml of deionized water.

(4) The pH of the extraction system was adjusted by passing 0.5 ml of 0.01 *M* acetic acid (pH 3.3) through the column.

(5) The residual water was removed from the column by 60 μl of methanol, and the column was dried by passing 6 ml of air through it.

(6) The acidic and the neutral drugs were eluted into the collection tube with 3.5 ml of acetone–chloroform (1:1) at a flow-rate of 1 ml/min (fraction A).

(7) After manually replacement of a labelled collection tube in the collection rack, 2 ml of 2% ammoniated ethyl acetate were added to the column at a flow-rate of 0.5 ml/min to elute the basic drugs (fraction B).

The two fractions (A and B) were transferred to separate evaporation tubes, and 100 μl of chromatographic standard solution were added to each. Both fractions were evaporated in a water-bath at 40°C under a gentle stream of nitrogen until *ca.* 100 μl of solvent remained in the tubes. Of each fraction, 2 μl were injected into the

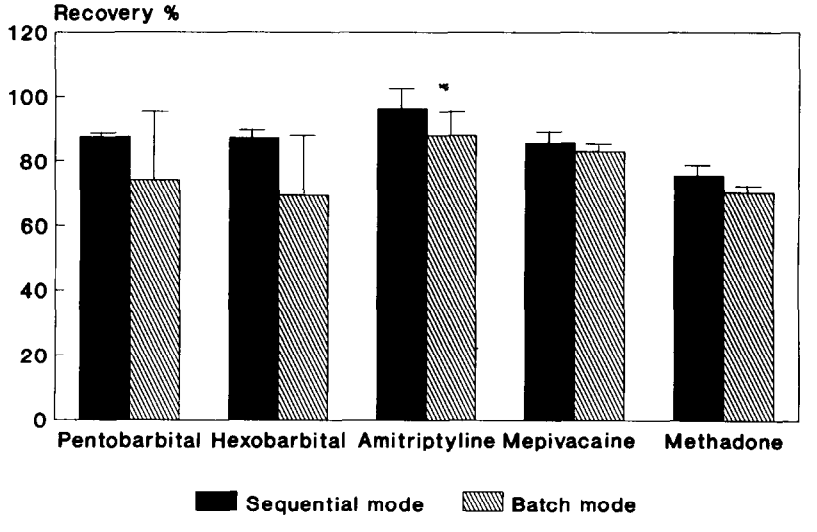

Fig. 3. Recoveries (mean and S.D.) of drugs from human urine in the two extraction modes of the ASPEC system (n = 3).

gas chromatograph. The ratio of the peak heights of the tested drugs to that of the chromatographic standard, prazepam, was used for quantitation.

RESULTS AND DISCUSSION

In order to evaluate the ASPEC system for screening, the drugs in this study were chosen to represent various stuctural characteristics and classes as well as to cover a relatively wide range of GC retention indices [14] to ensure different volatilities.

The ASPEC system is designed to carry out the extraction of a series of samples in either a batch or a sequential mode. In the batch mode, all of the SPE columns in the same SPE rack are treated batchwise: the next step of the extraction procedure will not take place until the previous one has been completed on all the samples in the same rack. In the sequential mode, the entire extraction is performed sample by sample: the ASPEC accomplishes the complete extraction process of the first sample, and then continues with the next sample. It was found that the batch mode was not suitable for a relatively large sample volume (in this study the volume of the buffered sample was 4 ml). Since the extraction could be performed on only one column at a time, this resulted in too long a time between the first column and the last column, so that the columns dried before the next sample application. As a result, all tested drugs showed significantly lower recoveries as well as poor reproducibilities (Fig. 3). In the batch mode for pentobarbital and hexobarbital the relative standard deviations exceeded 25%, for example. Therefore, the sequential mode was employed in the study.

In most of the applications of automatic SPE [10–12], the actual assay of the drugs was achieved by reversed-phase high-performance liquid chromatography (HPLC). In these cases the column drying step was not important, because the compounds of interest were eluted by a water-miscible organic solvent such as methanol, acetonitrile or the mobile phase of the HPLC system. Because of its good separation efficiency, high sensitivity and inertness, capillary GC is a particularly valuable technique for drug screening [15], and was therefore used in this study. Since residual water may influence the elution of drugs, and can damage the GC column, it has to be removed from the SPE column before elution of the drugs with a water-immiscible organic solvent. Unlike in manual SPE vacuum systems, positive air displacement is used as the drying force in the ASPEC system. In order to get a "completely" dry eluate, different volumes of air for drying SPE column were tested and 6 ml of air was found to be adequate.

Since the built-in operation programs of the ASPEC [16] were used for the extraction, allowing a maximum addition of elution solvent of 1 ml at a time, the elution of fraction A was performed by passing three 1-ml and one 0.5-ml aliquots of acetone–chloroform through the column, and fraction B was eluted with two 1-ml volumes of ammoniated ethyl acetate. The extraction method described here is in fact a semi-automatic process, because the collection tubes had to be changed manually to collect the two fractions separately so that the drugs could be classified into two pH-dependent groups. This fa-

TABLE I

RECOVERIES AND PRECISION DATA OF ELEVEN DRUGS FROM PLASMA

Plasma spiked with 2 μg/ml of each drug.

Drug	Recovery (n = 3) (%)		Relative standard deviation (%)
	Fraction A	Fraction B	
Allobarbital	77.8	N.D.[a]	1.48
Amitriptyline	N.D.	79.9	4.51
Codeine	N.D.	86.0	3.96
Dipipanone	N.D.	89.2	1.39
Hexobarbital	80.4	N.D.	3.25
Ketazolam	79.1	N.D.	3.04
Mepivacaine	N.D.	88.6	2.80
Methadone	N.D.	89.2	2.70
Methamphetamine	N.D.	73.2	4.64
Methylphenobarbital	96.3	N.D.	4.16
Pentobarbital	82.2	N.D.	4.05

[a] N.D. = not detected.

cilitates the identification process in unknown samples [6].

The absolute recovery data for eleven selected drugs from spiked plasma are shown in Table I and, in general, are quite satisfactory. The recoveries for various classes of drugs range from 73% for methamphetamine to 96.3% for methylphenobarbital, with relative standard deviations of less than 5%. The recoveries of the tested drugs are acceptable for screening and the reproducibilities are better than in the manual procedure. Moreover, Table I shows that, by using a single Clean Screen column, acidic and neutral drugs were extracted from plasma by the hydrophobic functional groups of the solvent and eluted by acetone–chloroform, whereas the basic drugs remained on the column at this stage, owing to ion-exchange interactions with the benzenesulphonic acid group of the sorbent. The basic drugs were subsequently eluted by 2% ammoniated ethyl acetate. Fig. 4 depicts typical results for spiked plasma, where chromatogram A represents the extract of fraction A, and chromatogram B that of fraction B. As can be seen, both extracts (A and B) are clean under the conditions used. The peak at the end of the chromatogram of fraction A is caused by endogenous cholesterol, which did not interfere with the GC analysis of most toxicologically relevant drugs [7].

CONCLUSION

The procedure for drug screening developed for manual SPE can be performed semi-automatically using the ASPEC automatic SPE system. With a single mixed-mode SPE column (Clean Screen), various classes of drugs were isolated from biological fluids. Acceptable recoveries of the tested drugs (73–96%) with low relative standard deviations (less than 5%) were obtained. In order to get satisfactory results, the extraction should be performed in the sequential mode rather than in the batch mode. Advantages of the automated procedure over the manual one are a better reproducibility (*e.g.* for methamphetamine the relative standard deviation in the automated procedure was 4.6% against 9.8% in the manual procedure, see ref. 7), and less labour. The

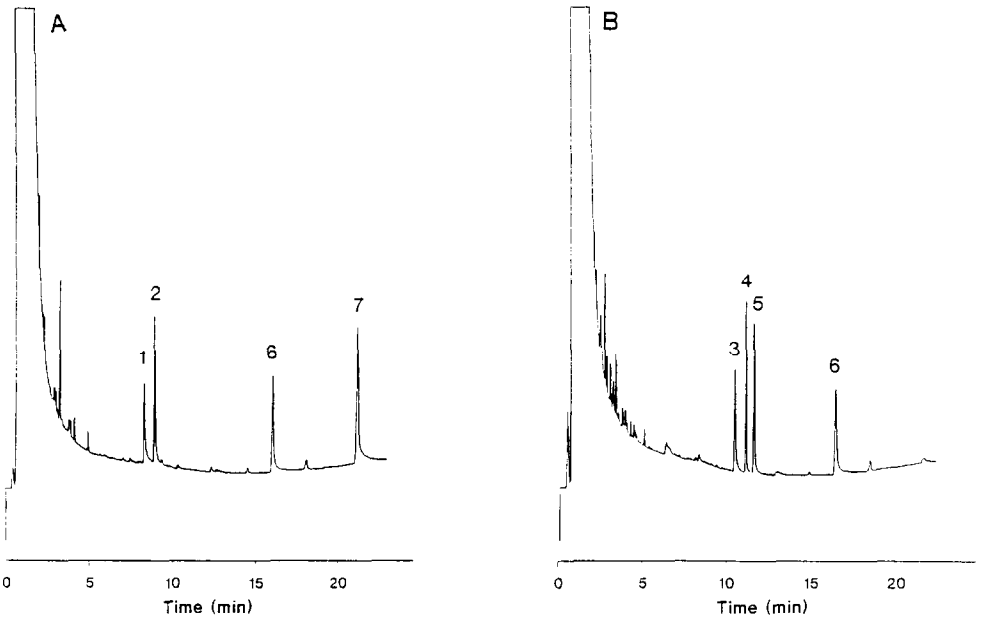

Fig. 4. Typical chromatograms of extracts of calf plasma, spiked with 2 μg/ml of each drug: (A) Fraction A; (B) fraction B. Peaks: 1 = pentobarbital; 2 = hexobarbital; 3 = mepivacaine; 4 = methadone; 5 = amitriptyline; 6 = prazepam (chromatographic standard); 7 = cholesterol.

manual procedure requires the full attention of the analyst during the extraction, whereas the automated procedure, once set up, can process 72 samples while the analyst has to change the collection tubes only once every 30 min. The method appears to be suitable for the screening of drugs from plasma as well as from urine. The disadvantage of this semi-automated SPE procedure is the need for regular operator intervention. In order to solve this problem the investigation of a fully automatic SPE procedure for drug screening is in progress.

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REFERENCES

1 O. Suzuki, T. Kumazawa, H. Seno and H. Hattori, *Med. Sci. Law*, 29 (1989) 243.
2 M. Mazhar and S. R. Binder, *J. Chromatogr.*, 497 (1989) 201.
3 B. K. Logan, D. T. Stafford, I. R. Tebbett and C. M. Moore, *J. Anal. Toxicol.*, 14 (1990) 154.
4 X.-H. Chen, J. Wijsbeek, J. Van Veen, J.-P. Franke and R. A. de Zeeuw, *J. Chromatogr.*, 592 (1990) 161.
5 B. C. Thompson, J. M. Kuzmack, D. W. Law and J. J. Winslow, *LC·GC Int.*, February (1990) 55.
6 X.-H. Chen, J. Wijsbeek, J.-P. Franke and R. A. de Zeeuw, *J. Forensic Sci.*, 37 (1992) 61.
7 X.-H. Chen, J. Wijsbeek, J.-P. Franke and R. A. de Zeeuw, *J. Anal. Toxicol.*, 16 (1992) 351.
8 R. D. McDowall, *J. Chromatogr.*, 492 (1989) 3.
9 E. L. Johnson, D. L. Reynolds, D. Scott Wright and L. A. Pachla, *J. Chromatogr. Sci.*, 26 (1988) 372.
10 H. T. Karnes, D. Farthing and E. Besenfelder, *J. Liq. Chromatogr.*, 12 (1989) 1809.
11 J. C. Pearce, R. D. McDowall, A. El Sayed and B. Pichon, *Int. Lab.*, November/December (1990) 34.
12 Ph. Hubert and J. Crommen, presented at the *2nd International Symposium on Pharmaceutical and Biomedical Analysis, York, April, 1990.*
13 E. Heebner, M. Telepchak and D. Walworth, *The Use of Novel Bonded Phase Absorbents for the Extraction of Abused Drugs from Biological Matrices*, Application note, Worldwide Monitoring Corporation, Horsham, PA, 1991.
14 A. C. Moffat, *Clarke's Isolation and Identification of Drugs*, The Pharmaceutical Press, London, 1986.
15 I. Ojanperä, I. Rasanen and E. Vuori, *J. Anal. Toxicol.*, 15 (1991) 204.
16 *ASPEC System User's Guide*, Gilson Medical Electronics, Villiers le Bel.