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Note

Solid-phase extraction for the screening of acidic, neutral and basic drugs in plasma using a single-column procedure on Bond Elut Certify

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The pretreatment of biological samples is a very important process before any identification in systematic toxicological analysis (STA). In recent years a large variety of solid-phase resins has been developed and evaluated [1–10]. It has been demonstrated that solid-phase extraction (SPE) can be a useful means for the isolation of drugs from biological matrices, yet the large majority of the publications has been geared towards the isolation of one drug or a limited number of related compounds. In STA, however, a screening analysis must be performed because no reliable information is available on the nature and/or the number of substances present. Hence a sample pretreatment step for STA cannot be optimized towards a given substance or a given class. Instead, it must compromise between an acceptable recovery of a great many different substances and an adequate removal of matrix compounds within a reasonably short period of time.

This paper describes an extraction procedure for the isolation of acidic, neutral and basic drugs from calf plasma using a single Bond Elut Certify column. This column contains a proprietary bonded silica sorbent, which exhibits a unique hydrophobic ion-exchange extraction mechanism and will retain acidic, neutral and basic drugs under the proper extraction conditions. The column was originally developed for the extraction of drugs with abuse potential from

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urine, using optimized procedures for different classes, such as amphetamines, barbiturates, opiates, etc. [11].

EXPERIMENTAL

Materials

Methamphetamine hydrochloride, hexobarbital, mepivacaine hydrochloride, trimipramine hydrochloride, levallorphan tartrate, nitrazepam and prazepam were obtained from commercial suppliers and were of pharmaceutical quality. Stock solutions were prepared by dissolving the appropriate amount of drug in methanol–ethyl acetate (1:1) to make the solutions equivalent to 2 mg/ml. They were stored at 4°C. Methanol, hexane, methylene chloride, ethyl acetate, glacial acetic acid, potassium hydroxide, potassium dihydrogenphosphate and diethylamine were of p.a. grade (Merck, Darmstadt, F.R.G.). Ammonia (33%) was extra-pure quality (Merck). Bond Elut Certify columns (Cat. No. LR 29904) were obtained from Analytichem International (Harbor City, CA, U.S.A.) and the Baker-10 SPE vacuum manifold system, which can accept ten SPE columns simultaneously, was purchased from J.T. Baker (Phillipsburg, NJ, U.S.A.).

Extraction procedure

Heparinized calf plasma was spiked with methamphetamine hydrochloride, hexobarbital, mepivacaine hydrochloride, trimipramine hydrochloride, levallorphan tartrate and nitrazepam, using the stock solution in methanol–ethyl acetate (1:1). The concentration of each drug in the plasma was 10 μg/ml, representing a toxicologically relevant as well as an analytical convenience. After adequate mixing and equilibration of the plasma samples, for each analysis 6 ml of 0.1 *M* phosphate buffer (pH 6.0) were added to 2 ml of spiked plasma.

Bond Elut Certify columns were inserted into the vacuum manifold cover. Each column was preconditioned by sequential washings with 2 ml of methanol and 2 ml of 0.1 *M* phosphate buffer under light vacuum ($6.82 \cdot 10^{-2}$ bar or 2 in.Hg on the meter). The vacuum was turned off when the phosphate buffer was close to reaching the top of the sorbent bed to prevent the sorbent running dry. Then 8 ml of diluted plasma sample were pipetted onto the column and drawn through completely at a flow-rate of ca. 1.5 ml/min (ca. $8.5 \cdot 10^{-2}$ bar or 2.5 in.Hg on the meter). The column was then washed with 1 ml of phosphate buffer mixed with 200 μl of methanol and subsequently with 1 ml of 1.0 *M* acetic acid. The column was dried under full vacuum ($5.1 \cdot 10^{-1}$ bar or 15 in.Hg on the meter) for 5 min, then 1 ml of hexane was added and the column was dried again under full vacuum for 1 min. The column outlet and the manifold basin were wiped with tissue, and an evaporation tube was placed into the manifold basin. Analytes were eluted from the column in two steps. The first

eluate, containing acidic and neutral drugs, was obtained by passing 4 ml of methylene chloride through the column at a flow-rate of ca. 1.7 ml/min (ca. $3.4 \cdot 10^{-2}$ bar or 1 in.Hg on the meter). The second eluate, containing the basic drugs, was obtained by passing 2 ml of ethyl acetate–33% ammonia (98:2) through the column at a flow-rate of ca. 0.6 ml/min (no vacuum). The eluates were combined and evaporated in a water-bath at 40°C under a gentle stream of nitrogen for ca. 4 min, then two drops of 2.0 *M* acetic acid in ethyl actate were added to the tube and the evaporation was completed to dryness. The residue was reconstituted in 100 μl of chromatographic standard solution (200 μg/ml prazepam in 0.2% diethylamine–ethyl acetate), of which 4 μl were injected into the gas chromatograph.

Gas chromatographic analysis

The analysis was carried out on a Hewlett-Packard (Avondale, PA, U.S.A.) Model 5880 A gas chromatograph equipped with a 5880 A GC terminal, and a flame ionization detector. The column was a fused-silica wide-bore capillary column, 25QC5/BP1 (25 m×0.53 mm I.D., film thickness 3.0 μm, SGE, Victoria, Australia). The oven temperature programme was 2 min at 80°C, 20°C/min to 215°C, 5°C/min to 285°C and 2 min at 285°C. The injector and detector temperatures were 275 and 310°C, respectively. The helium carrier gas flow-rate was 10.5 ml/min and the injection port was in the splitless mode.

Recoveries

Calibration curves were prepared with standard solutions of each drug. The concentrations were 100, 150, 170, 200, 220, 250 and 300 μg/ml and the concentration of prazepam (chromatographic standard) was fixed at 200 μg/ml. Each concentration was injected twice (4 μl). Peak-height ratios of drug to chromatographic standard were measured, and the calibration curves were obtained from least-squares linear regression. The regression lines were used to calculate the recoveries of the individual drugs from plasma.

RESULTS AND DISCUSSION

The drugs investigated in this pilot study were selected so as to represent various characteristics and classes as well as to cover a relatively wide range of GC retention indices to simulate different volatilities [12].

In order to properly pass the plasma through the columns it was found necessary to apply a 1:4 dilution with phosphate buffer. Smaller amounts of buffer caused clogging of the columns.

Fig. 1 shows the gas chromatograms of blank plasma (A) and a spiked plasma sample (B) under the same conditions. As can be seen, a fairly clean extract was obtained. The small peaks at ca. 3.5 and 8.5 min are due to the solvents and/or the cap septum of the vial.

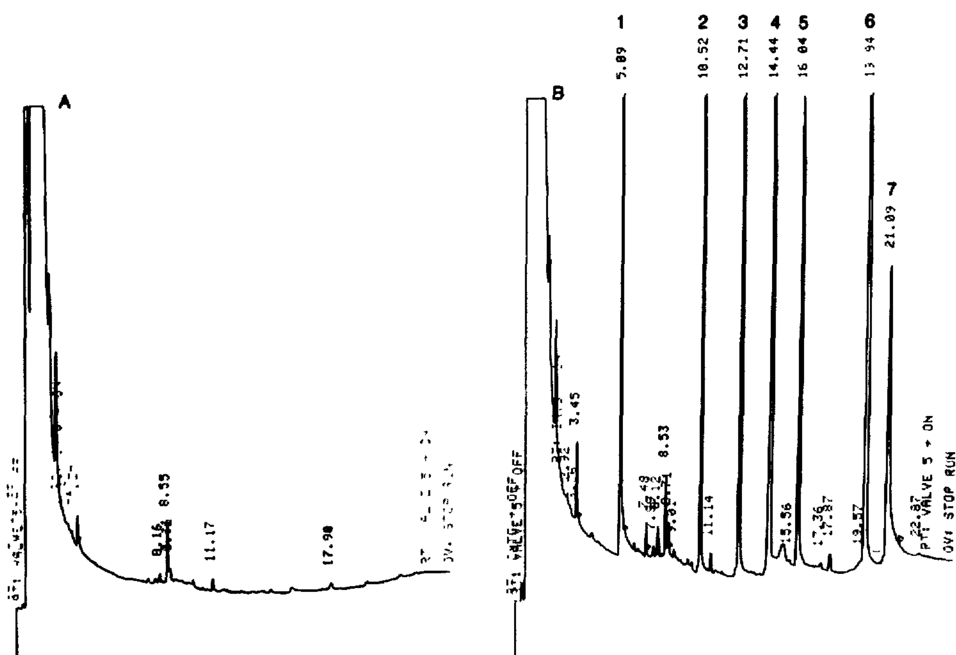

Fig. 1. Chromatograms of plasma samples. (A) Blank calf plasma; (B) calf plasma spiked with drugs, the concentration of each drug being 10 μg/ml. Peaks: 1 = methamphetamine; 2 = hexobarbital; 3 = mepivaciane; 4 = trimipramine; 5 = levallorphan; 6 = prazepam (chromatographic standard); 7 = nitrazepam.

It was very important to dry the SPE column before elution of the drugs because residual water in the column negatively affected the elution of some drugs. Moreover, the presence of water in the eluate prolonged the evaporation time, resulting in losses of the more volatile drugs. Elution with 1 ml of hexane was found to be adequate to displace the final traces of adsorbed water, without affecting drug recoveries. In the present procedure, methylene chloride was used for eluting the acidic and neutral drugs, and ammonia–ethyl acetate was used for eluting the basic drugs. In the evaporation step, two drops of acetic acid solution were added to the eluate to prevent the disappearance of the more volatile bases, such as amphetamines.

Good linear relationships between peak-height ratios and drug concentrations in the range 100–300 μg/ml were found for all drugs. The parameters of regression are listed in Table I.

Table II shows the recoveries from spiked calf plasma of the drugs under investigation with values between 86.0 and 112.9%. The reproducibilities are also listed in Table II, showing coefficient of variation of 10% or less ($n = 10$). The somewhat lower reproducibility for methamphetamine conceivably re-

TABLE I

LINEAR REGRESSION EQUATION PARAMETERS FOR THE VARIOUS CALIBRATION CURVES

Values are for a and b in the equation $y=ax+b$

Drug	a	b	Correlation coefficient (r)
Methamphetamine	0.0079	−0.408	0.997
Hexobarbital	0.0076	−0.076	0.997
Mepivacaine	0.0060	−0.034	0.999
Trimipramine	0.0054	−0.042	1.000
Levallorphan	0.0024	−0.053	0.999
Nitrazepam	0.0022	−0.172	0.995

TABLE II

RECOVERY AND REPRODUCIBILITY OF DETERMINATION OF DRUGS AFTER EXTRACTION FROM CALF PLASMA BY MEANS OF BOND ELUT CERTIFY COLUMNS

Drug	Mean recovery ($n=10$) (%)	Coefficient of variation (%)
Methamphetamine	93.3	10.27
Hexobarbital	86.0	6.37
Mepivacaine	98.2	2.23
Trimipramine	93.6	2.23
Levallorphan	112.6	4.80
Nitrazepam	101.7	5.27

flects the evaporation step. The high recovery of levallorphan of 112% cannot be explained at the moment.

The time needed for the conditioning of the columns plus the elution is ca. 30 min when ten columns are being used simultaneously. Evaporation of the combined eluates required some 30 min per sample. It should be noted that eluates can be collected separately so as to represent the fraction of acidic and neutral drugs and the fraction of basic drugs, respectively.

The present approach appears promising for the extraction of plasma samples in STA, offering good recoveries, reproducibilities and speed. Further studies are being carried out with additional drugs of toxicological relevance, as well as with regard to compatibility with other chromatographic techniques. Although the manufacturer recommends Bond Elut Certify columns to be used only once for urine, it remains to be seen if multiple use is possible for plasma samples.

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