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# Short Communication

## Reusability of Bond Elut Certify columns for the extraction of drugs from plasma

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ABSTRACT

The reusability of Bond Elut Certify columns for the extraction of toxicologically relevant drugs from plasma has been evaluated. Pentobarbital, hexobarbital, mepivacaine, trimipramine and clonazepam were selected as test drugs to represent various classes of drugs. The columns were regenerated immediately after an extraction by washing with methanol, hydrochloric acid (1%), water and methanol, sequentially. These regenerated columns were found to be reusable for plasma. More than 85% of the test drugs were recovered when the columns were used three times. However, the extraction power of the regenerated columns decreased slightly with the number of reuses, so that a column should not be used more than two or three times.

### INTRODUCTION

As demonstrated in our earlier papers [1–3], the mixed-mode solid-phase extraction (SPE) columns with hydrophobic and cation-exchange interactions are the best choice to prepare samples of biological fluids for drug screening in systematic toxicological analysis (STA). Various classes of drugs can effectively be extracted from plasma, whole blood and urine, and separated into two groups according to their physicochemical properties on a single mixed-mode SPE column. However, the columns are still relatively expensive for routine work for many laboratories. In addition, cost is one of the factors for evaluating a new analytical method. For economic reasons, some scientists have tried to reuse the SPE materials. The reusability of polymeric SPE columns has been documented by Patel *et al.* [4]. Because polymer-based SPE materials are stable over the entire pH range (from 1 to 14), the columns can easily be regenerated. Juergens [5] has reported that the bonded-silica SPE cartridges could be reused up to ten times, but no details were given.

In this study, we investigated the reusability of Bond Elut Certify columns for the extraction of plasma samples in STA. The examination was carried out by testing the recoveries of five selected drugs obtained from regenerated columns.

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## EXPERIMENTAL

### *Materials*

Methanol, ethyl acetate, acetone, chloroform and potassium dihydrogenphosphate were from Merck (Darmstadt, Germany) and were of analytical grade. Ammonia (33%) was of extra-pure quality (Merck). Hydrochloric acid (36%) was of analytical grade and was from Brocacef (Maarssen, Netherlands). The 0.1 *M* phosphate buffer (pH 6.0 ± 0.1), 0.01 *M* acetic acid (pH 3.3) and 2% ammonia in ethyl acetate were prepared as described previously [1]. Hydrochloric acid solution (1%) was prepared by diluting 5.6 ml of 36% hydrochloric acid in 200 ml with deionized water.

Pentobarbital, hexobarbital, mepivacaine hydrochloride, trimipramine hydrochloride, clonazepam and prazepam were of pharmacopoeial quality and were obtained from commercial suppliers. Stock solutions (1 mg/ml) of individual drugs 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 200 $\mu$g/ml. The test drugs were chosen so as to represent various toxicologically relevant drug classes, as well as to cover a relatively broad range of gas chromatographic (GC) retention indices to simulate different volatilities [6]. A concentration of 10 $\mu$g/ml was used for analytical convenience as well as to represent toxicologically relevant levels for most of the drugs.

Bond Elut Certify columns (130 mg of sorbent mass, 10 ml of column size) were supplied by Varian Sample Preparation Products (Harbor City, CA, USA). The Baker-10 SPE vacuum manifold system was purchased from J.T. Baker (Phillipsburg, NJ, USA).

### *Chromatographic equipment*

The quantitative analysis was performed on a Hewlett-Packard 5880A gas chromatograph (Avondale, PA, USA) equipped with a flame ionization detector, an HP 7671A automatic sampler and an HP 5880A GC terminal. The GC column was an HP-1 fused-silica wide-bore capillary (30 m × 0.53 mm I.D., 0.88-$\mu$m film thickness). The chromatographic operating conditions were initialized at 80°C with a 2-min hold. The temperature was then programmed from 80 to 215°C at 20°C/min, followed by an increase of 5°C/min to 285°C. The final temperature was held for 2 min. The injection port and detector temperatures were 275 and 310°C, respectively. The injection port was in the splitless mode.

### *Regeneration of Bond Elut Certify columns*

After completion of an extraction process, the Bond Elut Certify column was regenerated immediately as follows. (1) The column was washed with 2 ml of methanol followed by 4 ml of hydrochloric acid (1%). (2) The column was dried by applying vacuum (*ca.* 51 kPa) for 2 min. (3) Deionized water (10 ml) was passed through the column under light vacuum (*ca.* 7 kPa). (4) The column was dried again for 2 min. (5) Methanol (2 ml) was passed through the column and the column was dried again for *ca.* 5 min. (6) Both the luer tip and reservoir of the column were covered by plastic sealing film (Nescofilm, Nippon Shoji Kaisha, Osaka, Japan), and the column was stored in the dark until the next use.

### *Specimen pretreatment*

Citrated calf plasma (2 ml) spiked with appropriate drugs (10 $\mu$g/ml) was diluted with phosphate buffer (6 ml) in a glass sample tube. The mixture was vortex-mixed to ensure homogeneity.

### *Extraction of spiked plasma*

The extraction was performed with either Bond Elut Certify or regenerated Bond Elut Certify columns by installing them on a Baker-10 vacuum manifold. The extraction procedure was the same as described in our previous paper [1]. Briefly, the column was preconditioned with 2 ml of methanol followed by 2 ml of phosphate buffer (0.1 *M*, pH 6). The buffered sample was loaded onto the column and passed through completely (1.5 ml/min). After washing with 1 ml of deionized water, the column was acidified with 0.5 ml

of acetic acid solution (0.01 *M*, pH 3.3). The column was then dried by applying vacuum, and the residual water was removed with 50 μl of methanol. The first fraction (fraction A) containing acidic, neutral and weakly basic drugs, was obtained by elution with 4 ml of acetone–chloroform (1:1, 0.8 ml/min). The second fraction (fraction B), containing strongly basic drugs, was obtained by elution with 2 ml of 2% ammoniated ethyl acetate (0.5 ml/min). The two fractions were collected separately. To each fraction, 100 μl of chromatographic standard solution (prazepam, 200 μg/ml) were added. Both fractions were evaporated in a water-bath 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 gas chromatogarph.

*Recovery study*

Standard calibration curves were prepared with standard solutions of each drug. The concentrations were 100, 150, 170, 200, 200, 250 and 300 μg/ml and the concetration of prazepam (chromatographic standard) was fixed at 200 μg/ml. The peak height ratios of drugs to prazepam were measured, and the calibration curves were generated from least-squares linear regression. The regression lines were used to calculate the absolute recoveries of individual drugs from spiked plasma.

## RESULTS AND DISCUSSION

*Regeneration procedure*

Some endogenous plasma constituents were retained on the column after extraction. These endogenous components covered the functional groups of the sorbent, which reduced the extraction potential of the column when the column was reused directly without further cleaning. Therefore, it was essential to regenerate the column with suitable solvents.

The main functional groups of the sorbent of Bond Elut Certify columns are the hydrophobic and cation-exchange groups [7]. Because of the different properties of these two types of functional group. various solutions were used in the regeneration procedure. Methanol was first applied to the column to remove the endogenous components retained by the hydrophobic functional groups of the sorbent. It was observed that coloured endogenous components were removed by methanol, and that the sorbent regained its original white colour. Hydrochloric acid (1%) was used to regenerate the cation exchange groups of the sorbent. The residual acidic solution on the column was removed by passing 10 ml of deionized water through the column, and the column was finally cleaned with methanol again. The columns had to be regenerated immediately after use, otherwise it was difficult to clean them. In order to prevent non-specific adsorption of compounds from the environment [8], the regenerated columns were sealed with sealing film, and stored in the dark until the next use.

To check the cleanliness of the regenerated columns, an extraction of phosphate buffer solution was used instead of plasma. The chromatograms (Fig. 1) of blank phosphate buffer extracts on a regenerated Bond Elut Certify column show that both fraction A and fraction B are quite clean under the given analytical conditions. Neither remaining drugs, nor significant endogenous components of the previous plasma sample are present in the chromatograms.

*Potential of the regenerated columns*

The potential of the regenerated Bond Elut Certify columns was tested by extracting the five test drugs from plasma five times on the same column within one day. The columns were regenerated immediately after each extraction. The regeneration procedure was carried out four times. The mean recoveries together with the standard deviations of each extraction are given in Table I. As can be seen, the recoveries of pentobarbital, hexobarbital and mepivacaine tended to decrease (from 100% down to less than 80%) with each reuse. The recoveries of trimipramine and clonazepam remained virtually constant, near 100%.

The decreasing recoveries of some drugs may be attributable to two reasons. First, the columns were not totally clean, with some endogenous components still remaining after regeneration

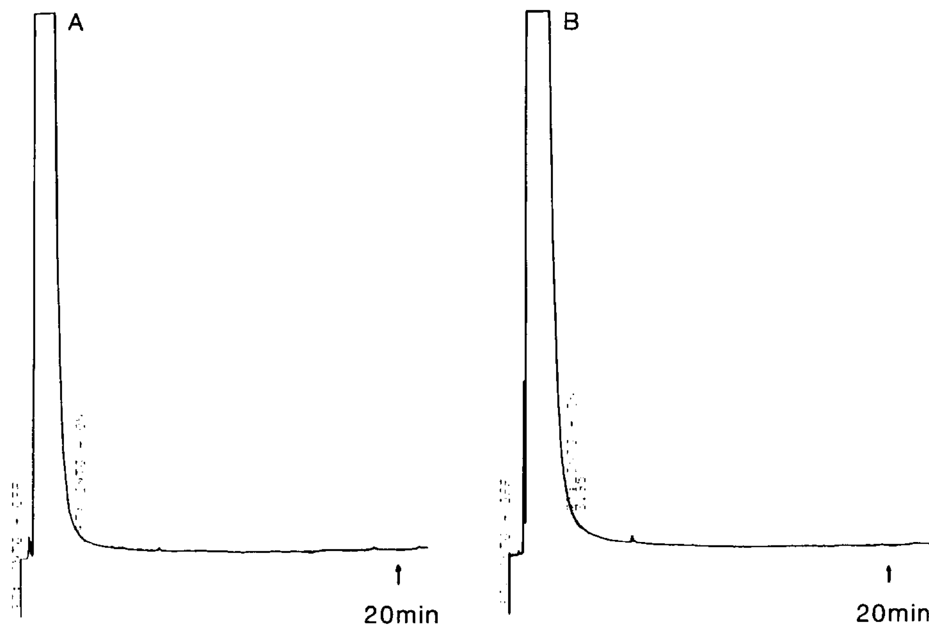


Fig. 1. Chromatograms of blank buffer extracts obtained from a regenerated Bond Elut Certify column. (A) Fraction A, acetone–chloroform (1:1); (B) fraction B, 2% ammoniated ethyl acetate.

and shielding active sites of the sorbent. Second, the use of 1% hydrochloric acid for regenerating the cation exchange functional groups slightly damaged the bonded silica sorbent during the regeneration process. However, the results in Table I demonstrate that the extraction potential of the columns was acceptable for at least one reuse. Further studies with a larger selection of drugs and at different concentrations are necessary to get a detailed insight in the reusability of these columns. Fig. 2 shows typical chromatograms of extracts obtained from a regenerated SPE column that had been used once.

It is of interest to mention that the sealed regenerated Bond Elut Certify columns could be stored for at least six months. As illustrated in

TABLE I

RECOVERIES OF FIVE TEST DRUGS FROM SPIKED PLASMA

Plasma was spiked with 10 μg/ml of each drug.

| Drug | Recovery (mean ± S.D., $n$ = 4) (%) | | | | |
|---|---|---|---|---|---|
| | New | First reuse | Second reuse | Third reuse | Fourth reuse |
| Pentobarbital | 97.6 ± 6.0 | 94.7 ± 9.8 | 85.3 ± 3.9 | 80.3 ± 11.9 | 74.0 ± 5.9 |
| Hexobarbital | 94.0 ± 9.1 | 90.0 ± 8.1 | 86.9 ± 2.0 | 83.8 ± 4.9 | 78.2 ± 1.4 |
| Mepivacaine | 100.1 ± 2.6 | 96.9 ± 4.3 | 93.1 ± 4.5 | 88.6 ± 7.5 | 79.2 ± 3.7 |
| Trimipramine | 101.5 ± 4.1 | 107.8 ± 4.9 | 106.1 ± 2.3 | 99.0 ± 6.6 | 96.9 ± 5.7 |
| Clonazepam | 97.4 ± 9.0 | 102.4 ± 8.1 | 100.4 ± 7.3 | 100.4 ± 5.3 | 96.6 ± 2.3 |

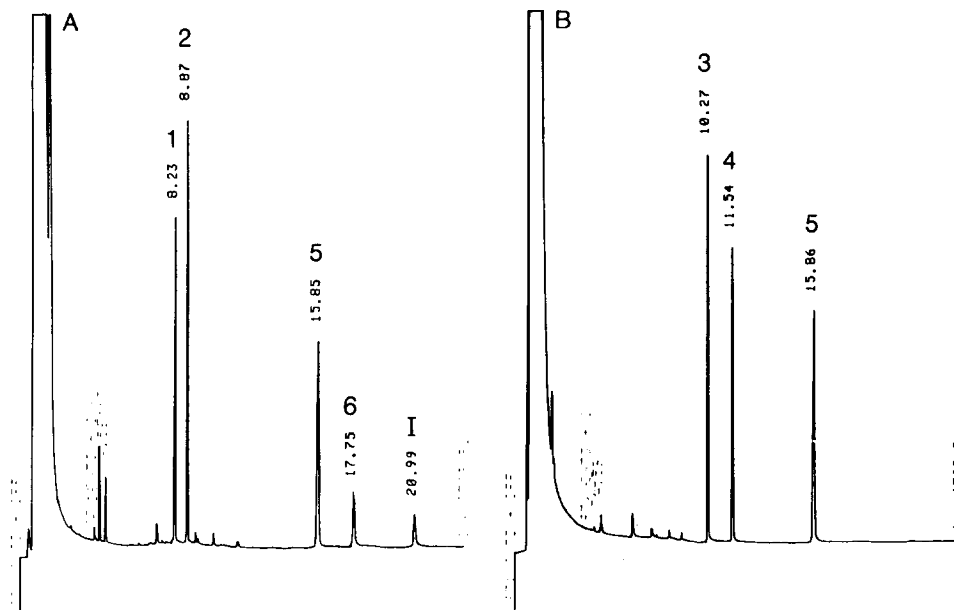


Fig. 2. Chromatograms of the extracts from spiked plasma obtained from a regenerated Bond Elut Certify column that had been used once. The plasma was spiked with 10 μg/ml of each drug. (A) Fraction A, acetone–chloroform (1:1); (B) fraction B, 2% ammoniated ethyl acetate. Peaks: 1 = pentobarbital; 2 = hexobarbital; 3 = mepivacaine; 4 = trimipramine; 5 = prazepam (chromatographic standard); 6 = clonazepam; I = cholesterol.

Fig. 3, quite comparable results were obtained by reusing columns that had been used once and regenerated six months previously. The recoveries of the five test drugs ranged from 83.5% for hexobarbital to 101.6% for trimipramine, with standard deviations less than 8.5%. In addition, it was found that the present regeneration method could be used as an extra pretreatment procedure

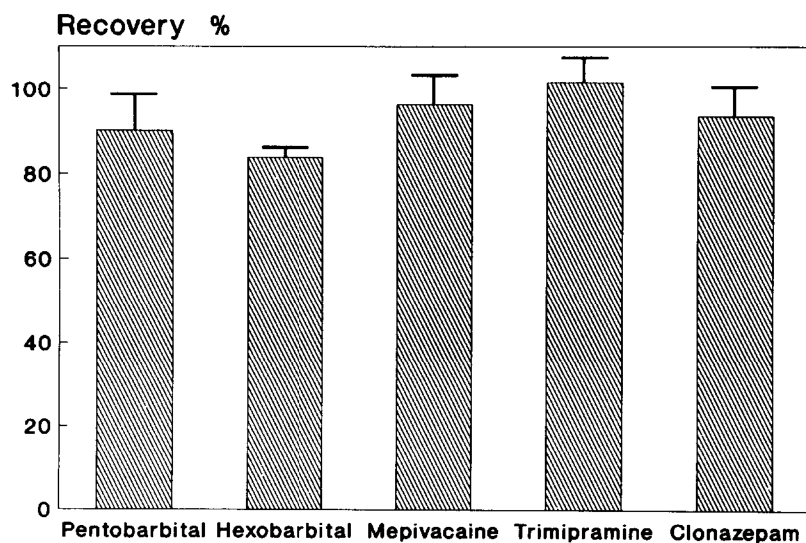


Fig. 3. Recoveries of five test drugs from spiked plasma (10 μg/ml) obtained from regenerated Bond Elut Certify columns. The columns had been stored for six months after having been used once and regenerated immediately afterwards ($n$ = 5).

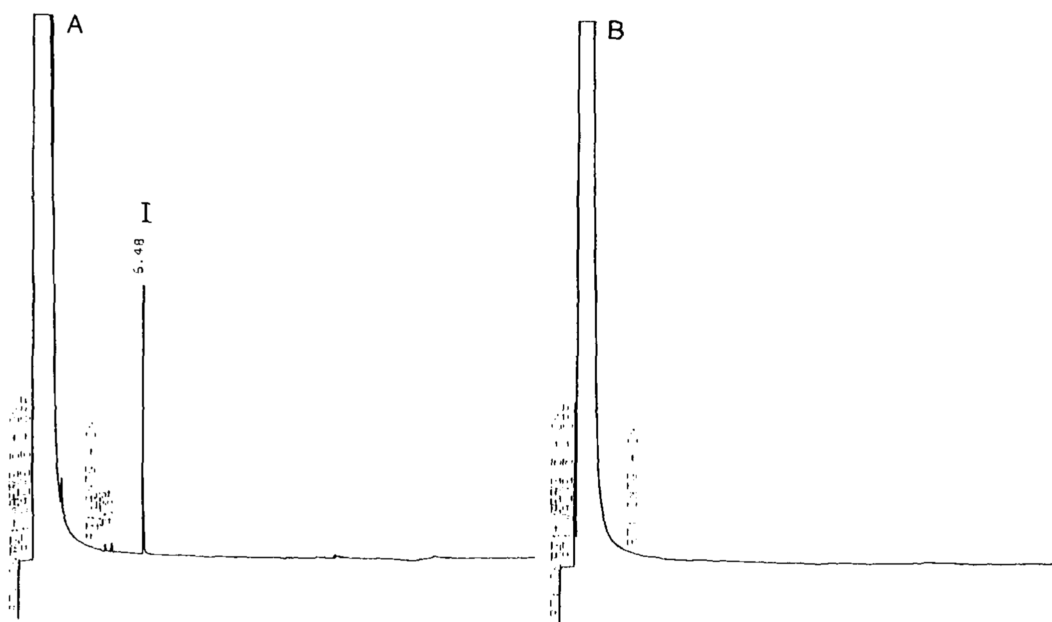


Fig. 4. Chromatograms of fraction B blank phosphate buffer, (A) without an extra column pretreatment step and (B) with an extra column pretreatment step. Peak I = impurity.

for new columns that had shown to release an impurity in the basic fraction. The latter was due neither to the samples nor to the solutions used in the extraction, but to the column materials. An example is shown in Fig. 4, which shows chromatograms of fraction B obtained from blank phosphate buffer (A) without an extra pretreatment procedure, and (B) with an extra pretreatment procedure. As can be seen, the impurity peak at 6.48 min in A is absent from B. This impurity was not batch-related, but was seen occasionally in columns taken from the same box of 50 units.

## CONCLUSION

This study indicates that Bond Elut Certify columns could be reused for analyzing plasma samples after regeneration without substantial losses in drug recoveries. Because the extraction power of the regenerated column decreased slightly with the number of reuses, it is recommended that the column is used no more than twice. After regeneration the extraction potential and cleanliness of the columns should be checked. The extraction of a blank buffer solution, a blank plasma and a spiked plasma should be carried out to ensure that the columns are still fit for the purpose intended.

## REFERENCES

1 X.-H. Chen, J. Wijsbeek, J.-P. Franke and R. A. de Zeeuw, *J. Forensic Sci.*, 37 (1992) 61.
2 X.-H. Chen, J.-P. Franke, J. Wijsbeek and R. A. de Zeeuw, *J. Anal. Toxicol.*, 16 (1992) 351.
3 X.-H. Chen, J.-P. Franke, K. Ensing, J. Wijsbeek and R. A. de Zeeuw, *J. Chromatogr.*, 613 (1993) 289.
4 R. M. Patel, J. R. Benson, D. Hometchko and G. Marshall, *Int. Lab.*, April (1990) 38.
5 U. Juergens, *J. Chromatogr.*, 371 (1986) 307.
6 R. A. de Zeeuw, J.-P. Franke, H. H. Maurer and K. Pfleger, *Gas Chromatographic Retention Indices of Toxicologically Relevant Substances on Packed or Capillary Columns with Dimethylsilicone Stationary Phases*, VCH, Weinheim, 3rd ed., 1992.
7 M. S. Mills, E. M. Thurman and M. J. Pedersen, *J. Chromatogr.*, 629 (1993) 11.
8 *Bond Elut Certify*™ *Instruction Manual*, Analytichem International, Harbor City, CA, 1989.