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Short Communication

Study of lot-to-lot reproducibilities of Bond Elut Certify and Clean Screen DAU mixed-mode solid-phase extraction columns in the extraction of drugs from whole blood

Xiao-Hua Chen*, Jan-Piet Franke, Jaap Wijsbeek and Rokus A. de Zeeuw

University Centre of Pharmacy, Department of Analytical Chemistry and Toxicology, Antonius Deusinglaan 2, 9713 AW Groningen (Netherlands)

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ABSTRACT

The lot-to-lot reproducibilities of Bond Elut Certify and Clean Screen DAU columns are described. The recoveries of five test drugs obtained from twelve lots of Bond Elut Certify columns ranged from 84 to 104% with standard deviations of less than 9%. The recoveries of five test drugs obtained from six lots of Clean Screen DAU columns ranged from 81 to 103% with standard deviations of less than 7%. The 95% confidence intervals of the means as obtained by ANOVA demonstrate that there are no significant differences between the tested lots of Bond Elut Certify and Clean Screen DAU columns. Comparison of the two brands shows that both Bond Elut Certify and Clean Screen DAU columns are well acceptable for routine drug screening in systematic toxicological analysis, with a slightly higher overall recovery for the former.

INTRODUCTION

In recent years, more and more analysts have shifted their attention from traditional liquid–liquid extraction (LLE) to solid-phase extraction (SPE), because the latter has some significant advantages [1,2]. SPE has not only been applied to isolate individual drugs or groups of structurally related drugs [3–6], but also utilized to extract various classes of drugs with different structures from biological specimens [7–10]. The latter application is especially useful for drug screening in systematic toxicological analysis (STA).

Initially, the lot-to-lot reproducibility appeared not so important, since one can purchase the same lot of SPE columns for a short project. In a long run, however, if a developed SPE procedure based on a given lot of SPE columns is to be used for routine work, or if this procedure is to be adapted by other laboratories, the lot-to-lot reproducibility becomes a very important factor. However, it should be noted that analysts have expressed their concern on different occasions with regard to lot-to-lot reproducibilities of SPE materials and HPLC materials. So far, little in-

* Corresponding author.

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formation appears to be available on the comparability of similar SPE materials from different manufacturers and on the lot-to-lot reproducibilities of a given SPE material from a single manufacturer. In this study the behavior and the lot-to-lot reproducibilities of two commercial brands of mixed-mode bonded silica SPE columns, Bond Elut Certify and Clean Screen DAU, were investigated with regard to their utility for drug screening in STA.

EXPERIMENTAL

Materials

Methamphetamine hydrochloride, pentobarbital, hexobarbital, mepivacaine hydrochloride, trimipramine 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 capped 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.

All reagents were of analytical grade (Merck, Darmstadt, Germany). Phosphate buffer (0.1 *M*, pH 6.0), 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 [8].

Twelve lots of Bond Elut Certify columns (130 mg sorbent mass, 10 ml column volume) were kindly supplied by Varian Sample Preparation Products (Harbor City, CA, USA). Six lots of Clean Screen DAU columns (130 mg sorbent mass, 1 ml column volume) were obtained from Worldwide Monitoring (Horsham, PA, USA). Both Bond Elut Certify and Clean Screen DAU were mixed-mode columns, containing hydrophobic and cation-exchange functional groups.

Instrumentation

A Hewlett-Packard 5890A Series II gas chromatograph (Avondale, PA, USA), equipped with a Hewlett-Packard 7673 automatic sampler and a flame ionization detector, was used. The analytical column was a HP-1, fused-silica, wide-bore capillary column (30 m × 0.53 mm I.D., 0.88 μm film thickness). The oven temperature was programmed from 80°C (2 min hold) to 215°C at 20°C/min, and then increased to 280°C at 5°C/min. The final temperature was held for 2 min. The injection port and detector temperatures were set at 275 and 300°C, respectively. The injection port was in the splitless mode.

A Vac-Elut 24 vacuum manifold system was purchased from Varian Sample Preparation Products. An SC-200-22 sonication bath was purchased from Sonicor Instrument (Farmingdale, NY, USA). A Megafuge 1.0 centrifuge was obtained from Heraeus Sepatech (Osterode/Harz, Germany).

Extraction from whole blood

Calf blood (1 ml) spiked with the five test drugs was sonicated in a sonic bath for 15 min at room temperature. To the sample were added 6 ml of 0.1 *M* phosphate buffer (pH 6.0). After vortex-mixing for 30 s, the diluted sample was centrifuged at 6000 *g* for 7 min and the supernatant was used for further extraction. The extraction was performed with either the Bond Elut Certify column or the Clean Screen DAU column. The extraction procedure used in this study was same as described in our previous paper [8]. A schematic overview is given in Fig. 1.

Calculations

The ratio of the peak heights of test drugs to that of prazepam was used for quantitation. The absolute extraction yield was determined by comparing the response ratio of the extract with the calibration graph obtained from pure substances in the concentration range 5–40 μg/ml with a constant 20 μg/ml concentration of prazepam.

RESULTS AND DISCUSSION

Reproducibility of Bond Elut Certify columns

In this investigation twelve lots of Bond Elut Certify columns were tested by extracting five selected drugs from whole calf blood. The twelve

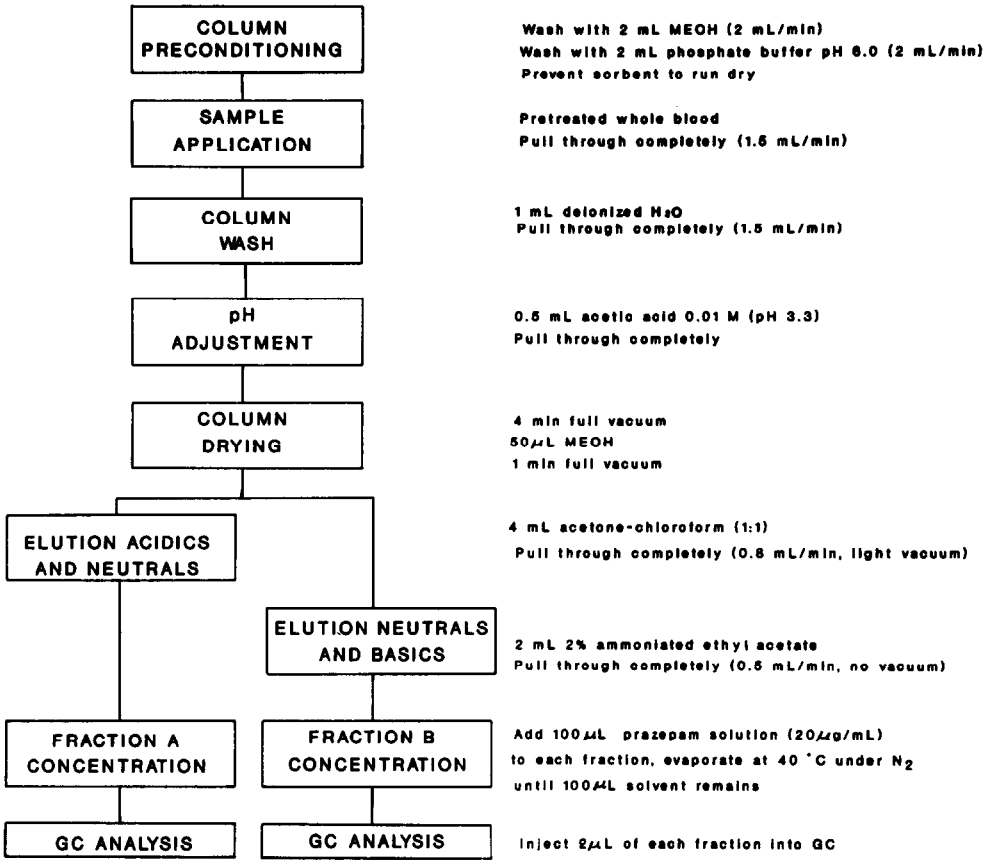

Fig. 1. Extraction scheme for whole blood on the mixed-mode SPE columns.

lots of Bond Elut Certify columns were analyzed in two groups (group I: lots 1–6; group II: lots 7–12) and tested over a one-year period. Table I (Nos. 1–12) shows the recoveries and standard deviations of the five test drugs obtained from both groups. It is interesting to mention that although the two groups of columns were tested over a twelve-month period, the data obtained from groups I and II are quite comparable. The recoveries of the individual drugs obtained from the individual lots varies from 84 to 104% with standard deviations of 9.2% or less. The mean recoveries of the individual drugs of each group ranged from 91 to 102% with standard deviations of less than 6%. The mean standard deviations of lots are less than 6%. Analysis of variance (ANOVA) was applied to the data set. The 95% confidence intervals of the means of the different lots are depicted in Fig. 2, which shows excellent reproducibility between the lots. Only lot 7 gave in average a somewhat higher recovery. The significance level (P) was 0.0164.

Reproducibility of Clean Screen DAU columns

The reproducibilities of the Clean Screen DAU columns were studied by extracting the same test drugs from whole calf blood. Six lots of these columns were tested, and the results are given in Table I (Nos. 13–18). The recoveries of the test drugs ranged from 81% for methamphetamine to 103% for trimipramine with standard deviations of less than 7%. The mean recoveries of individual drugs of the six lots ranged from 84 to 92% with standard deviations of 7% or less. The mean

TABLE I

ABSOLUTE RECOVERIES OF FIVE DRUGS FROM WHOLE CALF BLOOD OBTAINED FROM BOND ELUT CERTIFY AND CLEAN SCREEN DAU COLUMNS

No.[a]	Recovery (mean ± S.D., n = 3) (%)					
	Methamphetamine	Pentobarbital	Hexobarbital	Mepivacaine	Trimipramine	Mean[b]
1	99.9 ± 4.3	91.2 ± 5.6	90.9 ± 5.9	93.1 ± 2.3	101.9 ± 0.6	95.4 ± 5.1
2	94.5 ± 9.2	95.9 ± 1.4	91.9 ± 2.0	93.9 ± 3.3	101.6 ± 0.8	95.6 ± 3.7
3	91.7 ± 4.8	95.0 ± 1.9	92.1 ± 0.9	90.9 ± 2.0	103.6 ± 3.5	94.7 ± 5.2
4	100.1 ± 3.9	92.7 ± 1.9	87.7 ± 2.6	92.3 ± 0.1	101.8 ± 0.7	94.2 ± 5.9
5	93.2 ± 6.9	91.7 ± 1.9	89.6 ± 2.0	86.7 ± 3.7	101.7 ± 1.6	92.6 ± 5.7
6	91.5 ± 5.8	93.1 ± 4.0	95.1 ± 4.0	87.7 ± 2.8	102.5 ± 2.3	94.0 ± 5.5
Mean[c]	95.2 ± 3.9	93.3 ± 1.8	91.2 ± 2.5	90.8 ± 3.0	102.2 ± 0.8	
7	93.1 ± 2.7	100.9 ± 1.0	99.1 ± 3.5	100.7 ± 3.1	101.0 ± 2.3	99.0 ± 3.4
8	89.4 ± 2.3	100.8 ± 2.2	98.1 ± 7.4	93.7 ± 4.0	94.3 ± 2.6	95.3 ± 4.4
9	90.5 ± 2.9	98.8 ± 2.0	95.5 ± 3.7	83.9 ± 0.3	91.7 ± 3.9	92.1 ± 5.6
10	93.5 ± 7.3	101.1 ± 3.2	94.2 ± 3.6	91.1 ± 6.6	97.6 ± 4.6	95.5 ± 3.9
11	87.3 ± 3.9	100.3 ± 2.3	92.2 ± 7.8	86.7 ± 3.5	92.5 ± 2.3	91.8 ± 5.5
12	95.0 ± 6.2	96.5 ± 7.4	94.2 ± 3.6	88.7 ± 8.0	96.3 ± 2.8	94.1 ± 3.2
Mean[c]	91.5 ± 2.9	99.7 ± 1.8	95.6 ± 2.6	90.8 ± 5.9	95.6 ± 3.5	
13	83.2 ± 2.1	84.0 ± 1.9	85.7 ± 2.1	82.8 ± 2.2	88.2 ± 2.7	84.8 ± 2.2
14	85.9 ± 4.9	86.7 ± 1.7	84.8 ± 4.3	83.5 ± 2.8	88.7 ± 3.8	85.7 ± 2.0
15	85.7 ± 4.7	95.1 ± 0.8	91.4 ± 1.2	83.7 ± 0.7	90.2 ± 4.7	89.2 ± 4.6
16	84.3 ± 4.2	90.5 ± 1.2	89.1 ± 1.5	82.3 ± 3.7	88.5 ± 1.1	86.9 ± 3.5
17	81.2 ± 3.0	88.8 ± 6.8	88.9 ± 5.7	82.9 ± 4.2	91.0 ± 1.8	86.6 ± 4.3
18	100.2 ± 0.5	98.6 ± 1.3	98.2 ± 2.4	90.7 ± 5.4	102.5 ± 1.4	98.0 ± 4.4
Mean[c]	86.8 ± 6.8	90.6 ± 5.4	89.7 ± 4.8	84.3 ± 3.2	91.5 ± 5.5	

[a] 1–6 represent the first group (lots 1–6) of Bond Elut Certify columns; 7–12 represent the second group (lots 7–12) of Bond Elut Certify columns; 13–18 represent lots 1–6 of Clean Screen DAU columns.

[b] Mean of lot.

[c] Mean of drug.

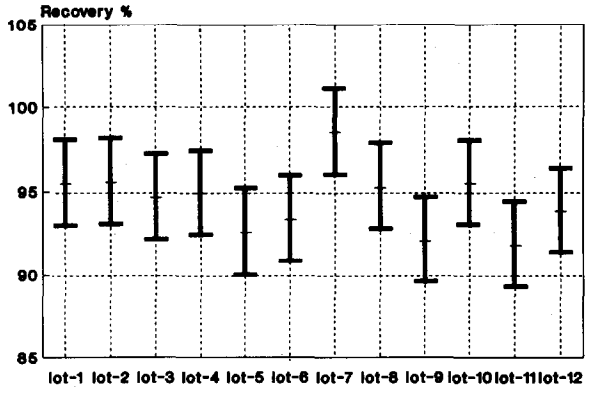

Fig. 2. Comparison of the twelve lots of the Bond Elut Certify columns. The bars indicate 95% confidence intervals of the means of the recoveries of the five drugs.

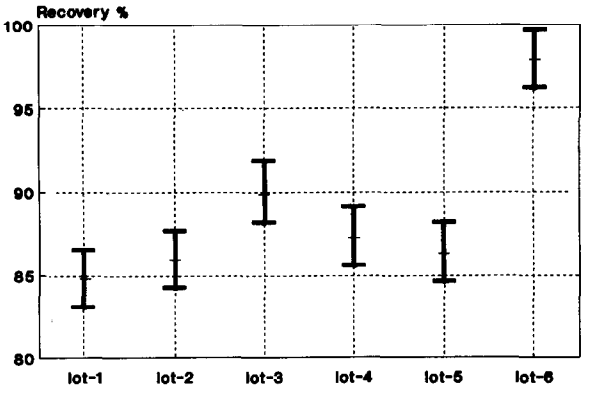

Fig. 3. Comparison of the six lots of the Clean Screen DAU columns. The bars indicate the 95% confidence intervals of the means of the recoveries of five drugs.

standard deviations of lots ranged from 2 to 5%. Fig. 3 shows the 95% confidence intervals of the means as obtained with ANOVA for six lots of Clean Screen DAU columns. As with the Bond Elut Certify columns the reproducibility between lots is good. However, one lot (lot 6) gave a significantly ($P < 0.0001$) higher recovery.

Comparison of Bond Elut Certify and Clean Screen DAU columns

In order to compare these two different brands of mixed-mode SPE columns, six lots of Bond Elut Certify columns (group II) and six lots of Clean Screen DAU columns were tested under the same analytical conditions within two weeks. Table I shows that both brands provide excellent recoveries for screening purposes for all five test drugs. These results are consistent with our earlier observations [8]. Yet, the overall recoveries of Bond Elut Certify appeared to be slightly but significantly higher ($P < 0.0001$) than those of Clean Screen DAU.

CONCLUSIONS

The results described in this report demonstrate that the lot-to-lot reproducibilities of both Bond Elut Certify and Clean Screen DAU columns are quite good. In principle, both commercial mixed-mode SPE columns showed no significant differences between lots. Although the overall recoveries obtained with the Clean Screen DAU columns (88.7%) were slightly lower than those with the Bond Elut Certify columns (94.5%), both brands of mixed-mode SPE columns are well acceptable for routine drugs screening in STA.

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