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Applicability of various brands of mixed-phase extraction columns for opiate extraction from blood and serum

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Abstract

Four commercially available types of mixed-phase solid-phase extraction (SPE) columns (Bond Elut Certify, Isolute Confirm HCX, Chromabond Drug and Bakerbond Narc–2) were examined in order to compare the extraction efficiencies and chromatographic purity of extracts. The absolute recovery of morphine, 6-monoacetylmorphine and codeine was examined in blood and serum (ten samples each at two concentration levels), using SPE columns of the same batch. GC–MS (ion trap) and HPLC with amperometric detection were used for quantitation. A distinct variability in extraction recovery was observed among the same batches of all brands of SPE columns. All extracts were chromatographically pure and no interfering peaks were observed, neither in GC–MS nor in HPLC examinations, but in some extracts large peaks of plasticizers were identified. The measurements of flow velocities of the same samples of blood or serum through the SPE columns of the same batch showed very large variability of random character. The morphometric analysis of particles was performed for two batches of each sort of SPE columns by means of an image analysing system. Symmetrical distribution of particle size was observed only in Chromabond MN Drug packing, while in other cartridges large fractions of fine particles and nonhomogenous distribution were found. Only in one case the morphometric findings were pretty concordant with the data available from the manufacturer; in two cases, observed data varied considerably from that expected, and in one case no information was available at all. The study showed generally that there was room for improvement in the quality of mixed-phase SPE columns.

Keywords: Morphine; 6-Monoacetylmorphine; Codeine

1. Introduction

Reliable and reproducible isolation of toxicologically relevant compounds from biological matrices is the first, the most important and the most critical step in systematic toxicological analysis. Among different extraction methods currently used in analytical toxicology, solid-phase extraction (SPE) has shown a dynamic development. Thus, various methodological and technical aspects of SPE have been comprehensively reviewed [1–3], and the number of publications has been rapidly growing in the last decade [3].

SPE may be regarded as a particular kind of column chromatography. Therefore, the optimization of extraction conditions, taking into account the

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properties of all three interacting factors, i.e. analyte, sorbent and eluent has been the subject of numerous studies [4–6]. Further studies have covered the problems of prediction of retention and recoveries of some model drugs [7–10]. Finally, the applicability of SPE in on-line automatic isolation procedure has been demonstrated (i.e. [11–13]).

According to the recent literature and to everybody's experience, SPE is widely used in forensic toxicological analysis, for both dedicated and "general unknown" procedures, mainly in the treatment of biological fluids of low viscosity, like urine or serum. The application of SPE cartridges for isolation of drugs from various biological tissues appeared feasible also and was reviewed by Scheurer and Moore [14]. The first studies demonstrated high purity of biological extracts obtained after SPE, especially in comparison with liquid–liquid extraction [15,16]. Comparative studies were done on the application of various reversed-phase (RP) (C_1, C_8 and C_{18}) SPE cartridges for isolation of drugs of abuse with subsequent HPLC separation with UV detection [17,18].

Besides RP other SPE sorbents, like cyanopropyl [19] or cation-exchange, were used for successful isolation of toxicologically relevant compounds [20–22].

In the late 1980s, mixed-phase SPE cartridges, containing reversed-phase and cation-exchange sorbents, were commercially introduced. Standard extraction procedures for isolation of various groups of drugs of abuse from urine were developed and supplied by the manufacturers. Several authors adapted mixed-phase SPE for the isolation of drugs of abuse from serum and then whole blood [23–30].

Very recently, a novel extraction strategy for the SPE was proposed, based on consecutive extraction with hydrophobic and ion-exchange column [31]. Such approach allowed to optimize the separate extraction stages individually, which is virtually impossible using mixed-phase packings.

However, it was suggested in some studies that SPE, in spite of its obvious advantages, also has some serious drawbacks. The most important problem, which was raised several times and in regard to various commercial products, is the reproducibility of the quality of the packing material. Very different recoveries, up to complete failure of the extraction, were observed for the same or different batches of cartridges [14]. Phtalate interference and removal of sorbent particles during extraction were also mentioned [14], as well as different flow velocities through columns of the same batch [20]. Comparative studies on extraction of opiates and other drugs from serum and blood, using various types of SPE cartridges, showed low reproducibility of results, particularly for cation-exchange and mixed-phase columns, with morphine recoveries from blood ranging from 12 to 90% [32,33].

Despite these problems, in recent years, several manufacturers have launched mixed-phase SPE cartridges, claiming in some cases high standards of quality control for their products and wide applicability in forensic toxicological analysis. The recommended use of these products, however, is generally limited to urine. Therefore, in the present study, it was decided to perform a comparative test on the isolation of toxicologically relevant drugs from blood and serum using different mixed-phase cartridges. Morphine, codeine and 6-monoacetylmorphine (6-MAM) were chosen as model drugs, due to their obvious forensic relevance. The quality of extracts was assessed using two detection methods: gas chromatography-mass spectrometry (GC–MS) and high-performance liquid chromatography with electrochemical detection (HPLC–ED). The ultimate purpose of the study was to find out whether all examined extraction columns meet the same standards in terms of substance recovery and chromatographic purity of the extract.

In a pilot phase of the present study, the morphine recoveries of ten mixed-phase cartridges (same brand and batch-number) from the same blood sample were determined using the method of Chen et al. [27], and the results obtained varied from 45 to 90%.

Therefore, a more simple procedure was chosen which has been used in the last few years for opiate isolation from blood and serum samples. The procedure was validated using serum samples spiked with morphine in the range of 50 to 1000 ng/ml as calibration standards and deuterated morphine (for GC–MS determinations) of hydromorphone (for HPLC determinations) as internal standards. The accuracy of this method met the quality standards of The German Society of Forensic and Toxicological Chemistry.

2. Experimental

2.1. Materials

2.1.1. Extraction columns

The following commercially available mixed-phase SPE cartridges were used: Bond Elut Certify (Analytichem/Varian, Harbor City, CA, USA), Isolute Confirm HCX (International Sorbent Technology, Hengoed, UK), Chromabond MN Drug (Macherey and Nagel, Düren, Germany) and Bakerbond Narc-2 (J.T. Baker, Philipsburgh, NJ, USA). The cartridges were filled with 125–130 mg of packing material.

2.1.2. Serum and blood samples

Serum and citrated blood were obtained from the local blood bank and checked for the absence of drugs by means of GC–MS before use. For recovery study, four spiked standards (100 ml each) were prepared: serum "low": 0.05 mg/l morphine and 0.1 mg/l codeine; serum "high": 0.2 mg/l morphine, 0.5 mg/l codeine and 0.2 mg/l 6-monoacetylmorphine (MAM); blood "low": 0.05 mg/l morphine, 0.1 mg/l codeine and 0.2 mg/l MAM; blood "high" 0.2 mg/l morphine, 0.5 mg/l codeine and 0.2 mg/l MAM. Ten samples of each spiked standard were subjected to extraction and further examination; five for GC–MS and five for HPLC.

To study the matrix effects, ten samples of serum and ten of blood without any additions were used as blanks.

For the study of recovery and removal of matrix interferences only cartridges from the same batches were used, i.e. Bond Elut Certify Batch No.292455, Isolute Confirm HCX Batch No. 5199504 CB, Chromabond Drug Batch No. 595 and Bakerbond Narc-2 Batch No. H-33573.

2.2. Instrumentation and methods

2.2.1. Sample preparation

A Visiprep vacuum manifold (Supelco, Bellefonte, PA, USA) was used for testing all the cartridges.

SPE cartridges were washed with 5 ml methanol, 5 ml water and 5 ml borate buffer 0.05 *M*, pH 8.5. Samples of blood or serum (1 ml) were mixed with 1 ml borate buffer 0.05 *M*, pH 8.5, vortexed and centrifuged for 5 min at 12 000 *g*. The supernatant was then applied on the preconditioned columns and passed through in about 5 min. The columns were then washed successively with water (2 ml), acetate buffer 0.1 *M*, pH 4.0 (1 ml) and methanol (2 ml). After drying for 5 min under full vacuum, the columns were eluted with 2 ml of freshly prepared dichlormethane–isopropanol–concentrated ammonia (80:20:2) under gravity force.

For GC–MS determinations, 100 ng morphine-d_3 (Sigma, St. Louis, MO, USA) was then added as an internal standard and the extract was evaporated under nitrogen. The residue was dissolved in 50 μl pentafluoropropionyl anhydride, incubated for 60 min at 60–70°C and then dried again. The residue was finally reconstituted with 20 μl of ethyl acetate and 1 μl was injected onto the GC–MS system.

For HPLC determinations the eluate was evaporated under nitrogen and reconstituted with 100 μl of mobile phase, containing 20 ng hydromorphone as an internal standard (Sigma); 10 μl were injected onto the HPLC system.

For the study of absolute recoveries, the methanolic standard solution containing 100 ng morphine, codeine and MAM each (absolute amounts) was mixed with appropriate internal standards and subjected to GC–MS and HPLC examinations without extraction. The recoveries of the drugs were calculated from the ratios drug/internal standard in extracted sample versus the ratios drug/internal standard in standard drug solution.

2.2.2. Gas chromatography–mass spectrometry

GC–MS examinations were performed by means of a Perkin-Elmer 8420 gas chromatograph with ion trap detector (Perkin-Elmer, Norwalk, CT, USA) on a fused-silica OV-1 column (Macherey and Nagel, Düren, Germany) (10 m×0.25 mm ID) using the following temperature program: 1 min at 150°C, 20°C/min to 250°C, 5 min at 250°C. The following ions were used for quantitation as target and qualifier ions, respectively: morphine 119 and 414, morphine-d_3 122 and 417, codeine 282 and 445, MAM 414 and 473.

2.2.3. High-performance liquid chromatography

HPLC measurements were carried out using a Type L-6000 pump (Merck Hitachi, Darmstadt,

Germany), Nucleosil 100-5 C_{18} AB column (Macherey and Nagel, Düren, Germany; 250 mm×4.6 mm) and Model 1049 A programmable amperometric detector (Hewlett-Packard, Palo Alto, CA, USA), set at 850 mV versus Ag/AgCl reference electrode. This potential allowed the detection of morphine and MAM, but not of codeine. The mobile phase consisted of acetonitrile and 5 m*M* citrate buffer, pH 5.0, containing 20 m*M* lithium perchlorate (12:88). The flow-rate was 0.8 ml/min. Hydromorphone was used as an internal standard for drug quantitation.

2.2.4. Reproducibility of flow velocity

Twenty cartridges of the same batch from each manufacturer were washed consecutively with 2 ml of methanol and 2 ml of water. A 100-ml volume of serum and blood was diluted with water (1:1) and centrifuged; 1-ml samples of supernatant were applied individually on each cartridge (ten serum samples and ten blood samples) under the same negative pressure. The time in which 1 ml eluent was obtained was measured.

2.2.5. Morphometric analysis

For these experiments, two different batches of each SPE product were used: Bond Elut Certify Batch No. 292455 (examined in duplicate) and 291766, Isolute Confirm HCX Batch No. 5199504 CB (examined in duplicate) and 5199510 CC, Chromabond Drug Batch No. 595 (examined in duplicate) and 995 and Bakerbond Narc-2 Batch No. H33573 (examined in duplicate) and J12551.

The packing material from each column was evacuated in the following manner: The outlet part of the SPE column was cut off with a scalpel and the lower frit was carefully removed. The whole packing material was then divided in five equal portions in ascending direction and dispersed consecutively on a cleaned microscopic glass slide without cover slip. From each portion, 200 particles were then measured, i.e. the total number of measured particles for each column was 1000.

The morphometric analysis of particles was performed by means of a bright field microscopy using a Leitz PL Fluotar 10×/0.30 objective (Leitz, Wetzlar, Germany). Microscopic images were captured with a CCD camera (Hamamatsu Photonics, Hamamatsu, Japan) and digitized into a 736×552 pixel image so that 100 μm were represented by 76 pixel. Further image processing operation and the final measurements were done by means of Image Analyses System KS 400 (Kontron Elektronik, Eching, Germany). The projection area of each particle on the plane was measured and transformed to the circular form. The diameter of this circle (DCIRCLE) was used as a measure of particle size. The optical resolution of the system did not allow measurement of particles smaller than 10 μm.

3. Results and discussion

3.1. Recoveries

Table 1 and Table 2 show the absolute recoveries of opiates from blood and serum as observed in HPLC and GC–MS assays. For both analytical methods, very relevant variability in the recovery

Table 1
Percent recoveries (mean values ±S.D. from five determinations) of morphine and 6-monoacetylmorphine extracted from serum and blood and determined by means of HPLC

	Bond Elut Certify	Confirm HCX	Bakerbond Narc-2	Chromabond-Drug
Serum-mo 50	53±16	30±22	41±6	51±8
Serum-mo 200	66±11	37±25	34±22	67±7
Serum-MAM	74±5	79±40	34±27	73±14
Blood-mo 50	49±34	56±28	68±31	73±14
Blood-mo 200	48±27	36±20	49±20	69±11
Blood-MAM	45±25	66±34	41±18	55±14
MSD	22	29	22	12

MSD=mean standard deviation of absolute recovery, calculated for each type of SPE cartridge.

Table 2
Percent recoveries (mean values ±S.D. from five determinations) of morphine, codeine and 6-monoacetylmorphine extracted from serum and blood and determined by means of GC–MS

	Bond Elut Certify	Confirm HCX	Bakerbond Narc-2	Chromabond-Drug
Serum-mo 50	48±10	17±11	36±6	31±2
Serum-mo 200	44±13	49±30	101±142	69±16
Serum-cod 100	124±22	46±23	55±9	49±13
Serum-cod 500	39±10	47±22	81±17	67±15
Serum-MAM	41±15	43±16	59±17	50±12
Blood-mo 50	106±104	67±17	104±51	64±37
Blood-mo 200	45±18	47±16	90±47	93±21
Blood-cod 100	86±59	71±15	70±38	53±36
Blood-cod 500	97±57	70±29	64±32	76±12
Blood-MAM	24±27	6±4	15±3	12±1
MSD	44	18	29	20

MSD=mean standard deviation of absolute recovery, calculated for each type of SPE cartridge.

values were noted (from some 8% to over 100%), which was neither related to the kind of biological fluid, nor to the type and concentration of the analyte. Also, the differences in the composition, ionic strength etc. of the sample cannot explain the variability, since the same samples of blood and serum were used throughout the whole study. The recovery obtained with Chromabond Drug cartridges appeared more reproducible in HPLC examination, since its mean standard deviation (MSD) was approximately two times smaller than for other SPE columns. In GC–MS examination, the results obtained with Confirm HCX and Chromabond Drug cartridges were more reproducible than the other ones.

Generally, the comparison of HPLC and GC–MS data suggests that the latter method gives less reproducible results. This was particularly distinct for the extracts obtained with Bond Elut Certify and Bakerbond Narc-2 columns. The possible reason of this difference may be the derivatization step applied in GC–MS, which may add to the variability. Closer inspection of GC–MS findings revealed that the variabilities of peak areas for test substances and internal standard were in some samples uncorrelated, i.e. low areas of sample peaks were associated with unusually high areas of internal standard peak or vice versa. It seems therefore, that the use of deuterated analog as an internal standard did not automatically assure a higher reproducibility of results. In the validation experiments, performed on non-extracted

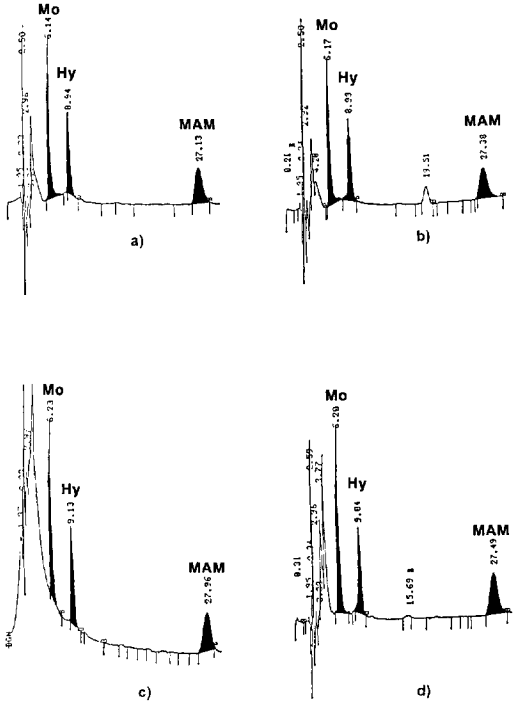

Fig. 1. HPLC chromatograms of serum extracts spiked with morphine (Mo) and 6-monoacetylmorphine (MAM) to the concentration of 0.2 mg/l and extracted with (a) Bond Elut Certify, (b) Confirm HCX, (c) Bakerbond Narc-2 and (d) Chromabond Drug. Hydromorphone (Hy), 2 ng/10 μl, was added to the reconstituted extract as internal standard.

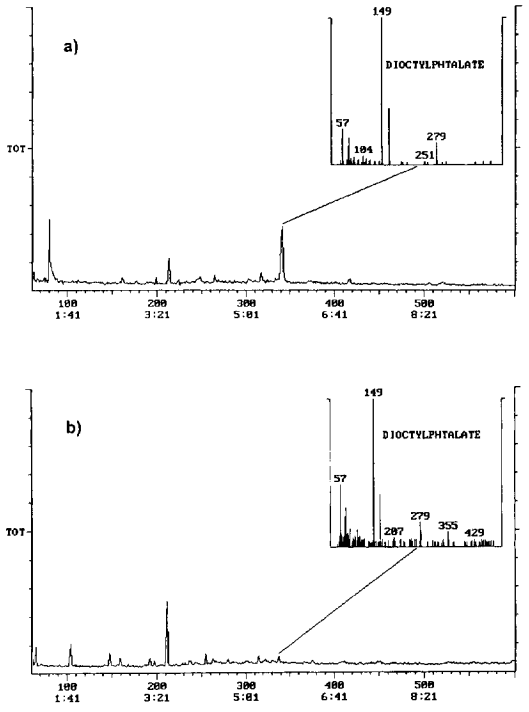

Fig. 2. Total-ion GC–MS chromatograms of blank serum samples extracted with Bond Elut Certify (a) and Isolute Confirm HCX (b) columns, showing matrix peaks and the mass spectrum of a plasticizer.

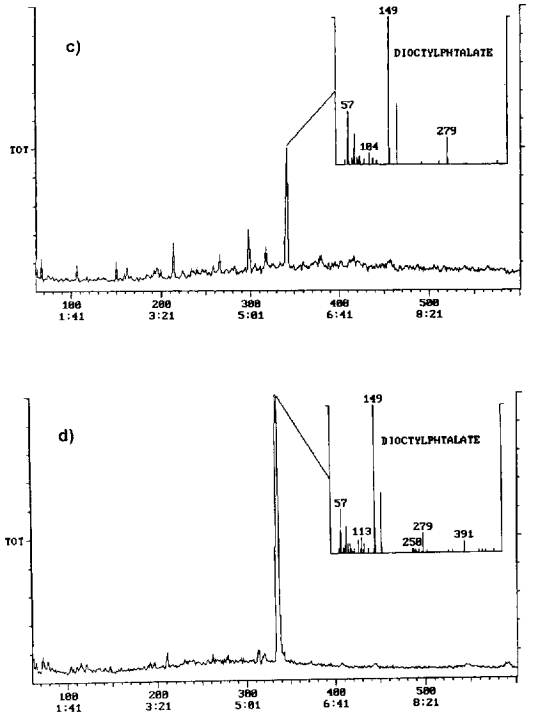

Fig. 3. Total-ion GC–MS chromatograms of blank serum samples extracted with Bakerbond Narc-2 columns (c), and Chromabond-Drug (d) showing matrix peaks and the mass spectrum of a plasticizer.

drugs, a linear relationship between peak ratios (sample:internal standard) and concentration over the range of 50 to 1000 ng/ml was noted.

3.2. Matrix effects

All examined extracts were sufficiently clean for both HPLC and GC–MS. Fig. 1 shows typical HPLC chromatograms of blood samples, spiked with morphine, hydromorphone and 6-MAM and extracted with SPE cartridges. The extracts obtained with Bond Elut Certify and Chromabond Drug cartridges were almost completely free of interfering matrix peaks in HPLC examinations.

Fig. 2 and Fig. 3 show typical GC–MS chromatograms of serum extracts for each extraction cartridge. The total ion chromatograms of the same serum sample extracted with particular cartridge showed distinct differences. The extracts from Chromabond Drug columns showed large peaks of plasticizer. These peaks were smaller in the extracts from Bond Elut Certify and Bakerbond Narc-2 cartridges and almost absent in extracts from Confirm HCX columns. In the last extract, however, another large matrix peak (scan 210) was noted. The chromatograms of particular diagnostic ions (m/z 414, 417 and 445) have demonstrated the absence of interfering matrix peaks in each extract (Fig. 4 and Fig. 5).

Several studies were already undertaken on the application of SPE for isolating morphine from a biological matrices using mixed-phase Bond Elut Certify [27,30–32]. In the paper by Chen et al. very clean extracts were observed, using HPLC with electrochemical detection, and this finding was confirmed in our study for all examined cartridges; also morphine recovery of above 80±3% was reported, using radioactive counting of [^{3}H]morphine [27]. In another study from the same research group [29] five acidic and basic test drugs (pentobarbital, hexobarbi-

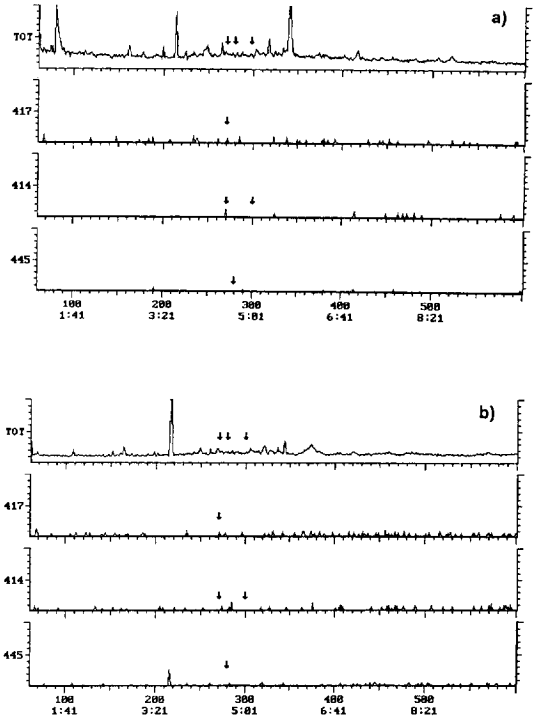

Fig. 4. GC–MS chromatograms of blank serum samples extracted with Bond Elut Certify columns (a) and Isolute Confirm HCX (b). Total ion chromatograms and ion traces of PFPA-derivatives of morphine and MAM (m/z 414), morphine-d_3 (m/z 417) and codeine (m/z 445). The arrows show the retention times of morphine/morphine-d_3, MAM and codeine.

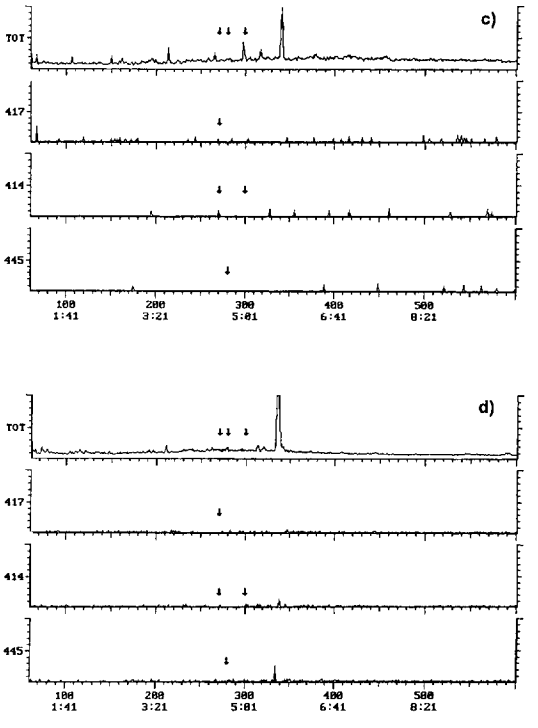

Fig. 5. GC–MS chromatograms of blank serum samples extracted with Chromabond-Drug (c) and Bakerbond Narc-2 (d) columns. Total ion chromatograms and ion traces of PFPA-derivatives of morphine and MAM (m/z 414), morphine-d_3 (m/z 417) and codeine (m/z 445). The arrows show the retention times of morphine/morphine-d_3, MAM and codeine.

tal, methamphetamine, mepivacaine and trimipramine, unknown concentrations) were extracted by means of Bond Elut Certify and Clean Screen DAU (Worldwide Monitoring, Horsham, PA, USA) mixed-phase columns, showing very high (84–104%) and very reproducible (C.V. 0.5–9%) recoveries. These findings are contrary to the results obtained in the present as well as in our previous studies [32,33]. The sample pretreatment in the method of Chen et al. [27,29] included a sonication step, what makes a direct comparison difficult. On the other hand, however, the method of Chen [27] was used in the pilot phase of this study and in the other study, and very variable absolute recoveries were observed. Also, Zweipfenning et al. [30] have observed erratic recoveries of underivatized morphine (171.3±66% or 56.3±26.8 at the concentration of 0.5 mg/l and 0.25 mg/l, respectively), 6-monoacetymorphine (57.6±33.3% and 26.4±3.4) and nalorphine (135±120%) from blood, extracted with Bond Elut Certify columns and examined by means of GC–NPD. After silylation more consistent (C.V. ca. 13%) and higher recoveries were obtained, using GC–MS. However, the authors have calculated the latter recoveries only from selected samples, showing satisfactory ion chromatograms of the target ions and qualifier ions as well. The samples, showing deviations in the ratios of peak areas target/qualifier were rejected [30].

3.3. Flow velocities

Serum and blood (100 ml) were diluted with water (1:1), centrifuged, and applied individually in 1-ml portions on cartridges of the same batch number

Table 3
Reproducibility of flow velocity (s/ml) through the SPE cartridges of the same batch. The same serum and blood samples were used for all measurements

	Bond Elut Certify		Confirm HCX		Bakerbond Narc-2		Chromabond-Drug	
	Serum	Blood	Serum	Blood	Serum	Blood	Serum	Blood
1	274	97	171	81	81	103	86	75
2	71	600	78	610	60	136	45	79
3	115	8	228	95	164	22	41	166
4	216	85	239	72	102	211	11	183
5	91	72	462	71	57	167	484	73
6	101	46	83	87	61	51	97	87
7	307	49	660	45	83	40	85	105
8	211	96	91	82	221	23	47	60
9	111	127	63	69	85	93	203	296
10	25	98	165	75	65	145	115	95
Mean	152	128	224	129	98	99	121	122
S.D.	93	169	194	170	53	65	138	73

under the same negative pressure. Table 3 shows the time (in seconds) the samples took to be pulled through the columns. For all cartridges, dramatic differences in flow velocities were observed, which were not related to the kind of biological fluid or packing material and were obviously of random

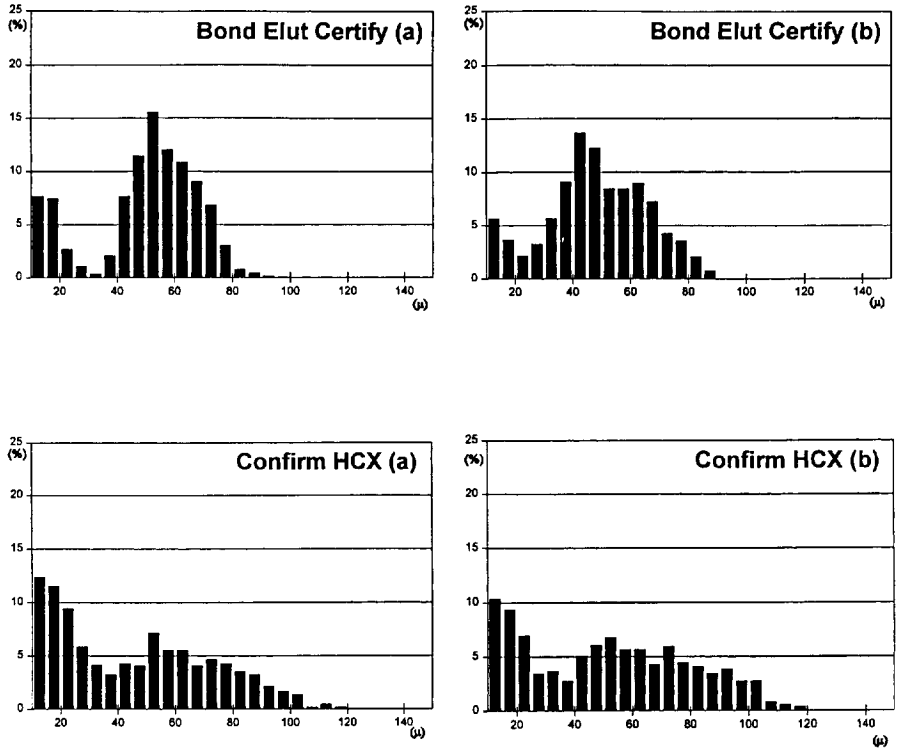

Fig. 6. Particle size distribution of two batches of Bond Elut Certify and Isolute Confirm HCX column packings (data from 1000 measurements for each column).

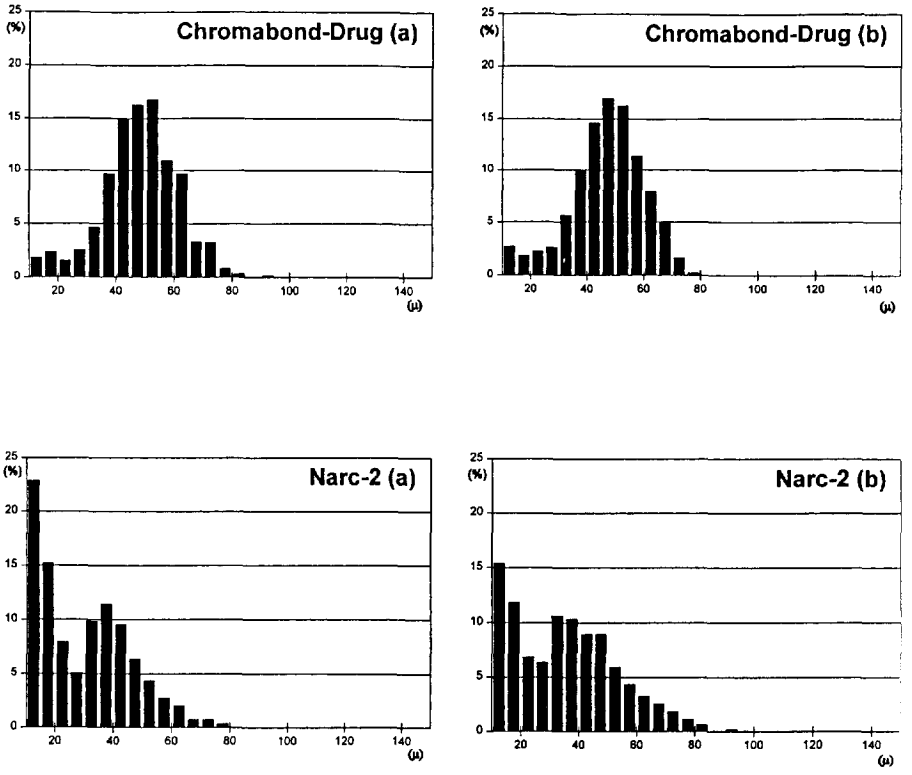

Fig. 7. Particle size distribution of two batches of Chromabond-Drug and Bakerbond Narc-2 column packings (data from 1000 measurements for each column).

character. These findings are similar to the observations of Tebbett, who applied a single hemolyzed and centrifuged blood sample on 10 Bond Elut SCX columns of the same batch. Of these 10 samples, 7 passed the column without difficulty, 1 passed through under positive pressure, and the remaining 2 columns were blocked [20].

The different flow velocities were most probably related to different particle size distribution in the different cartridges, and especially to the number of fine particles. These particles may sediment onto the bottom filter and block the flow partially or totally. Different flow velocities may also explain, to some extent, the large variability of absolute recoveries of drugs. Irrespective of blocking the column, the presence of fine particles may indicate that a fraction of the silica particles in the packing was damaged, exposing free silanol groups. As a consequence, an irreversible adsorption of analytes on silica might occur.

3.4. Morphometric analysis

Fig. 6 and Fig. 7 show the distribution of particle size in two batches of the four examined SPE cartridges. The most narrow and symmetrical distribution was observed in Chromabond Drug columns, whereas other packings presented a nonhomogenous distribution of particles with a large fraction of small-sized grains. Typical microscopic images of all packings are depicted in Fig. 8.

Table 4 presents the statistical evaluation of morphometric examinations. No differences between the results from two batches of the same packing type were observed. The Chromabond Drug column packing showed the smallest scatter of particle size. For comparison sake, the morphometric data obtained in the study were expressed in the same way as particle size distribution was reported by the particular manufacturer. The results of this comparison are presented on the Table 5. Only in the

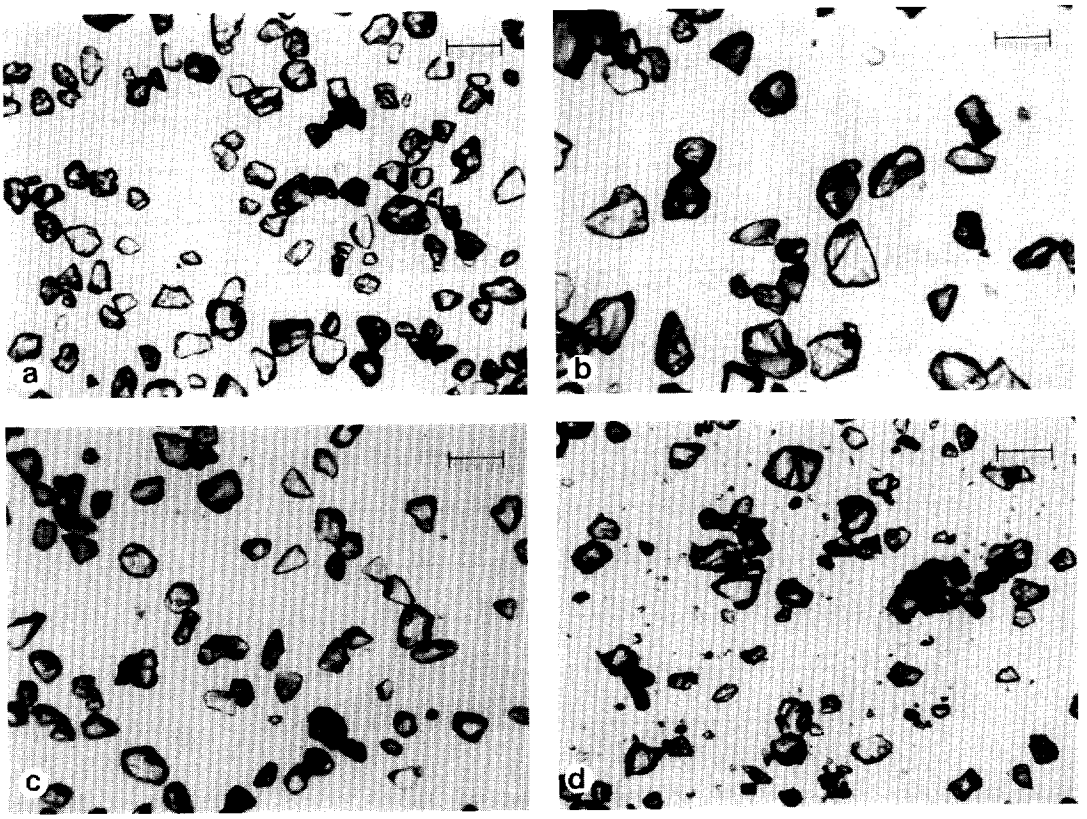

Fig. 8. Typical microscopic images of the examined column packings. (a) Bond Elut Certify, (b) Isolute Confirm HCX, (c) Chromabond-Drug and (d) Bakerbond Narc-2.

Table 4
Statistical evaluation of particle size distribution expressed as DCIRCLE (diameter of the circle equivalent area) for the 1st batch (a-duplicate measurement) and 2nd batch (b-single measurement) of each SPE column

SPE column	Mean value (μm)	±S.D. (μm)	Median value (μm)	Maximum[a] (μm)
Bond Elut Certify (a)	50	19	53	98
Bond Elut Certify (a)	49	22	52	144
Bond Elut Certify (b)	48	18	48	127
Confirm HCX (a)	46	27	44	147
Confirm HCX (a)	54	31	57	131
Confirm HCX (b)	52	28	52	117
Bakerbond-Narc-2 (a)	30	16	29	78
Bakerbond-Narc-2 (a)	35	18	34	90
Bakerbond-Narc-2 (b)	35	18	35	96
Chromabond-Drug (a)	48	13	49	96
Chromabond-Drug (a)	48	13	48	89
Chromabond-Drug (b)	47	13	48	102

[a] The minimal value for each analysis was set at 10 μm, due to the resolution of optical system.

Table 5
Comparison of observed particle size distribution (PSD) with the available data from the manufacturers; a1 and a2=first batch (duplicate measurement), b=second batch

SPE column	PSD (manufacturer)	PSD a1 (measured)	PSD a2 (measured)	PSD b (present data)
Bond Elut Certify	No data	No comparison possible	No comparison possible	No comparison possible
Confirm HCX	6.5%>75 μm 93% 30–75 μm 0.05%<10 μm avg. 61 μm	17.3%>75μm 43.4% 30–75 μm – avg. 46 μm	31.4%>75μm 35.6% 30–75 μm – avg. 54 μm	23.5%>75 μm 46% 30–75 μm – avg. 52 μm
Bakerbond-Narc-2	16% <31 μm avg. 50 μm	53% <31 μm avg. 30 μm	44%<31 μm avg. 35 μm	43%<31 μm avg. 35 μm
Chromabond-Drug	95%>30μm 50%>39.85 μm 5%>51.06 μm avg. 45 μm	92%>30μm 78%>39.85 μm 43%>51.06 μm avg. 48 μm	91%>30μm 76%>39.85 μm 40%>51.06 μm avg. 48 μm	90%>30μm 75%>39.85 μm 39% >51.06 μm avg. 47 μm

case of Chromabond Drug packing were similar results observed. For other two products (Isolute Confirm HCX and Bakerbond Narc-2) a distinct discrepancy was noted. The manufacturers of Bond Elut Certify did not supply any information concerning quality control, therefore no comparative data could be generated.

4. Conclusions

The present study showed that the absolute recoveries of opiates from blood or serum were very variable for all examined mixed-phase SPE cartridges. Some of brands were slightly better than others. Also, large differences in flow velocities for the same batch of the same commercial products were observed. The observed differences in absolute recoveries among cartridges of the same brand and batch make any quantitation without internal standardization unreliable.

The morphometric examinations of SPE column packings have demonstrated a large scatter of particle size, and an important fraction of fine particles in some products. This may cause clogging of the SPE cartridges and consequently impair the recovery. The cartridges packed with most homogenous particles showed also the most reproducible recoveries.

Blood and serum extracts appeared very pure in HPLC–ED examination. Also in GC–MS examination no relevant interferences were observed, but some extracts revealed large peaks from a plasticizer.

Finally, the study showed that there is a need for improvement in the quality of SPE cartridges: the products were supplied without quality control certificates or with very sparse ones, which were not always concordant with the reality. Generally, this resembles the situation of analytical HPLC in mid 1980s, when various commercial brands, or even various batches of the same packing material showed large differences in selectivities.

The doubts and critical opinions concerning the not always satisfactory quality of SPE columns, which are often heard in scientific discussions, are seldom reported in systematic studies. This may be caused by several factors: in many cases, the compounds in question are easily extractable, the amount of the sample is not critical or the concentration is sufficiently high. In the present study, drugs were chosen which are difficult to isolate and which occur in relatively low concentrations in complicated biological matrix. The multitude of methods for the isolation of morphine from biological fluids may serve as evidence of these difficulties (e.g. [34]). Last but not least, the psychological factor should not be neglected; it is not easy to present a study showing some negative results or pitfalls. The opposite is much more attractive. Nevertheless, critical reviews of SPE products are indispensable, as was

proven in the case of analytical HPLC columns in the previous decade.

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