*Journal of Pharmaceutical & Biomedical Analysis*
Vol. 9, No. 2, pp. 129–139, 1991
Printed in Great Britain

0731–7085/91 $3.00 + 0.00


# Evaluation of solid-phase extraction of basic drugs from human milk*

M. MOORS and D.L. MASSART†

*Vrije Universiteit Brussels, Pharmaceutical Institute, Laarbeeklaan 103, B-1090 Brussels, Belgium*

**Abstract**: This article evaluates the use of commercially available cyanopropyl and octadecyl sorbents for the extraction of basic drugs from breast milk. Twenty drugs were selected from different pharmacological groups (beta-blocking agents, antidepressants, anxiolytic sedatives and neuroleptics, antihistamines, alkaloids and an anthelmintic) and subjected to a general solid-phase extraction (SPE) procedure described earlier for plasma samples. This SPE method was developed on a cyanopropyl cartridge and consisted of a conditioning step with methanol and water, the adsorption of the deproteinized matrix, washing with water and/or methanol, and finally the elution of the basic compounds with 0.1% propylamine in methanol. The extracts were further analysed by reversed-phase liquid chromatography (RP-LC). The application of SPE to human milk samples utilized cyanopropyl and octadecyl cartridges. The latter can be applied more generally because it better retains the basic compounds. For 14 out of 17 drugs extracted from breast milk, recoveries of >70% were obtained. Standard deviations were, with the exception of three drugs, in the same range as those observed for plasma samples, i.e. 2–8%.

The development of a strategy for SPE of drugs from human milk was difficult. For a number of drugs, in particular those present in human milk at low concentrations and/or detected in a non-selective way, matrix compounds interfered with the subsequent LC analysis. Therefore, SPE on CN or $C_{18}$-sorbent for the analysis of basic compounds in breast milk was found to be useful as one of the steps in an extraction procedure, but not as a single technique. A major drawback of SPE is the batch-to-batch variation of the sorbents.

**Keywords**: *Solid-phase extraction; liquid chromatography; breast milk.*

## Introduction

Drugs taken by the mother during the lactation period may pass the blood–milk barrier and accumulate in the breast milk. The amount of drug ingested by the infant through breast feeding depends on the drug properties (e.g. the $pK_a$, solubility, molecular weight and half-life), as well as mother- and child-related pharmacokinetic and pharmacological factors (e.g. the protein binding, milk composition, pH of the breast milk, etc.). The degree of transfer of drugs from the blood stream into the breast milk is expressed as a milk-to-plasma ratio (M/P). Determination of a M/P ratio demands the availability of analytical methods for quantifying the drug in both plasma and breast milk. The number of articles describing analytical methods for the determination of drugs in breast milk are few when compared with other biological fluids like plasma and urine. Wilson *et al.* [1] reviewed the different techniques used in the quantification of drugs in breast milk: chromatographic techniques (GC, LC and TLC), radioimmunoassay, electrophoresis, spectrophotometric and enzymatic techniques. When the analysis has been performed by LC, the detection systems used were UV-spectroscopy, fluorimetry or electrochemical detection. The majority of the described extraction and purification procedures consisted of liquid–liquid extraction, while only a few articles mentioned the use of solid-phase extraction (SPE). For the extraction of mycotoxins from milk, the use of $C_{18}$-cartridges has been described [2–4]. Chiou *et al.* [5] extracted the antiparisitic agent ivermectin from plasma and human milk using a $C_2$-cartridge, after which the drug was derivatized and a further SPE was carried out on a diol-sorbent. Ross *et al.* [6] used a $C_{18}$-cartridge for the extraction of bile acids from human milk and SPE on silica has been used as one of the sample handling steps in a method for quantifying vitamin D in breast milk [7]. The advantages of SPE compared with liquid–liquid extraction have been described extensively [8–11] and many applications can be found in the literature with biological fluids such as plasma.

* Presented at the "Second International Symposium on Pharmaceutical and Biomedical Analysis", April 1990, York, UK.

† Author to whom correspondence should be addressed.

The aim of this work was to investigate systematically whether commercially available sorbents can be used for extracting drugs from breast milk samples. It is a continuation of the work of Musch and Massart [8, 9], who studied the use of the cyanopropyl-sorbent for the extraction of basic drugs from plasma. They developed a general strategy on the CN-sorbent that used a mixture of phosphate buffer and methanol or propylamine in methanol as the eluent.

## Experimental

*Chemicals and reagents*

The water used to prepare all solutions and buffers was filtered, double distilled and deionized by means of a Milli-Q water purification system (Millipore, Bedford, USA). Methanol, acetonitrile p.a., sodium dihydrogen phosphate monohydrate p.a. and *ortho*-phosphoric acid 85% p.a. were purchased from E. Merck (Darmstadt, FRG). Propylamine was obtained from Fluka A.G. (Buchs, Switzerland). All vials were silanized with Aquasil® (Pierce Chemicals Co., Rockford, IL, USA).

*Preparation of solutions*

Stock solutions (1000 ppm) were prepared by dissolving 50 mg of the drug in 50 ml water or methanol depending on the solubility of the drug. The stock solutions were kept in the refrigerator at 4°C. Standards of different concentrations were prepared daily by appropriate dilutions of the stock solutions.

Phosphate buffer (pH 3, $\mu = 0.05$) was prepared by dissolving 6.8995 g $NaH_2PO_4 \cdot 1H_2O$ per litre $H_2O$, to which 8 ml of 1 M $H_3PO_4$ was added.

*Apparatus*

The SPE was performed on cyanopropyl or octadecyl cartridges (J.T. Baker Chemical Co., Philipsburg, USA) using a Baker-10 or Baker-21 Vacuum Manifold. The cartridges contained 100 mg sorbent and had a reservoir of 1 ml.

A Perkin–Elmer Series 10 Liquid Chromatograph pump was used. Chromatography was performed in a reversed-phase mode on a 5-μm Lichrosorb® cyanopropyl or octadecyl column, 125 or 250 × 4 mm i.d. and protected with a guard column (30 × 4 mm). The mobile phase consisted of a pH 3 phosphate buffer, $\mu = 0.05$, and an appropriate amount of the organic modifier acetonitrile. The flow rate was 1 ml $min^{-1}$. Samples were injected into a Rheodyne injector fitted with a 100-μl loop. The chromatograms were recorded on an Ankersmit A41 2-channel recorder and integrated by means of a Merck–Hitachi D-2000 Chromato-Integrator or an Intersmat Data-Processor IC-R3A. Two detection systems were used: a Perkin–Elmer LC 90 UV-spectrophotometric detector with a pathlength of 1 cm and a Perkin–Elmer LS-4 fluorescence spectrometer.

*Extraction procedure of basic drugs from breast milk*

A 1-ml aliquot of breast milk was spiked with 100 μl of an appropriate concentration of an aqueous solution of a drug, and vortex-mixed. The milk was deproteinized by adding dropwise 3 ml of acetonitrile with continuous vortex-mixing. After centrifugation, the liquid was separated from the protein pellet by decanting into a vial. The acetonitrile, used for deproteinization, was removed by evaporation at a temperature of 60°C under a stream of nitrogen, until approximately 750 μl of the aqueous phase was left. One millilitre water was added to increase the volume and this sample was subjected to the SPE on a 1 ml CN- or $C_{18}$-cartridge. The cartridge was conditioned with 2 ml methanol, followed by 2 ml water, while the vacuum pressure was kept at ±30 kPa. The sample was subsequently passed through the column under vacuum to allow the adsorption of the drug. The wash step consisted of 5 ml water or 3 ml water followed by 2 ml water–methanol (1:1), depending on the drug to be analysed. The elution of the drug was performed with 1 ml of 0.1% propylamine in methanol. The eluent was evaporated to dryness at 60°C under a stream of nitrogen. The residue was dissolved in 1 ml of the mobile phase used in the subsequent LC analysis.

## Results and Discussion

The drugs selected in this study belong to varying pharmacological groups (Table 1) but they are all basic compounds with 10–21 carbon atoms. Thus, a set of drugs comparable with the one used by Musch and Massart [8, 9] in their investigations for the SPE of basic drugs from plasma was obtained.

**Table 1**
Experimental LC and detection conditions

| Group | Drug | % Acetonitrile in mobile phase | $k'$ | UV-detection $\lambda_{max}$ (nm) | UV-detection LDC (ppm)‡ | Fluorescence detection $\lambda_{ex}$ | Fluorescence detection $\lambda_{em}$ | Fluorescence detection LDC (ppm)‡ |
|---|---|---|---|---|---|---|---|---|
| Beta-adrenoceptor blocking agents | Propranolol | 10 | 1.5 | 289 | 0.1 | 290 | 340 | 0.005 |
| | Metoprolol | 1 | 1.5 | 224 | 0.075 | 254 | 305 | 0.075 |
| | Alprenolol | 10 | 1.0 | 270 | 0.2 | 275 | 308 | 0.05 |
| | Acebutolol | 2.5* | 3.7 | 235 | 0.2 | 254 | 460 | 4 |
| | Prenalterol | 0 | 0.4 | 225 | 0.03 | 230 | 330 | 0.03 |
| | Atenolol | 0 | 0.7 | 275 | 0.25 | 230 | 300 | 0.0125 |
| | Practolol | | 1.0 | 247 | 0.2 | — | — | — |
| Tricyclic antidepressants | Carbamazepine | 20 | 1.3 | 285 | 0.04 | — | — | — |
| | Imipramine | 30 | 1.8 | 254 | 0.025 | — | — | — |
| Benzodiazepine | Diazepam | 15 | 1.6 | 254 | 0.025 | — | — | — |
| Phenothiazines | Prochlorperazine | 0 | 1.4 | 254 | 0.05 | — | — | — |
| | Chlorpromazine | 25 | 1.7 | 254 | 0.025 | — | — | — |
| | Promethazine | 25* | 3.7 | 254 | 0.1 | — | — | — |
| Alkaloids | Papaverine | 15* | 2.8 | 249 | 0.05 | — | — | — |
| | Yohimbine | 10 | 1.1 | 220 | 0.015 | 285 | 363 | 0.004 |
| | Vincamine | 15* | 2.6 | 230 | 0.125 | — | — | — |
| | Quinine sulph. | 10 | 1.1 | 250 | 0.01 | 354 | 420 | 0.01 |
| | Quinidine sulph. | 7.5 | 1.0 | 249 | 0.04 | 248 | 434 | 0.0015 |
| $H_2$-antihistamines | Cimetidine | 0/20† | 0.8/2.0** | 220 | <0.05 | — | — | — |
| Anthelmintic | Thiabendazole | 2.5 | 1.6 | 300 | 0.015 | — | — | — |

*Chromatographed on a CN-column of 125 mm length instead of 250 mm.
†Chromatographed on a $C_{18}$-column.
‡Experimentally determined lowest detectable concentration.

*Chromatography and detection*

The drugs were chromatographed in a reversed-phase (RP) mode on a cyanopropyl stationary phase with pH 3 phosphate buffer, $\mu = 0.05$. The percentage of organic modifier added was selected according to the rules for the determination of the starting mobile phase composition in RP with buffer, described in the expert system LABEL [12]. Minor changes in the amount of acetonitrile were sometimes necessary to allow elution of the drugs with a capacity factor between 1–5. The percentage of acetonitrile used and the corresponding $k'$ for the drugs studied are shown in Table 1. If a more selective detection system could be used for the drug (e.g. fluorescence), a $k'$ as low as 1 was sometimes sufficient to separate the peak of interest from the matrix peak. This was the case for prenalterol and atenolol. In case of UV-detection, and especially at low wavelength, a higher $k'$ was required. Therefore, milk extracts of cimetidine were chromatographed on a $C_{18}$ stationary phase to increase the retention; with a mobile phase composition of acetonitrile–phosphate buffer (20:80), a capacity factor of 2.0 was attained. Table 1 also lists the $\lambda_{max}$ for UV-detection and the excitation and emission wavelength for compounds with intrinsic fluorescence capacity, determined in the mobile phase.

If both detection systems were applicable for a compound, the most sensitive one was chosen. This was judged by comparing the experimentally determined lowest detectable concentrations (LDC), i.e. the concentration of an aqueous solution giving rise to a UV-absorption of $2.5 \times 10^{-4}$ units or a fluorescence intensity of 1 unit (Table 1). Because of its selectivity, fluorescence detection is preferred if the lowest detectable concentration is approximately the same with both detectors.

*Solid-phase extraction (SPE)*

The investigation on the use of SPE for milk samples was carried out by checking each subsequent step of the procedure described above for possible losses or specific problems.

*Deproteinization.* The precipitation of the proteins, performed by dropwise addition of 3 ml acetonitrile, caused no major losses. For all drugs, recoveries of approximately 100% were demonstrated.

*Adsorption.* After deproteinization, the milk was pulled through a conditioned cyanopropyl cartridge, ideally to adsorb the drug completely. Based on the information regarding SPE of basic drugs from plasma [8, 9], the same sorbent (100 mg cyanopropyl) was used. The average percentage of drug adsorbed from deproteinized human milk, which consisted of milk–acetonitrile (1:3, v/v), was only 18.2%. For quinine sulphate, the highest adsorption was obtained, namely 81%. Practolol, metoprolol and papaverine were not retained at all. The results are shown in Table 2.

The adsorption of 5 ppm solutions prepared in a water–acetonitrile mixture (1:3, v/v) was 100% for the majority of the drugs. Exceptions were carbamazepine, thiabendazole, papaverine, diazepam and cimetidine.

The following experiment suggested that for most drugs, the incomplete adsorption from deproteinized milk was caused by both a competition with the minerals present in milk and by the eluotropic strength of the 3 ml acetonitrile used for deproteinization. Aqueous solutions containing increasing amounts of $Ca^{2+}$ or $Na^{+}$ and $K^{+}$ were spiked with 5 $\mu g\ ml^{-1}$ imipramine. To one series of spiked salt solutions, 3 ml acetonitrile per ml water was added to simulate the deproteinization of a milk sample; to another series of spiked salt solutions, 3 ml of water was added. In both series the salt concentrations were the same but the solvent composition differed, water versus water–acetonitrile (1:3, v/v). The samples were subjected to the adsorption step. As can be seen from Table 3, the adsorbed fraction of imipramine from the series of salt solutions containing no acetonitrile was 100%. However, when acetonitrile was added, the adsorbed fraction decreased with increasing salt concentration and only from the water–acetonitrile (1:3, v/v) mixture (without minerals) was 100% adsorption obtained.

Dilution of the deproteinized milk sample with 2 and 6 ml water reduced the salt concentration as well as the acetonitrile fraction, and generally an improvement in the fraction adsorbed was seen (Table 2). An alternative method was to evaporate the acetonitrile used for the deproteinization. The volume of the remaining aqueous sample was increased by the addition of 1 ml water which was subsequently pulled through the cartridge. This procedure resulted in an adsorbed fraction of $\geq 90\%$ for all drugs except cimetidine, which

**Table 2**
Drug adsorption on a cyanopropyl cartridge (1 ml capacity–100 mg sorbent) from different media

| Drug | % Adsorbed from water–acetonitrile (1:3) | Spiked conc. in milk (ppm) | % Adsorbed from: deprot. breast milk | deprot. milk diluted with 2 ml water | deprot. milk diluted with 6 ml water | deprot. milk and acetonitrile evaporated |
|---|---|---|---|---|---|---|
| Propranolol | 100 | 5 | 18 | 80 | 96.8 | 98 |
| Metoprolol | 100 | 10 | 0 | 35 | 19 | 94 |
| Alprenolol | 100 | 10 | 8 | 48 | 34 | 98.5 |
| Acebutolol | 100 | 5 | 35 | 14.5 | 32.5 | 98 |
| Prenalterol | 100 | 0.5 | — | — | — | 100 |
| Atenolol | 100 | 0.5 | 5.3 | 31.2 | 0 | 90.4 |
| Practolol | 100 | 5 | 0 | 45 | 27 | 100 |
| Carbamazepine | 0 | 5 | — | — | — | 98 |
| Imipramine | 100 | 10 | 51 | 92 | 100 | 98 |
| Diazepam | 20 | 5 | 11 | 0 | 0 | 99.8 |
| Prochlorperazine | 100 | 5 | 29 | 73 | 100 | 94.8 |
| Chlorpromazine | 100 | 10 | 32 | 85 | 98 | 97 |
| Promethazine | 100 | 5 | 12 | 43 | 96.9 | 99.3 |
| Papaverine | 36.5 | 5 | 0.7 | 0 | 4.8 | 100 |
| Yohimbine | 100 | 5 | 7 | 22 | 44 | 98.7 |
| Vincamine | 100 | 5 | 3.6 | 33.6 | 63.1 | 98.3 |
| Quinine sulphate | 100 | 5 | 81 | 94 | 100 | 100 |
| Quinidine sulphate | 100 | 1 | 12 | 28 | 55 | 99.6 |
| Cimetidine | 15 | 5 | * | 30 | 0 | 71.5 |
| Thiabendazole | 28 | 5 | — | — | — | 100 |

*Not possible to determine: the peak of cimetidine co-elutes with the matrix peak in the chromatogram.

**Table 3**
Adsorbed fraction of imipramine on 100 mg cyanopropyl sorbent from salt solutions

| Adsorption medium | + 3 ml Acetonitrile (%) | +3 ml Water (%) |
|---|---|---|
| $CaCl_2 \cdot H_2O$ solutions: | | |
| 0 mequiv $l^{-1}$ $Ca^{2+}$ | 100 | 100 |
| $1.36 \times 10^{-3}$ mequiv $l^{-1}$ $Ca^{2+}$ | 50.7 | 100 |
| $1.36 \times 10^{-2}$ mequiv $l^{-1}$ $Ca^{2+}$ | 21.4 | 100 |
| $5.44 \times 10^{-2}$ mequiv $l^{-1}$ $Ca^{2+}$ | 17.1 | 100 |
| NaCl and KCl solutions: | | |
| 0 mequiv $l^{-1}$ $Na^+$–$K^+$ | 100 | 100 |
| 0.02 mequiv $l^{-1}$ $Na^+$–$K^+$ | 35.0 | 100 |
| 0.05 mequiv $l^{-1}$ $Na^+$–$K^+$ | 21.2 | 100 |
| 0.14 mequiv $l^{-1}$ $Na^+$–$K^+$ | 14.0 | 100 |

was only 71.5% retained (Table 2). However, cimetidine has only 10 carbon atoms in its structure and previous work has shown that the retention of smaller compounds with less than 11 C-atoms is problematic on the CN-sorbent [8].

To obtain better adsorption, the more apolar octadecyl sorbent was used. The adsorption experiments performed on CN-cartridges were repeated on $C_{18}$-cartridges for the five beta-blocking agents and the smaller molecule cimetidine. The results in Table 4 show that for the $C_{18}$-sorbent, the eluotropic strength of the adsorption medium must be decreased by diluting the deproteinized milk with water or by evaporating the acetonitrile, just as for the CN-sorbent. However, the drugs were 100% adsorbed on a $C_{18}$-cartridge after evaporating the acetonitrile.

For cimetidine, which was never totally adsorbed on the cyanopropyl-sorbent under any conditions, the $C_{18}$-sorbent could successfully be employed instead.

The results indicate that the $C_{18}$-sorbent is a better choice than the CN-sorbent when developing general SPE procedures.

*Wash step*. For most of the basic compounds retained on the CN-sorbent, 3 ml water could be used for rinsing without elution of the drug. Exceptions were thiabendazole, carbamazepine, diazepam, vincamine, alprenolol and yohimbine (Table 5). For these drugs, the alternative cartridges of 100 mg $C_{18}$-sorbent and 500 mg CN-sorbent were tested and all compounds were adsorbed completely on both types of cartridges with no elution of the drugs upon rinsing with water.

The use of methanol or water–methanol (1:1) was more variable and had to be checked for every compound. The percentage of drug eluting in the wash-step is presented in Table 5.

The advantage of the use of methanol in this step is that the final eluent is cleaner: the chromatogram of the extract shows less interfering matrix peaks. This is illustrated in Fig. 1, which shows the chromatograms of the extracts of 1 ml breast milk. In Fig. 1A, the CN-cartridge was not rinsed; in 1B, the column was washed with 3 ml water; and in 1C, a sequence of 1 ml water, 1 ml water–methanol (1:1) and 1 ml methanol was used. In chromatogram 1C, the matrix peak is clearly smaller than in A and B.

*Elution*. The eluents used were the same as

**Table 4**
Drug adsorption on an octadecyl cartridge (1 ml capacity–100 mg sorbent) from different media

| Drug | % Adsorbed from: deprot. breast milk | deprot. milk diluted with 2 ml water | deprot. milk diluted with 6 ml water | deprot. milk and acetonitrile evaporated |
|---|---|---|---|---|
| Propranolol | 10.6 | 46.3 | 100 | 100 |
| Practolol | 0 | 23.9 | 38.8 | 100 |
| Alprenolol | 50.7 | 58.3 | 100 | 100 |
| Metoprolol | 100 | 100 | 100 | 100 |
| Acebutolol | 100 | 100 | 75.1 | 100 |
| Cimetidine | — | — | — | 100 |

**Table 5**
Elution of basic drugs adsorbed on a CN-cartridge (A) or a $C_{18}$-cartridge (B) with different wash solvents

| Drug | % Elution with 3 ml water | % Elution with 2 ml methanol–water (1:1) | % Elution with 1 ml methanol–water (1:1) + 1 ml methanol |
|---|---|---|---|
| | | (A) CN-cartridge | |
| Propranolol | 0 | 0 | 0 |
| Metoprolol | 0 | 0 | 12.9 |
| Alprenolol | 8.9 | 0 | 12.9 |
| Acebutolol | 0 | 0 | 12.6 |
| Prenalterol | 0 | 46.9 | 40.6 |
| Atenolol | 0 | 16.7 | 59.1 |
| Practolol | 0 | 20.5 | 24.3 |
| Carbamazepine | 21.0 | 82.7 | 86.6 |
| Imipramine | 0 | 0 | 4.8 |
| Diazepam | 4.5 | 77.4 | 77.2 |
| Prochlorperazine | 0 | 0 | 52.9 |
| Chlorpromazine | 0 | 1.9 | 7.2 |
| Promethazine | 0 | 4.2 | 25.5 |
| Papaverine | 0 | 7.0 | 78.9 |
| Yohimbine | 2.6 | 0 | 26.7 |
| Vincamine | 2.9 | 0.9 | 82.7 |
| Quinine sulphate | 0 | 5.0 | 7.4 |
| Quinidine sulphate | 0 | 0 | 17.3 |
| Thiabendazole | 21.0 | — | — |
| | | (B) $C_{18}$-cartridge | |
| Propranolol | 0 | 0 | 0 |
| Metoprolol | 0 | 0 | 20.3 |
| Alprenolol | 0 | 0 | 79.3 |
| Acebutolol | 3.2 | 0 | 75.8 |
| Practolol | 0 | 0 | 0 |
| Carbamazepine | 0 | 82.5 | 93.0 |
| Diazepam | 0 | 1.1 | 83.0 |
| Cimetidine | 0 | — | — |
| Thiabendazole | 0 | 76.7 | 93.1 |

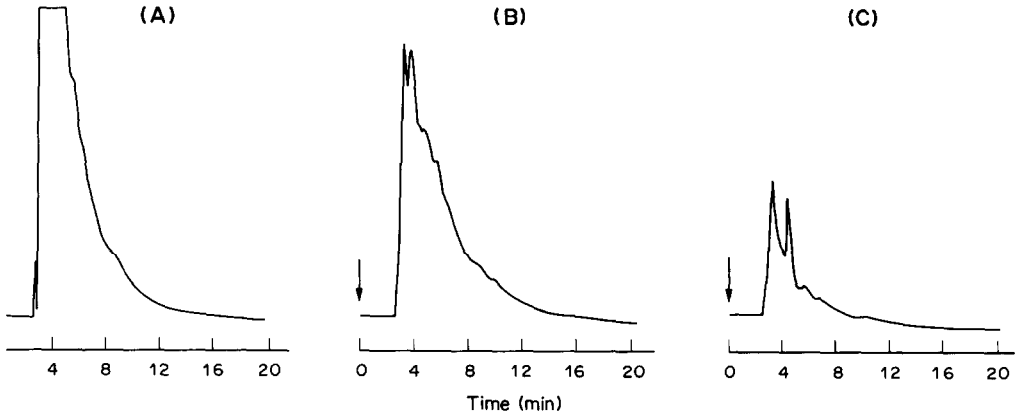


**Figure 1**
Extracts of 1 ml breast milk on a CN-cartridge. (A) no wash step, (B) 3 ml water wash, (C) 1 ml water–methanol (1:1) + 1 ml methanol wash. HPLC mobile phase:phosphate buffer–$CH_3CN$ (85:15). Detection: $\lambda$ = 254 nm, 0.005 AUFS.

described in the article by Musch and Massart [8, 9]: 1 ml methanol containing 0.1% propylamine or 1 ml of the mixture methanol–phosphate buffer pH 3, $\mu$ = 0.05. According to Musch and Massart [8, 9], methanol–phosphate buffer yielded better results for more polar drugs, whereas 0.1% propylamine in methanol was a more suitable eluent for apolar basic drugs. For the drugs under study, the latter gave satisfactory elution recoveries when extracting aqueous solutions on both CN- and $C_{18}$-cartridges. The recoveries obtained with methanol–phosphate buffer were generally in the same range or lower than those with

propylamine in methanol (exceptions were cimetidine and metoprolol on $C_{18}$; Table 6).

Extraction of spiked human milk samples on CN or $C_{18}$-cartridges with 0.1% propylamine in methanol as the eluent yielded mean recoveries between 50–95% (Table 7). The lowest recoveries were observed for chlorpromazine (50.6%), imipramine (59.3%) and atenolol (60.5%). All other recoveries were >70%. The chromatograms of a blank extract, an extract of a spiked milk sample and a standard solution for promethazine, metoprolol, prenalterol and carbamazepine are shown in Figs 2–5, respectively. These results show that the SPE method described for plasma samples, whether CN or $C_{18}$-cartridges were used, was applicable for human milk

**Table 6**
Drug recoveries from the SPE of aqueous samples

| Drug | Type of cartridge | % Recovery with 1 ml 0.1% PA in methanol | % Recovery with 1 ml methanol–phosphate buffer |
|---|---|---|---|
| Propranolol | $C_{18}$ | 87.5 | 69 |
| Metoprolol | $C_{18}$ | 80.3 | 90.4 |
| Alprenolol | $C_{18}$ | 71 | 37 |
| Acebutolol | $C_{18}$ | 94 | 80 |
| Prenalterol | $C_{18}$ | 96.4 | 101.1 |
| Atenolol | $C_{18}$ | 96.7 | 71 |
| Practolol | $C_{18}$ | 92 | 78 |
| Carbamazepine | $C_{18}$ | 99 | 43.5 |
| Imipramine | CN | 84.5 | 70 |
| Diazepam | $C_{18}$ | 93.5 | 0 |
| Prochlorperazine | CN | 91.5 | 37 |
| Chlorpromazine | CN | 84.5 | 60 |
| Promethazine | CN | 89.5 | 59 |
| Papaverine | CN | 86 | 69 |
| Yohimbine | CN | 102 | 91.8 |
| Vincamine | CN | 94.5 | 92.9 |
| Quinine sulphate | CN | 98 | 89 |
| Quinidine sulphate | CN | 101 | 87.2 |
| Cimetidine | $C_{18}$ | 82.6 | 95.2 |
| Thiabendazole | $C_{18}$ | 96 | 93 |

Eluent = 0.1% propylamine (PA) in methanol or methanol–phosphate buffer pH 3, $\mu = 0.05$.

**Table 7**
Recoveries on the SPE on CN or $C_{18}$ cartridges (100 mg sorbent) for basic drugs from breast milk

| Drug | Spiked concentration† | Recovery (%) ± standard deviation | Number of repetitions | Cartridge type | Wash solvents |
|---|---|---|---|---|---|
| Propranolol | 50 ng ml$^{-1}$ | 77.0 ± 7.5 | 6 | $C_{18}$ | Water–methanol |
| Metoprolol | 300 ng ml$^{-1}$ | 80.5 ± 4.2 | 7 | $C_{18}$ | Water–methanol |
| Alprenolol | 200 ng ml$^{-1}$ | 83.2 ± 11.2 | 7 | $C_{18}$ | Water–methanol |
| Acebutolol | 1 μg ml$^{-1}$ | 75.8 ± 7.8 | 4 | CN | Water |
| Prenalterol | 500 ng ml$^{-1}$ | 86.0 ± 3.3 | 7 | $C_{18}$ | Water |
| Atenolol | 200 ng ml$^{-1}$ | 60.5 ± 8.7 | 7 | $C_{18}$ | Water |
| Practolol | 1 μg ml$^{-1}$ | 77.3 ± 2.0 | 7 | $C_{18}$ | Water |
| Carbamazepine | 1 μg ml$^{-1}$ | 95.6 ± 1.9 | 6 | $C_{18}$ | Water |
| Imipramine | 1 μg ml$^{-1}$ | 59.3 ± 7.1 | 6 | CN | Water–methanol |
| Diazepam | 100 ng ml$^{-1}$ | 72.7 ± 5.6 | 7 | $C_{18}$ | Water |
| Chlorpromazine | 250 ng ml$^{-1}$ | 50.6 ± 6.8 | 6 | CN | Water–methanol |
| Promethazine | 500 ng ml$^{-1}$ | 71.9 ± 5.0 | 7 | CN | Water |
| Papaverine | 250 ng ml$^{-1}$ | 89.5 ± 4.5 | 7 | CN | Water |
| Yohimbine | 50 ng ml$^{-1}$ | 79.8 ± 5.5 | 7 | CN | Water–methanol |
| Quinine sulphate | 100 ng ml$^{-1}$ | 95.2 ± 5.4 | 7 | $C_{18}$ | Water–methanol |
| Quinidine sulphate | 1 μg ml$^{-1}$* | 83.5 ± 9.6 | 7 | CN | Water |
| Thiabendazole | 100 ng ml$^{-1}$ | 95.8 ± 2.0 | 7 | $C_{18}$ | Water |

Eluent = 0.1% propylamine in methanol.

*The residue, obtained after evaporation of the eluent, was dissolved in 2 ml mobile phase. Dilution was necessary to stay within the linear range of the fluorescence detector.

†The spiked concentrations correspond with those that can be expected to appear in breast milk, and if no data were found in literature on breast milk concentrations, amounts 5–10 times the LDC were added.

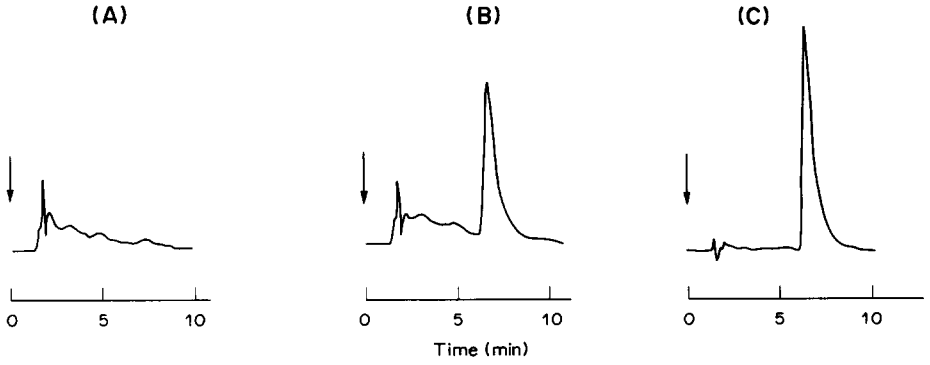


**Figure 2**
Chromatograms of (A) a blank extract, (B) an extract of breast milk spiked with 0.5 μg $ml^{-1}$ promethazine·HCl and (C) a 0.5 ppm standard solution. LC and detection conditions: see Table 1. Att. = 0.005 AUFS.

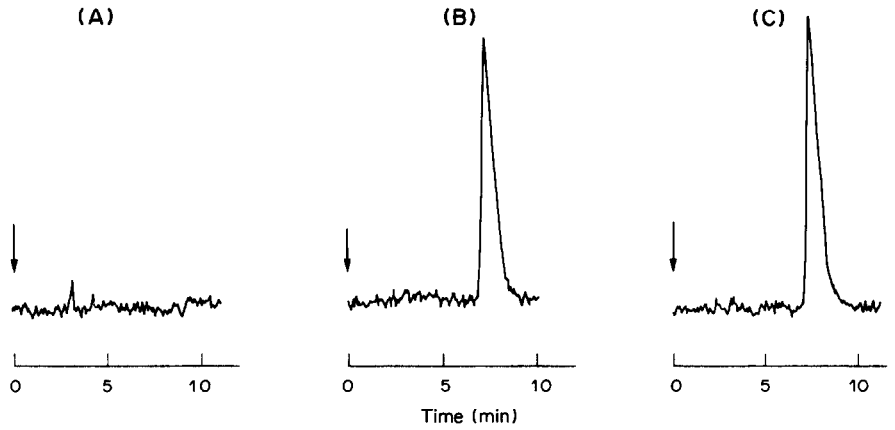


**Figure 3**
Chromatograms of (A) a blank extract, (B) an extract of breast milk spiked with 300 ng $ml^{-1}$ metoprolol and (C) a 0.03 ppm standard solution. LC and detection conditions: see Table 1. Att. = 10 fluorescence units/full scale.

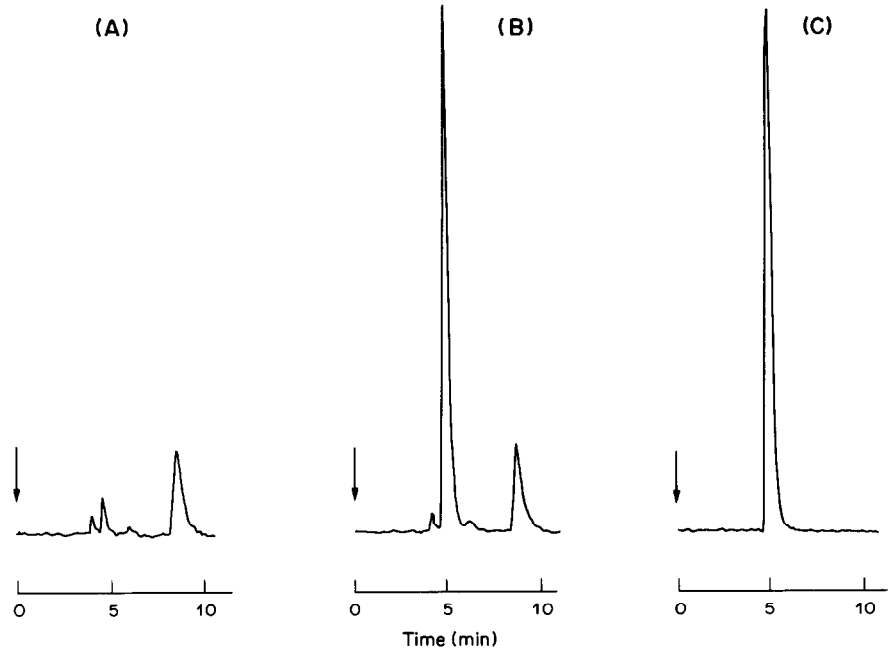


**Figure 4**
Chromatograms of (A) a blank extract, (B) an extract of breast milk spiked with spiked 500 ng $ml^{-1}$ prenalterol and (C) a 0.5 ppm standard solution. LC and detection conditions: see Table 1. Att. = 50 fluorescence units/full scale.

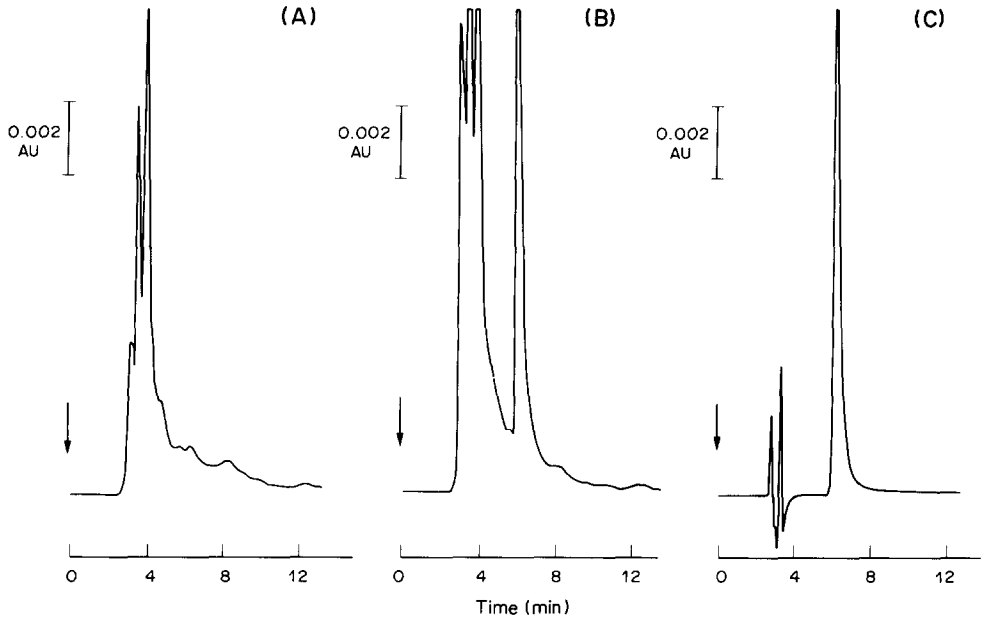


**Figure 5**
Chromatograms of (A) a blank extract, (B) an extract of breast milk spiked with 1 μg $ml^{-1}$ carbamazepine and (C) a 1 ppm standard solution. LC and detection conditions: see Table 1. Att. = 0.02 AUFS.

samples. However, there were some limitations and drawbacks which are discussed subsequently.

*Limitations and drawbacks of the use of SPE for human milk samples*

*Matrix interferences*. The quantitative determination in human milk by means of SPE and LC with UV or fluorescence detection was not possible for cimetidine, vincamine and prochlorperazine because of the presence of interfering matrix peaks in the chromatogram.

Difficulties were also encountered when analysing different breast milk samples. The composition of the breast milk varied during the lactation period and from one person to another such that the applied SPE yielded sufficiently clean extracts for some samples but not for others. Figure 6 illustrates the chromatograms of two different milk samples that were extracted and chromatographed under the same conditions. Chromatogram B shows more matrix interferences than chromatogram A. A quantitative determination of a compound with a retention time of 12 min will give no problems for breast milk A but becomes impossible for breast milk B, because the extract is not clean enough. Thus, the appli-

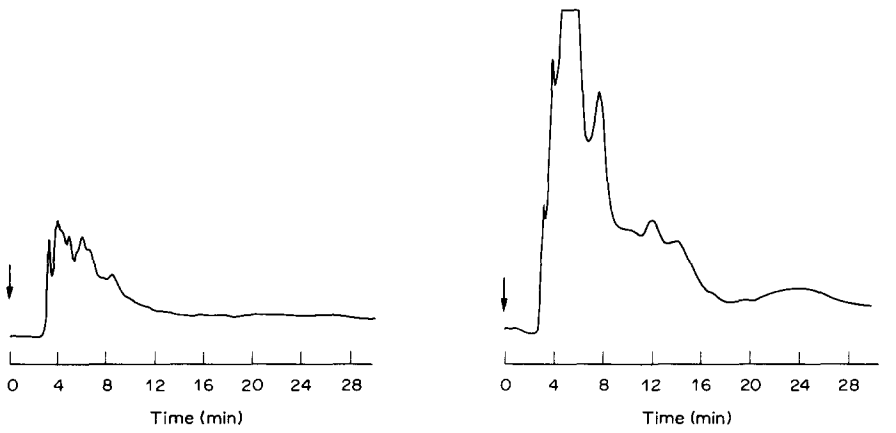


**Figure 6**
Chromatograms of extracts of two different milk samples. SPE on a CN-sorbent; Detection, λ = 254 nm; LC mobile phase, phosphate buffer pH 3 (μ = 0.05)–acetonitrile (3:1, v/v).

cation of SPE on non-selective sorbents such as $C_{18}$ or CN is limited because it does not yield highly purified extracts. Whether matrix compounds still present in the SPE extract will actually interfere in the chromatogram depends on the selectivity and sensitivity of the detector and/or the concentration of the analyte. Using a variable wavelength UV-detector or a fluorescence detector, it is estimated that only concentrations in the high ppb to ppm range can be determined.

*Sources of variation in recovery*

*(a) Repeatability.* Preliminary experiments indicated that manipulations such as the deproteinization step, the evaporation of the eluent and the dissolution of the residue in the mobile phase were unlikely to cause large variations. The standard deviations of the recoveries of basic drugs extracted from plasma ranged between 2 and 8% [8, 9]. The standard deviation remains within that range when extracting from breast milk, except for alprenolol, quinidine sulphate and atenolol for which higher values of 11.2, 9.6 and 8.7%, respectively, were observed.

*(b) Between-batch variation.* A milk sample, spiked with 250 ng $ml^{-1}$ papaverine, was extracted on six CN-cartridges of one batch and six CN-cartridges of another batch. The mean recoveries, 60.1 ± 6.49% and 49.1 ± 6.26%, differed significantly (*t*-test, $P = 0.014$). It is assumed that the between-batch variation in recovery is caused by a varying fraction of silanols on the sorbent and would be comparable with the between-batch variation for LC columns. It implies that an SPE method developed on one batch of cartridges, and that yielded good results, may need to be adapted when a new batch is used.

## Conclusion

A general extraction scheme for basic drugs from breast milk is more difficult to develop than for plasma samples because of the limitations of SPE for this more complex matrix. Whether the SPE extract will be clean enough for the subsequent LC analysis is information that should be obtained on the basis of the detection parameters for the analyte (e.g. detector selectivity, $\epsilon_M$, the analyte concentration and some LC parameters (e.g. injection volume). The difference in milk composition between different samples remains an uncontrolled factor. For "dirty" samples, deproteinization and non-selective SPE alone are insufficient as purification methods. Additional steps, such as a liquid–liquid extraction or a second more selective SPE are needed.

The $C_{18}$-sorbent is preferred to the CN-sorbent because it better retains a wider range of drugs. Because of the batch-to-batch variation, ideally all samples should be analysed on the same batch on which the SPE procedure was optimized. The varying batch-to-batch recoveries are attributed to the residual silanolic functions since the endcapped fraction of bonded phases varies between batches and the residual silanols play an important part in the binding mechanism of basic drugs. However, this needs further investigation.

*Acknowledgements* — The authors thank Mrs K. Decq for skillful technical assistance and the FGWO for financial support.

## References

[1] J.T. Wilson, R.D. Brown, D.R. Cherek, J.W. Dailey, B. Hilman, P.C. Jobe, B.R. Manno, J.E. Manno, H.M. Redetzki and J.J. Stewart, *Clin. Pharmacokinetic.* **5**, 1–66 (1980).
[2] J. Ferguson-Foos and J.D. Warren, *J. Assoc. Off. Anal. Chem.* **67**, 1111–1114 (1984).
[3] N. Takeda, *J. Chromatogr.* **288**, 484–488 (1984).
[4] S.P. Swanson, A.M. Dahlem, H.D. Rood, L.-M. Cote, W.B. Buck and T. Yoshizawa, *J. Assoc. Off. Anal. Chem.* **69**, 41–43 (1986).
[5] R. Chiou, R.J. Stubbs and W.F. Bayne, *J. Chromatogr.* **416**, 196–202 (1987).
[6] P.E. Ross, A. Hill, A. Ramsay and L. Chalmers, *Proc. Symp. Anal. Steroids*, Szeged, Hungary, 239–243 (1984).
[7] I. Ballester, E. Cortes, M. Moya and M.J. Campello, *Clin. Chem.* **33**, 796–799 (1987).
[8] G. Musch and D.L. Massart, *J. Chromatogr.* **432**, 209–222 (1988).
[9] G. Musch and D.L. Massart, *J. Pharm. Biomed. Anal.* **7**, 483–497 (1989).
[10] R.D. McDowall, *J. Chromatogr.* **492**, 3–58 (1989).
[11] I. Liska, J. Krupcik and P.A. Leclerq, *J. High Resol. Chromatogr.* **12**, 577–590 (1989).
[12] M. De Smet, A. Peeters, L. Buydens and D.L. Massart, *J. Chromatogr.* **457**, 25–42 (1988).

*[Received for review 5 April 1990; revised manuscript received 29 June 1990]*