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Determination of Tricyclic Antidepressants in Human Breast Milk by Capillary Electrophoresis

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A method for the determination of several tricyclic antidepressants (imipramine, desipramine, amitriptyline, nortriptyline, clomipramine, norclomipramine, doxepine and nordoxepine) in breast milk has been developed. This assay consists of a common extraction process in an organic phase, which is evaporated until dried and finally reconstituted in the appropriate buffer for injection in a capillary electrophoresis system. The capillary electrophoresis method used is an "acetonitrile stacking" method previously reported for determining these drugs in serum samples. The method developed was applied to the analysis of these compounds in human breast milk at different concentration levels (50, 100 and 200 ppb of the TCAs hydrochlorides). An interference study of some ansiolitic drugs such as lorazepam and alprazolam was made.

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Introduction

The United Nations International Children's Emergency Fund (UNICEF), the World Health Organization (WHO) and Paediatric Academics around the world have endorsed breast milk as the ideal form of nutrition for the newborn and they emphasize the physical and emotional benefits on the baby. These facts and the increase of the incidence of the immediate postpartum psychiatric disorders[1] have focused attention on the dilemma regarding the use of psychotropic medications in postpartum women in the recent years. To define the risk-benefit assessment of the antidepressant therapy and to reduce nursing infant exposure by discarding the breast milk during these times, data from breast milk studies are of primary importance.[2,3]

Breast milk is a difficult matrix for psychotropic drug analysis because of its high protein and lipid concentrations and the variability of its composition throughout the postpartum period and throughout the individual feeding period. For these reasons, few methods are reported in the literature for the determination of psychotropic drugs in breast milk. In the case of the tricyclic antidepressants, which are the interest of this paper, some methods using HPLC with UV detection are found in the literature for single analytes and also with their metabolites,[4,5] whereas there are few for several compounds, among them the paper of Hostetter and coworkers.[6] In this case, two liquid–liquid successive extractions of the sample, followed by a solid phase extraction in C18, are necessary to determine amitriptyline, imipramine, doxepin and their metabolites in breast milk. Good recovery at concentrations of 50 and 200 ng/mL, and good reproducibility are reported by the authors. On the other hand, selective serotonin reuptake inhibitor antidepressants have been analyzed in breast milk, using a novel system for the extraction of these analytes from the sample by a liquid-phase microextraction (LPME).[7] The lipid content of the milk needed a preextraction step before the LPME system.

With respect to the determination of tricyclic antidepressants (TCAs) by CE, to our knowledge, no article on TCAs's determination in breast milk samples by CE has been published so far. In other biological matrices (as serum, plasma and urine) and in pharmaceuticals, several methods have been published in the literature.[8-12]

In this paper, a CE procedure to the determination of the several TCAs: imipramine, amitriptyline, clomipramine, and doxepine and their metabolites desipramine, nortriptyline, norclomipramine and nordoxepine in breast milk is presented. This assay consists of a common extraction process in organic phase, evaporation of this until dried and, finally, reconstitutions in the sample buffer, prior the injection in the CE system to the separation and quantification. An "acetonitrile stacking" capillary electrophoresis method, previously reported[8] for the determination of these drugs in serum samples, is utilized.

Experimental

Drugs, chemicals, solutions

The TCA hydrochlorides were obtained from Sigma (St. Louis, MO) and 100 μg/mL standard solutions of these compounds were prepared by dissolving the appropriate amounts in water. These solutions were kept refrigerated in the dark and working solutions of lower concentrations were freshly prepared by appropriate dilution.

Analytical-reagent grade triethanolamine (TEA) from Riedel-de-Haën (Seelza, Germany), HPLC-grade acetonitrile and sodium hydroxide from Merck (Darmstadt, Germany); analytical-reagent grade hydrochloric acid from Scharlau

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(Barcelona, Spain) and analytical-reagent grade sodium chloride, isopropanol and isoamyl alcohol from Panreac (Barcelona, Spain) were used.

Breast milk used in the experiments was obtained through a period of 6 months, starting the fourth month from the partum.

CE method

All CE experiments were conducted in a capillary electrophoresis system (Hewlett-Packard Española Instruments, Model HP^{3D}CE, Madrid, Spain) equipped with a diode array detector and a temperature control device. Analytes were monitored at 214 nm. The separation runs were carried out at normal polarity and constant voltage of 30 kV, which was reached following a ramp of 0.2 min, using a fused silica capillary with 50 µm i.d. Samples were injected hydrodynamically at 50 mbar for 185 s. The temperature of the capillary was adjusted at 25°C and the tray of samples was adjusted at 20°C. The separation buffer was constituted by 300 mM TEA, 40% (v/v) of acetonitrile, at pH 8.25 adjusted with hydrochloric acid 1 M. For stacking-CE, the sample was prepared in 60% (v/v) of acetonitrile and 100 mM sodium chloride (named as buffer sample). At the beginning of each day, the capillary was rinsed for 5 min with high pressure water. Between injections, the capillary was rinsed with water for 3 min, sodium hydroxide 0.2 M for 2 min, high pressure water for 5 min and separation buffer for 10 min.

Procedure for the analysis of breast milk

One milliliter of breast milk was introduced in a 5-mL centrifuge tube. Next, 1 mL of solution containing NaCl 1 g/L and NaOH 1.5 M was added and mixed. Following the addition of 3.00 mL of 1% isoamyl alcohol in hexane, the tube was capped and mixed for 15 min. After centrifugation for 7 min, 2.60 mL of the organic phase were taken with a pipette and transferred into a balloon. The organic phase was evaporated in a rota-evaporator, reconstituted with 0.50 or 1.00 mL of the buffer sample that was composed of 60% v/v acetonitrile and 100 mM NaCl, and the solution was injected in the CE system.

Results and Discussion

Development of the procedure for TCAs determination in human breast milk

Different methods were assayed with the aim of determining these compounds (imipramine, desipramine, amitriptyline, nortriptyline, clomipramine, norclomipramine, doxepine and nordoxepine) in human breast milk by using a simple and fast procedure.

First of all, a simple and fast procedure reported in the literature[8] for the determination of the TCAs in serum was tried. According to this, a portion of breast milk spiked with the TCAs, was enriched with NaCl to get a final concentration of 100 mM, and a volume of acetonitrile was added to reach a 60% v/v proportion. The acetonitrile allows the precipitation of the proteins. After mixing and centrifugation, the supernatant was analyzed. Low recoveries were obtained when compared with the corresponding standards of the TCAs at the same concentrations. It means that in the protein precipitation a portion of TCAs is co-precipitated. For that reason, the precipitate is extracted another time with acetonitrile joining the two liquid phases. These were dried, reconstituted with buffer sample and analyzed; but low recoveries were found anyway. Also, the addition of acetic acid to guarantee the positive charge of the analytes before the extraction was tried, but good results were not obtained.

Table 1 Comparison of procedures

Analyte	Isopropanol/hexane, *%R*	Isoamyl alcohol/hexane, *%R*
Desipramine	86.2	99.6
Nortriptyline	93.3	112.0
Imipramine	82.0	94.8
Amitriptyline	82.7	97.4
Clomipramine	71.2	83.4
Doxepine	90.7	100.0

Concentration of TCAs: 200 ppb.

The next procedure tried was based on the Hostetter and coworkers[6] method; it was the following: a volume of breast milk spiked with TCAs was firstly extracted with 1% isopropanol in hexane in order to eliminate fatty substances. The organic phase was eliminated and the aqueous phase was made basic by adding a mixture composed of NaOH and NaCl, in order to cause the TCAs to be neutral. A second extraction with 1% isopropanol in hexane was therefore made. The organic phase was evaporated and reconstituted with the buffer sample. Good recoveries were not obtained. In order to make the organic phase less polar and to avoid the possible TCA extraction into the isopropanol/hexane phase during the first extraction, the extraction with pure hexane was tried maintaining the second part of the procedure as previously. However, acceptable recoveries were not obtained.

Finally, taking into account the extraction procedure reported by Queiroz and coworkers[12] for the determination of amitriptyline, imipramine and their metabolites in plasma samples, a similar procedure was tried. In this way, an aliquot of spiked breast milk was made basic after the addition of a solution containing NaCl 1 g/L and NaOH 1.5 M and extracted with 1% isopropanol in hexane. The organic phase was evaporated until a viscous liquid was remaining in the residue. Next, a volume of buffer sample was added to dissolve the analytes. It was found that the volume of the sample buffer used for the reconstitution had influence in the results and 1.00 mL was checked to be sufficient to dissolve the analytes. After the reconstitution, the viscous phase is over the aqueous, so, it is necessary to be careful when the aqueous phase is taken. Also, this procedure was tried with two different alcohols, isoamyl alcohol and isopropanol. Better results were obtained by using isoamyl alcohol, as shown in Table 1.

Validation of the procedure for the determination of TCAs in human breast milk

The validation of the proposed procedure was made with different samples of human breast milk, spiked with TCAs at different concentration levels. These samples were analyzed by using the proposed procedure. Three different concentration levels for each analyte were analyzed in triplicate. Electropherograms of TCAs at 50, 100 and 200 ppb concentration levels in human breast milk are presented in Fig. 1, and the results obtained in the analysis of the spiked breast milk, expressed as recoveries (*%R*), are shown in Table 2. Satisfactory results were obtained for all analytes at 100 and 200 ppb by using the corrected peak area. For the samples spiked at the lowest concentration level (50 ppb), only 0.50 mL of sample buffer were used to the reconstitution, in order to reach a final concentration above the detection limits. This can explain the low recovery values obtained for imipramine,

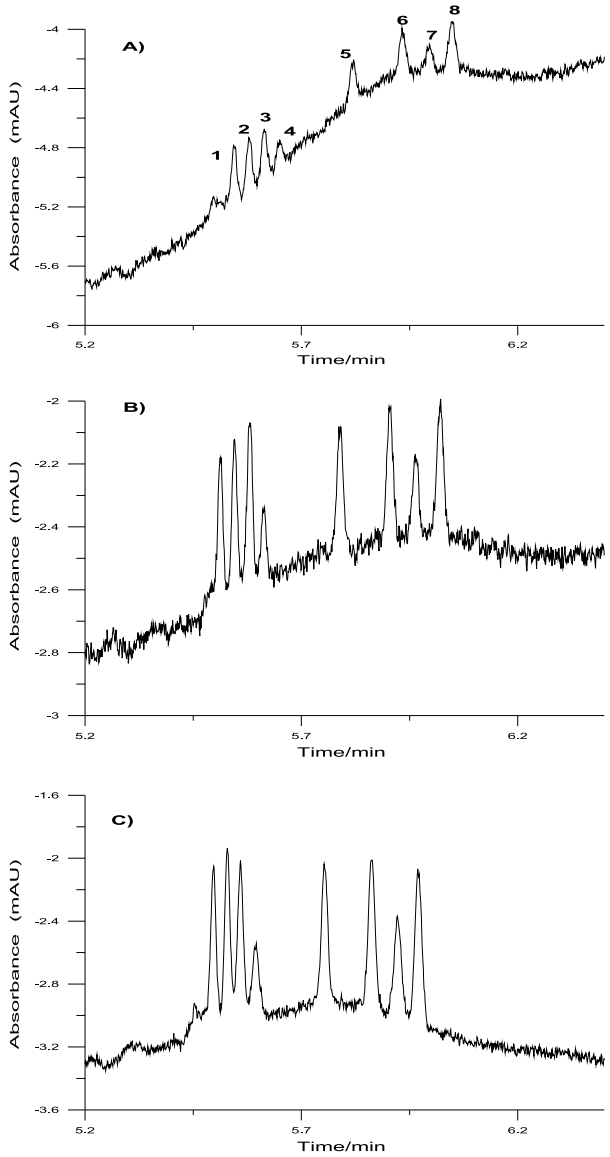

Fig. 1 Electropherograms of TCAs at (A) 50 ppb, (B) 100 ppb, and (C) 200 ppb concentration levels in human breast milk. 1, Desipramine; 2, nortriptyline; 3, nordoxepine; 4, norclomipramine; 5, imipramine; 6, amitriptyline; 7, doxepine; 8, clomipramine.

amitriptyline, clomipramine and doxepine. This problem could be avoided by using a more sensitive detection in a bubble capillary, as described for the analysis of TCAs in serum,[8] which would allow the use of 1 mL sample buffer for the reconstitution. The migration times of the analytes are also shown in Table 2.

Interference studies

Two different ansiolitic drugs that could be co-administrated with tricyclic antidepressant were studied in order to check if they could interfere in TCAs determination.

The drugs assayed were lorazepam and alprazolam, from the pharmaceuticals Orfidal (Wyeth) and Trankimazin (Pharmacia & Upjohn), respectively. In each case the treatment was the same: two tablets were weighed and grounded to fine powder. Accurately weighted aliquots were dissolved in methanol by sonicating for about 30 min, the mixtures were filtered, the filtrates transferred to volumetric flasks and diluted to the mark with methanol. Standards of the TCAs spiked with each one of

Table 2 Recoveries (*%R*) obtained in the analysis of spiked breast milk samples

Analyte ($t_m \pm s$[a], min)	50 ppb		100 ppb		200 ppb	
	%R	% RSD	*%R*	% RSD	*%R*	% RSD
Desipramine (5.51 ± 0.02)	89.8	4.7	96.5	9.5	90.8	9.7
Nortriptyline (5.54 ± 0.02)	82.0	5.9	93.6	9.5	88.2	9.2
Nordoxepine (5.58 ± 0.02)	90.8	8.3	88.5	4.7	87.8	6.1
Norclomipramine (5.61 ± 0.02)	85.8	11.0	99.3	7.2	101.2	9.0
Imipramine (5.79 ± 0.03)	52.0	9.0	90.1	10.0	73.9	1.4
Amitriptyline (5.89 ± 0.03)	40.4	6.0	74.3	2.9	71.7	1.2
Clomipramine (5.95 ± 0.03)	35.0	1.6	62.3	1.7	59.3	9.0
Doxepine (6.00 ± 0.04)	63.6	8.0	81.4	1.5	82.5	6.8

a. t_m, Migration time (n = 6).

the ansiolitic drugs, both at 200 ppb, were analyzed. No peaks from the ansiolitic assayed were observed in the electropherograms. That means that there is no interference of lorazepam or alprazolam in TCAs determination in human breast milk, in the proposed conditions.

Conclusions

A new method for determining tricyclic antidepressant (imipramine, desipramine, amitriptyline, nortriptyline, clomipramine, norclomipramine, doxepine and nordoxepine) in breast milk has been developed. The sample procedure consists in a simple liquid-liquid extraction with isoamyl alcohol in hexane, evaporation of the organic phase and reconstitution with a NaCl and acetonitrile mixture. The last step is necessary for the preconcentration "on-line" of the sample in the CE system. In this way it is possible to determine TCAs at levels as low as 50 ppb in breast milk.

No interferences were detected in the presence of the ansiolitic drugs, lorazepam and alprazolam, which could be co-administrated with tricyclic antidepressants.

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