Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
32 (2003) 1061–1066
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

HPLC determination of tramadol in human breast milk

Vojko Kmetec*, Robert Roškar

Department of Biopharmaceutics and Pharmacokinetics, Faculty of Pharmacy, Aškerčeva 7, 1000 Ljubljana, Slovenia

Received 24 April 2002; received in revised form 22 January 2003; accepted 24 January 2003

Abstract

Tramadol is a centrally acting analgesic used for prevention and treatment of moderate to severe pain. It is estimated that 0.1% of the administered dose passes into breast milk causing potentially unwanted effects in nursing babies. Pharmacokinetically, breast milk is supposed to be a separate compartment into which the drug is excreted-mainly by passive diffusion. Due to a complex composition of breast milk, a suitable sample preparation procedure is needed with a subsequent chromatographic analysis for drug determination. Among several sample cleanup procedures tested we chose the liquid–liquid extraction procedure using *n*-hexane as an organic phase with back extraction into aqueous phase since it was considered the most suitable and the most compatible with the subsequent HPLC analysis. The precision and the reproducibility of the method were improved approximately two times by using metoprolol as an internal standard thus making the method also more robust with regard to a variable composition of milk samples. These characteristics, together with low detection limit and short analysis time, proved that the developed method is suitable for monitoring of tramadol in human breast milk.

Keywords: Tramadol; Breast milk; Liquid–liquid back-extraction; HPLC

1. Introduction

Tramadol/2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol/ is a synthetic, centrally acting analgesic agent (Fig. 1a). It has mild opioid agonist properties and activates monoaminergic spinal inhibition of pain. Unlike other opioids, tramadol has no clinically relevant cardiovascular or respiratory depressant activity. Furthermore, it does not have a prostaglandin inhibitory effect. It is used as racemate, whose two enantiomeres function in a complementary manner enhancing efficacy and improving tolerability. Administered orally, rectally or parenterally, tramadol was proven to be effective and well tolerated analgesic agent for prevention and treatment of moderate to severe pain of various origin, including the pain associated with labor. The dosage of tramadol should be adjusted to the intensity of pain and the response of an individual patient. The possible risks to the neonates and infants should always be carefully considered, regardless of the fact that

* Corresponding author. Tel.: +386-1-4769-500; fax: +386-1-4258-031.
E-mail address: vojko.kmetec@ffa.uni-lj.si (V. Kmetec).

0731-7085/03/$ - see front matter
doi:10.1016/S0731-7085(03)00209-7

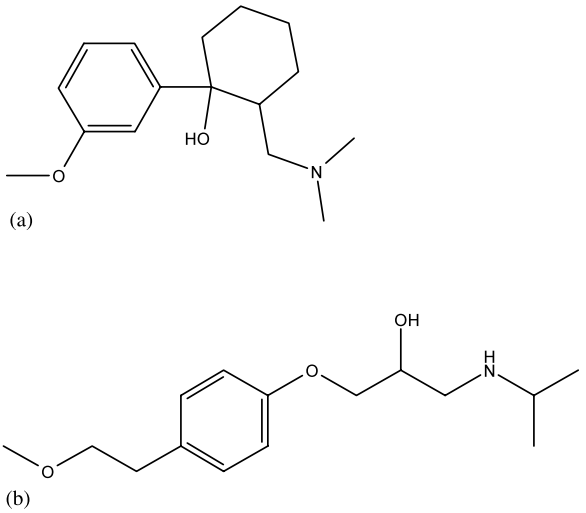

Fig. 1. Chemical structures of tramadol (a) and metoprolol (b).

usually low drug concentrations may be found in the milk. It is estimated that 0.1% of the original dose of tramadol passes into milk. Drug excretion into breast milk occurs primarily by passive diffusion depending on molecular weight, drug ionization, lipid solubility and binding, protein binding and mammary blood flow [1–4].

Water is the main component of breast milk. Lactose levels are quite constant representing the principal osmotically active compound in milk. The protein content of mature milk shows little diurnal or feed-to-feed variation. The lipid content of milk, however, varies considerably within a feed, between feeds and between individuals. Milk is more acid than plasma, pH values varying from 6.6 to 7.0 [5–8].

Safety, scientific reasons and regulatory requirements demand that the extent of drug excreted into breast milk should be evaluated. A suitable procedure for preparation of sample with subsequent analytical method should be, therefore, developed for reliable drug determination in this complex and variable biological matrix. High protein, fat and carbohydrate contents and especially varying amounts of lipids considerably affect the method performance, demanding a special procedure for sample preparation. Due mainly to high protein and lipid content, milk sample preparation differs from those for plasma or urine. On the one hand, a protein fraction of milk contains numerous hydrophilic proteins which are difficult to precipitate; on the other hand, lipid globules can retain the drug, thus making the subsequent analysis impossible. If not removed from the sample, lipids can also deteriorate the chromatographic column used for the analysis. A number of milk sample cleanup techniques have been described in literature. The most commonly used are direct injection, ultrafiltration or dialysis, protein precipitation, liquid–liquid extraction, solid-phase extraction and immunoaffinity extraction [9–11].

In this study, we present our method for tramadol determination in milk samples. We used the liquid–liquid extraction with back extraction technique for sample preparation which was followed by the reversed phase liquid chromatography for quantitative determination of tramadol.

2. Experimental

2.1. Materials

Tramadol hydrochloride and metoprolol tartrate (internal standard) were supplied by Krka d.d. (Novo mesto, Slovenia). All solvents and other chemicals used for chromatography and sample preparation were of analytical or of HPLC grade. Blank milk samples, as well as those obtained after treatment with tramadol were provided by the Postojna Hospital Center (Slovenia).

2.2. Preparation of standard solutions

Standard stock solutions of tramadol hydrochloride and of metoprolol tartrate were prepared in bidistilled water at the concentrations of 1 and 2 μg/ml, respectively. With further dilution in bidistilled water a series of standard water solutions of tramadol hydrochloride were prepared at the concentrations of 50, 100, 150, 300 and 500 ng/ml with constant internal standard concentration of 150 ng/ml. By dilution of standard stock solution of tramadol hydrochloride in homoge-

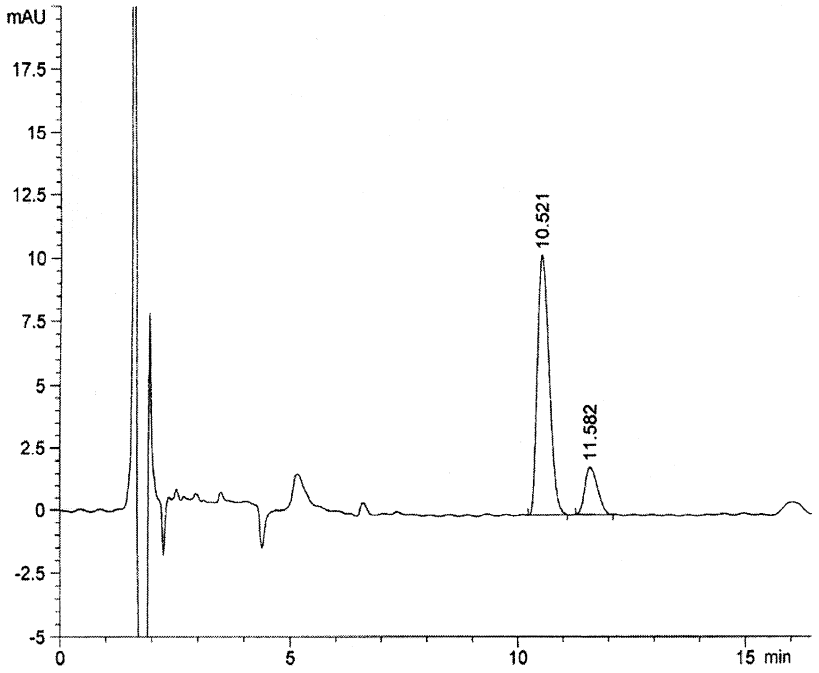

Fig. 2. Chromatogram of standard water solution of tramadol ($t_R = 10.5$ min; conc = 500 ng/ml) and metoprolol ($t_R = 11.5$ min; conc = 150 ng/ml).

nized blank breast milk a series of standard milk solutions were prepared with the same drug concentrations as above.

2.3. Extraction procedure

150 μl of standard stock solution of internal standard was added to 2 ml of milk sample or standard milk solution and vortexed for several seconds. 100 μl of 0.1 M NaOH was added and vortexed again for several seconds. After adding 10 ml of *n*-hexane, the mixture was sonificated for 10 min and then centrifuged at 5000 rpm for 5 min. The supernatant was transferred into glass vial; then 200 μl of 0.1 M HCl and 50 μl of mobile phase were added. This mixture was vortexed again for 1 min and the water phase injected into liquid chromatograph.

2.4. Chromatography

We used the Hewlett–Packard series HP1100 high performance liquid chromatograph (G1312A binary pump) controlled by HP Chemstation and equipped with thermostated autosampler (G1329A) and diode array detector (G1315A). The flow rate was set at 1 ml/min, the volume of injection at 100 μl and UV detection at a wavelength of 225 nm. A column Luna C18 (150 × 4.6 mm), particle size 3 μm with inline filter was used. The mobile phase consisted of aqueous solution of 0.05 M KH_2PO_4 and triethylamine adjusted to pH 3.5, methanol and acetonitrile in a ratio of 74:22:4 (v/v/v). The samples were thermostated at 15 °C.

3. Results and discussion

At the beginning of our study we applied the sample clean-up procedure used for tramadol determination in the plasma [12]. According to this procedure, milk samples spiked with tramadol were treated with 0.1 M NaOH for protein precipitation followed by extraction with ethyl acetate. The organic phase was evaporated with

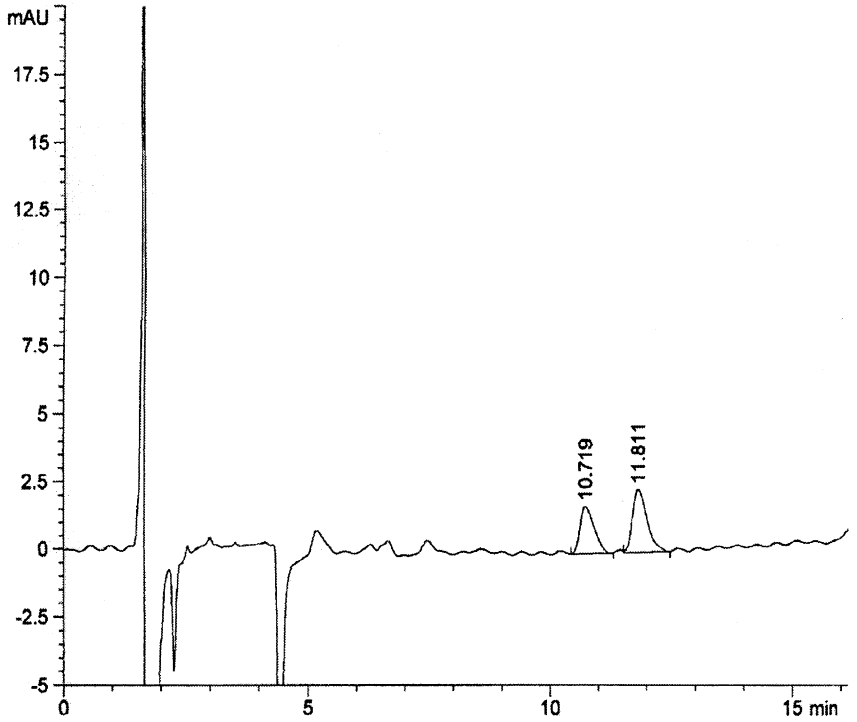

Fig. 3. Chromatogram of standard milk solution of tramadol (conc = 100 ng/ml) and metoprolol (conc = 150 ng/ml).

nitrogen and then reconstituted with the mobile phase. As foreseen, however, the reconstituted solutions were not homogenous due to the retained lipids and, therefore, not suitable for subsequent chromatographic analysis. The second approach, direct injection of filtered supernatant after protein precipitation and centrifugation also failed because of poor recoveries caused by the retention of tramadol in lipid globules. We did not test the solid phase extraction procedure since we had also foreseen the problems associated with retained milk lipids. Therefore, the liquid–liquid extraction procedure was upgraded with the second back extraction step into aqueous phase. In this case, however, *n*-hexane was found a better extraction solvent than ethyl acetate or chloroform.

Such sample preparation approach was also compatible with the subsequent reversed phase HPLC analysis since it helped avoiding the problems concerning retained lipids. Among several stationary phases tested, Luna C18 with 3 μm particles proved to be the most suitable. After optimization of the mobile phase, well-shaped peaks with favorable retention times were achieved. Preliminary chromatographic measurements showed that approximately two-fold better precision was obtained using the internal standard procedure compared with the external standard approach. In our case metoprolol was chosen as internal standard because of its structural similarities (Fig. 1b) with tramadol resulting in similar partition and response characteristics. In fact, both substances showed practically identical retention times on several chromatographic columns tested. The adequate resolution of their chromatographic peaks (Fig. 2) was, however, achieved on Luna C18 stationary phase, proving at the same time its separation power for future analyses. Figs. 2–4 show chromatograms of water standard solution, milk standard solution and real milk sample taken after tramadol administration.

The validation of the method proved that the developed internal standard procedure is sufficiently precise with the intra-day and inter-day precision better than 10% for three replicates in the

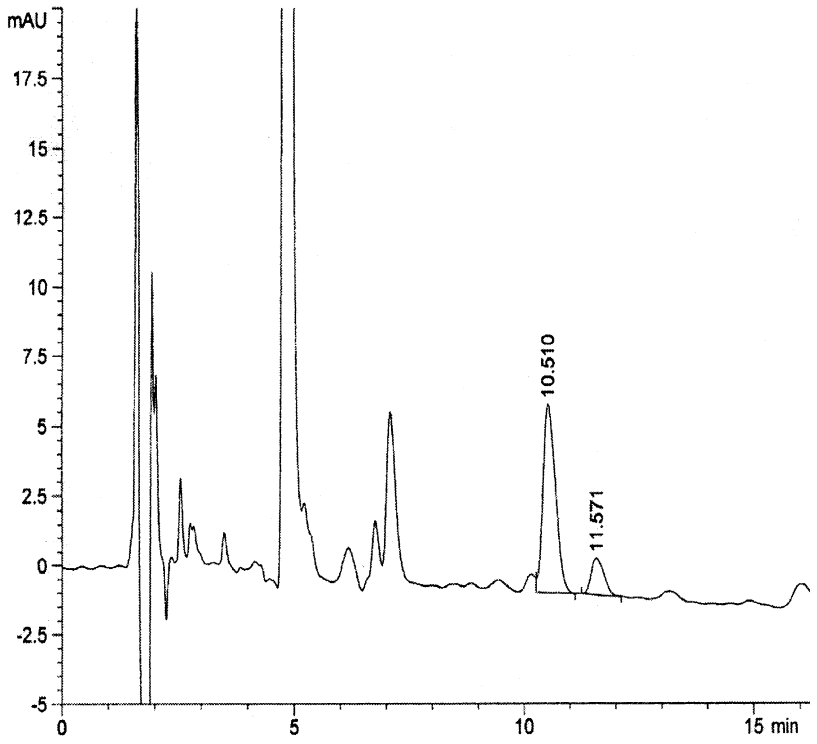

Fig. 4. Chromatogram of milk sample from a breast feeding mother after 50 mg i.v. dose of tramadol.

concentration ranging from 50 to 500 ng/ml. In this concentration range based on five concentration points the method was proved linear ($r = 0.99$). Recoveries for tramadol ranged from 55 to 65% at the concentration level of 150 ng/ml. Limit of quantification and detection estimated by the ICH guidelines (the slope–intercept method) were 38 and 12 ng/ml, respectively.

Applicability of the developed method was proven by analyzing several breast milk samples taken approximately 10 h after oral or parenteral (i.v.) administration of tramadol. Fig. 4 shows the chromatogram of milk sample after an i.v. dose of 50 mg tramadol; the responses after an oral dose have been approximately two times smaller. Adequate selectivity of the developed method, the ability of the method to detect concentrations of tramadol expected in human breast milk after therapy, as well as short retention times confirmed the suitability of the method for monitoring tramadol levels after treatment.

4. Conclusions

Drug excretion into breast milk should be evaluated in view of a possible risk to neonates and infants. Complex nature of milk demands application of special cleanup procedures for drug determination. For this purpose we developed a liquid–liquid extraction with subsequent back extraction, followed by the reversed phase chromatography. Data obtained by this approach show that the method is reliable and suitable for tramadol determination in human breast milk.

References

[1] L.J. Scott, C.M. Perry, Drugs 60 (1) (2000) 139–176.
[2] T.P. Gibson, Am. J. Med. 101 (1996) 47S–53S.
[3] C.R. Lee, D. McTavish, E.M. Sorkin, Drugs 46 (2) (1993) 313–340.
[4] K.A. Lehmann, Drugs 47 (Suppl. 1) (1994) 19–32.
[5] B. Bretanic, Slov. Pediatr. 5 (Suppl. 1) (1998) 24–37.

[6] G. Pons, E. Rey, I. Matheson, Clin. Pharmacokinet. 27 (4) (1994) 270–289.
[7] O. Spigset, S. Hagg, Poediatr. Drugs 2 (2000) 223–238.
[8] E.A. Shipton, Anaesth. Intensive Care 28 (4) (2000) 363–374.
[9] D.T. Rossi, D.S. Wright, J. Pharm. Biomed. Anal. 15 (1997) 495–504.
[10] J. Rampono, J.H. Kristensen, L.P. Hackett, M. Peach, R. Kohan, K.F. Ilett, J. Clin. Pharmacol. 50 (2000) 263–268.
[11] T. Stebler, T.W. Guentert, J. Chromatogr. 564 (1991) 330–337.
[12] G.C. Yeh, M.T. Sheu, C.L. Yen, C.H. Wang Y.W., Liu, H.O. Ho, J. Chromatogr. B 723 (1999) 247–253.