ELSEVIER

Journal of Chromatography B, 701 (1997) 140–145

JOURNAL OF CHROMATOGRAPHY B

Short communication

Simple liquid chromatographic method for the determination of naltrexone in human plasma using amperometric detection

Kok Khiang Peh, Nashiru Billa, Kah Hay Yuen*

School of Pharmaceutical Sciences, University of Science Malaysia, 11800 Penang, Malaysia

Received 21 April 1997; received in revised form 26 June 1997; accepted 27 June 1997

Abstract

A simple liquid chromatographic method using amperometric detection was developed for the determination of naltrexone in human plasma. Prior to analysis, naltrexone and the internal standard (naloxone) were extracted from plasma samples using a 9:1 mixture of chloroform and isopropyl alcohol. The mobile phase comprised 0.1 *M* disodium hydrogen orthophosphate (pH 3.5) and acetonitrile (85.5:14.5, v/v). Analysis was run at a flow-rate of 0.8 ml/min with the detector operating under oxidative mode at an applied potential of +0.95 V. The method is specific and sensitive with a detection limit of approximately 1 ng/ml at a signal-to-noise ratio of 3:1. Mean recovery value of the extraction procedure was about 93%, while the within day and between day coefficient of variation and percent error values of the assay method were all less than 10%. The calibration curve was linear over a concentration range of 1.5–100 ng/ml. © 1997 Elsevier Science B.V.

Keywords: Naltrexone

1. Introduction

Naltrexone is a long-acting, potent, non-addictive and effective narcotic antagonist [1–3], commonly suggested for the treatment of narcotic addiction [1]. Various analytical methods including thin-layer chromatography (TLC) [4], gas chromatography (GC) [5,6] and liquid chromatography (LC) [7–11] have been reported for its determination in biological fluids. The method based on TLC [4] may not be sufficiently selective and sensitive for routine measurement of the drug in plasma. On the other hand, the GC methods [5,6] were reported to be sensitive and selective but required extensive sample preparation and derivatisation before analysis. LC methods using amperometric detection were reported by Derendorf et al. [7] and O'Connors et al. [9]. Derendorf et al. [7] reported a detection limit of 2–5 ng/ml with their method, but a multiple extraction procedure was employed in the sample preparation. In the method of O'Connors et al. [9], a single extraction procedure with satisfactory recovery was employed, but the method was demonstrated with only one concentration level of 100 ng/ml of naltrexone spiked in plasma. Moreover, the accuracy and precision were not reported. More recently, LC methods using coulometric detection [10,11] have been reported. However, the coulometric cell can be easily contaminated, especially in the analysis of plasma samples. Once contaminated, the cell may need to be replaced as it is difficult to be dismantled for cleaning.

*Corresponding author.

0378-4347/97/$17.00
PII S0378-4347(97)00352-6

In this paper, we report a relatively simple, specific, and sensitive LC method for the determination of naltrexone in human plasma using amperometric detection. A one-step extraction procedure similar to that used by O'Connor et al. [9] was utilized in the sample preparation.

2. Experimental

2.1. Materials

Phosphoric acid 85% was purchased from Ajax Chemicals (Sydney, Australia). Disodium hydrogen orthophosphate anhydrous was purchased from BDH Chemicals (Poole, UK). Both naltrexone and naloxone HCl were obtained from Diosynth (Oss, The Netherlands). All other solvents used were of analytical reagent grade or of HPLC grade purchased from Mallinckrodt (KY, USA).

2.2. Instrumentation

The LC system comprised a Jasco PU-980 pump (Jasco, Tokyo, Japan), a DECADE digital electrochemical amperometric detector (Antec Leyden, Leiden, The Netherlands) and a Hitachi D-2500 Chromato-integrator (Hitachi, Tokyo, Japan). The amperometric detector contained a highly shielded oven compartment accommodating a pulse damper, a Rheodyne 7725i six-valve sample injector, a metaphase KR100-5-C18 (Biosains, Kuala Lumpur, Malaysia) column (250×4.6 mm I.D., 5 μm) fitted with a refillable guard column (Upchurch Scientific, Oak Harbour, WA, USA) packed with Perisorb RP-18 (30–40 μm, pellicular), a flow cell equipped with a glassy carbon working electrode and a Ag/AgCl reference electrode saturated with LiCl. The mobile phase consisted of 0.1 *M* disodium hydrogen orthophosphate adjusted to pH 3.5 with 85% phosphoric acid and acetonitrile (85.5:14.5, v/v). Analysis was run at a flow-rate of 0.8 ml/min with the detector operating under oxidative mode at an applied potential of +0.95 V with 10 nA and the oven temperature set at 30°C.

2.3. Sample preparation

A 500-μl aliquot of plasma sample was measured into a glass tube with a teflon-lined screw cap, followed by the addition of 50 μl of 250 ng/ml naloxone (internal standard) solution, 50 μl of 2 *M* sodium hydroxide and 4.5 ml of chloroform and isopropyl alcohol (9:1, v/v) extracting solvent. The mixture was vortexed for 1 min on a vortex mixer and centrifuged at 2000 *g* for 20 min. The organic layer was transferred into a reactivial and evaporated to dryness at 40°C under a gentle stream of nitrogen gas. The residue was reconstituted with 70 μl of mobile phase and 50 μl was injected onto the column.

2.4. Assay validation

Samples were quantified using peak height ratio of naltrexone over the internal standard. Standard calibration curves were constructed by spiking drug-free pooled plasma with a known amount of naltrexone at a concentration range of 1.5–100 ng/ml. These plasma standards were also used to determine the extraction recovery, within-day and between-day precision and accuracy (n=6) of the method. The recovery of the extraction procedure for naltrexone and the internal standard was calculated by comparing the peak height obtained after extraction with that of aqueous drug solution of corresponding concentration without extraction.

3. Results and discussion

Chromatograms obtained with blank plasma and plasma spiked with naltrexone and naloxone are shown in Fig. 1A and Fig. 1B. It can be seen that naltrexone and naloxone peaks were well resolved and free of interference from endogenous compounds in the plasma, with retention times of 9.42 and 6.98 min, respectively.

Extraction using chloroform alone produced several extra peaks in the chromatograms for both blank and spiked plasma. On the other hand, a mixture of chloroform and isopropyl alcohol (9:1, v/v) not only

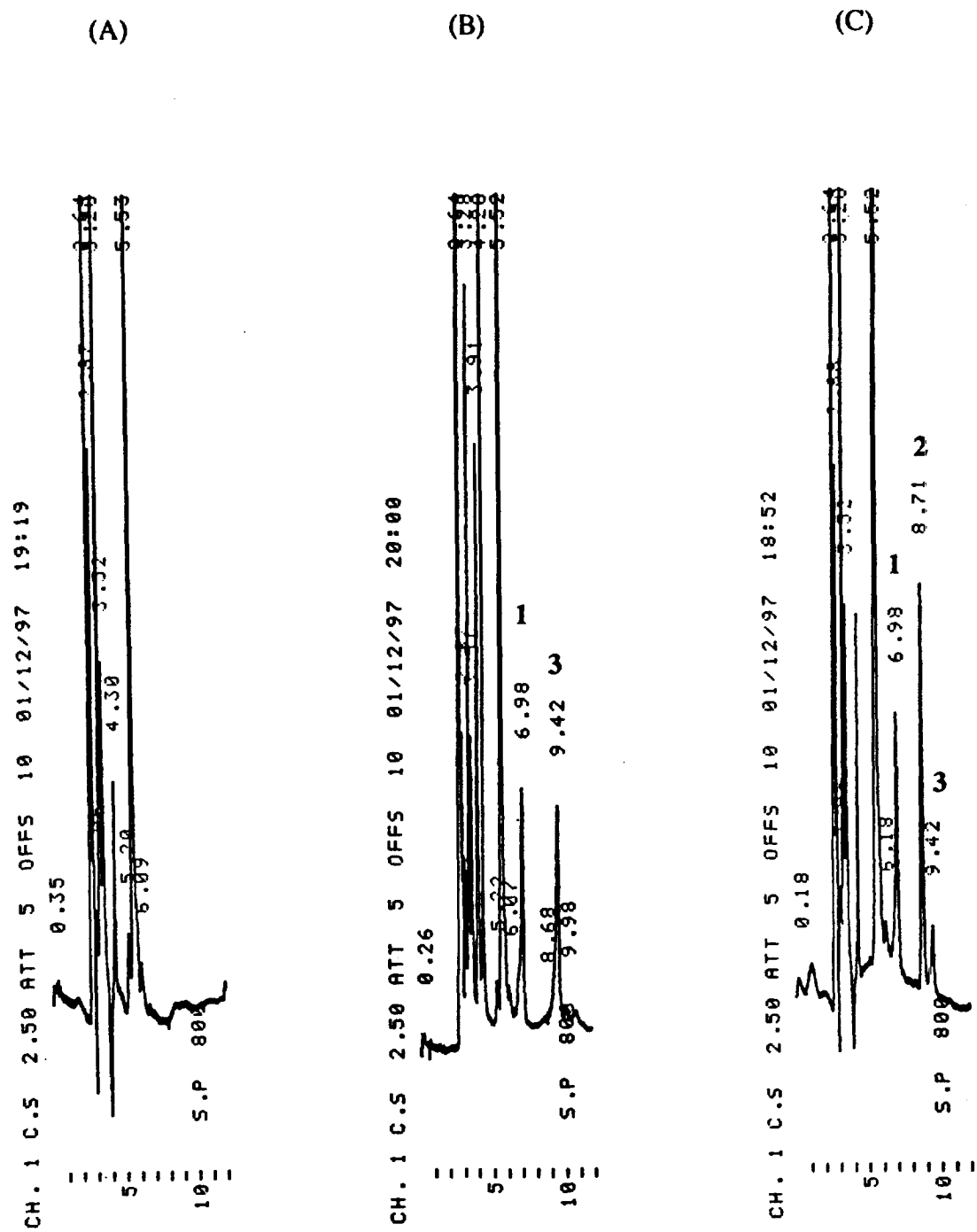

Fig. 1. Chromatograms for the analysis of naltrexone in plasma. (A) Blank plasma. (B) Plasma spiked with 250 ng/ml naloxone and 50 ng/ml naltrexone. (C) A volunteer plasma containing 7.1 ng/ml naltrexone 15 min after oral administration of 100 mg of naltrexone. *Y*-axis, attenuation=5; *X*-axis, chart speed=2.5 mm/min; 1, naloxone; 2, 6β-naltrexol; 3, naltrexone)

Table 1
Absolute recovery, within-day and between-day precision and accuracy ($n=6$)

Concentration (ng/ml)	Recovery		Within-day		Between-day	
	Mean (%)	C.V. (%)	Precision (CV%)	Accuracy (% error)	Precision (C.V.%)	Accuracy (% error)
3	95.3	1.7	8.7	9.6	9.9	9.1
12.5	91.1	8.3	4.7	2.4	5.9	4.3
50	92.6	8.2	8.8	1.0	3.3	1.3

provided relatively less peaks and cleaner chromatograms, but also a satisfactory recovery of 93 and 91% for naltrexone and naloxone (Table 1). Our values were closely comparable to those of O'Connors et al. [9] in which a similar extraction solvent mixture was used.

The extraction recovery, within-day and between-day accuracy and precision values are presented in Table 1. The coefficient of variation (C.V.) and percent error values of both the within-day and between-day precision and accuracy were all less than 10%. The standard calibration curve ($n=6$) was found to be linear over the concentration range used. A slope of 26.68 with an intercept of −0.29, and a correlation coefficient of 0.9996 were obtained.

A detection limit of 1 ng/ml was obtained at a signal-to-noise ratio of 3:1. This could be further improved by using either a larger volume sample loop or plasma sample. In comparison, Derendorf et al. [7] reported a detection limit of 2–5 ng/ml after using multiple extractions and a larger volume of plasma sample.

An applied potential of +0.95 V was selected mainly because, in addition to a sufficiently sensitive response, it provided reproducible signals and also reduced the down-time of the detector which was not obtainable at higher potentials. Although better sensitivity was obtained at higher potential, this was associated with a rapid decline in sensitivity of the detector. For example, a drop in detector response of about 50% was observed after injection of as few as 10 samples. However, at the potential used by us, we could run at least 60 samples before the detector response was decreased by half, and could be readily regenerated by cleaning the working electrode under a pulse mode. The cell potential was allowed to jump between +1 and −1 V for 15 min (E_1, +1000 mV; T_1, 1000 ms; E_2, −1000 mV; T_2, 1000 ms; E_3, 0 mV; T_3, 0 s). Thus, polishing of the working electrode which is a routinely used procedure for restoring the sensitivity of detector may not be necessary.

Saturated LiCl solution instead of saturated KCl solution was used to fill the Ag/AgCl reference electrode because the use of LiCl could reduce the drop-time of the detector sensitivity compared to that of KCl. Moreover, it has been suggested that the reference electrode should be filled with LiCl when a high percentage of organic modifier is present in the mobile phase in order to prevent clotting of the salt bridge which could lead to a drop in electrical contact between the working and reference electrodes [12].

The present method was applied to analyze plasma samples of 12 healthy adult male volunteers from a comparative bioavailability study of two different naltrexone HCl tablet preparations, namely Trexan and Narpan, the latter being a generic preparation. Fig. 1C shows a chromatogram obtained from a volunteer 15 min after dosing with 100 mg of naltrexone. The acetonitrile content in the mobile phase was found to be critical in separating naltrexone from its major metabolite, 6β-naltrexol (Fig. 1C). When the acetonitrile content was less than 14.5% v/v, the naltrexone peak was not well separated from 6β-naltrexol and the two peaks gradually superimposed to become a single peak in the chromatogram as the acetonitrile content was further reduced. On the other hand, at a higher concentration of acetonitrile (approximately 16% v/v and above), the compounds were eluted from the column too fast and the peaks were found to be overlapping. Thus, a concentration of 14.5% was found most satisfactory in the present study.

Fig. 2 shows the individual plasma concentration–time profiles of the volunteers obtained with the two preparations. It can be seen from the plasma

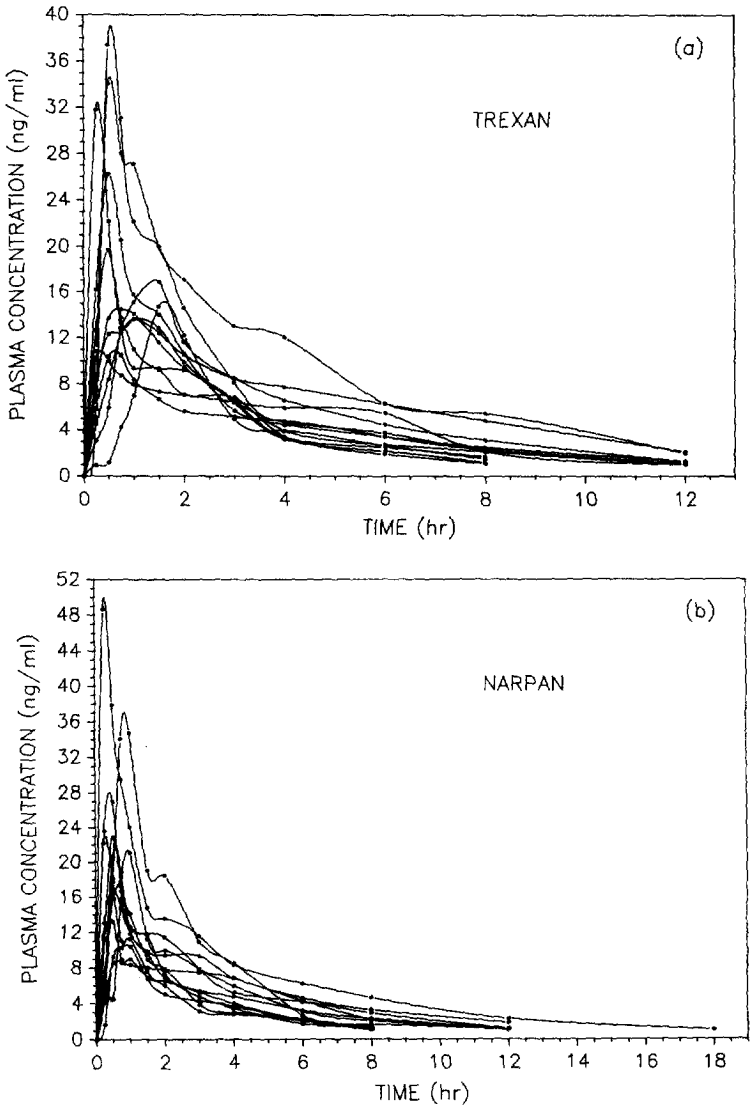

Fig. 2. Plasma naltrexone concentration versus time profiles from 12 volunteers following the oral administration of 100 mg of Trexan and Narpan.

profiles of both preparations that naltrexone could still be detected up to at least 8 h and, in all cases, the last detectable level was less than 10% of the peak plasma concentration.

In conclusion, the present LC method was simple, specific, sensitive, and suitable to be used for determination of plasma naltrexone in pharmacokinetic/bioavailability studies.

References

[1] Fact Sheet on Naltrexone, National Clearinghouse for Drug Abuse Information, National Institute on Drug Abuse, Rockville, MD, Series 38 (1), December 1978.
[2] W.R. Martin, D.R. Jasinki, Clin. Pharmacol. Ther. 14 (1973) 142.
[3] S. Taylor, R. Rodgers, R. Lynn, N. Gerber, J. Pharmacol. Exp. Ther. 213 (1980) 289.

[4] M.E. Wall, D.R. Brine, M. Perez-Reyes, Drug Metab. Dispos. 9 (1981) 369.
[5] D.M. Chinn, K.M. McGinnis, R.L. Foltz, Annual Meeting of the American Society for Mass Spectrometry, Boston, MA, May 8–13, 1983.
[6] K. Vereby, M.J. Kogan, A. De Pace, S.J. Mule, J. Chromatogr. 118 (1976) 331.
[7] H. Derendorf, A. El-Koussi, E.R. Garret, J. Pharm. Sci. 73 (1984) 621.
[8] E.R. Garret, A. El-Koussi, J. Pharm. Sci. 74 (1985) 50.
[9] E.F. O'Connors, S.W.T. Cheng, W.G. North, J. Chromatogr. 491 (1989) 240.
[10] N. Wetzelsberger, P.W. Lucker, W. Erking, Arzneim. Forsch. 36 (1986) 1707.
[11] P. Zuccaro, I. Altieri, P. Betto, R. Pacifici, G. Ricciarello, J. Chromatogr. 567 (1991) 485.
[12] DECADE Digital Electrochemical Amperometric Detector, 4th ed., Leiden, November 1995.