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Short Communication

Determination of naltrexone and 6β-naltrexol in plasma by high-performance liquid chromatography with coulometric detection

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ABSTRACT

A simple and reliable reversed-phase high-performance liquid chromatographic method with coulometric detection is described for the quantitation of naltrexone and its metabolite, 6β-naltrexol, in plasma samples of healthy volunteers who received orally 50 mg of naltrexone. The analytes and the internal standard, naloxone, are extracted with an octadecyl solid-phase extraction column before chromatography. The mobile phase is 0.01 *M* potassium phosphate (pH 3)–acetonitrile (85:15, v/v) and it is pumped at 0.8 ml/min. The coulometric detector is formed by two electrodes set at +0.20 V and +0.70 V, with a palladium reference electrode. The limit of quantitation observed was 5 ng/ml for both naltrexone and 6β-naltrexol. This method can be used to investigate pharmacokinetic parameters of different pharmaceutical preparations of this opioid antagonist.

INTRODUCTION

Naltrexone (NTX) is an effective and clinically useful narcotic antagonist, which has been proposed for the treatment of opiate dependence [1–3]. It is biotransformed to a less active metabolite, 6β-naltrexol (6β-NTX-OL), and both are excreted mainly into the urine [4,5].

Many techniques have been developed for the determination of these compounds and their metabolites in biological materials, such as thin-layer chromatography (TLC) [6], gas chromatography (GC) [7–9], and high-performance

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liquid chromatography (HPLC) with amperometric [10,11] or coulometric detection [12].

GC methods require extensive sample clean-up and derivatization procedures prior to assay, making these procedures time-consuming. HPLC techniques with amperometric detection require stable conditions for good baseline stability, frequent electrode cleaning and, consequently, a thorough clean-up of the sample.

Electrochemical detection has been improved since the appearance of coulometric detectors. They are claimed to be more sensitive and selective than amperometric ones, and with shorter maintenance times [13], even though an amperometric cell, when contaminated, can be easily dismantled, polished and restored whereas a coulometric cell usually needs to be replaced.

In the case of NTX and 6β-NTX-OL, HPLC with amperometric detection [10,11] did not present sufficient data to ensure good sensitivity, and HPLC with coulometric detection could not assay both substances [12]. In addition, lengthy liquid–liquid extraction is always required.

This paper describes a simple and reliable method for determination of both NTX and 6β-NTX-OL by HPLC with coulometric detection, using a rapid solid-phase extraction. The results obtained for extraction from human plasma are described, but the method has also been successfully applied to human urine.

EXPERIMENTAL

Materials

Naloxone, naltrexone and 6β-naltrexol were obtained from Simes (Milan, Italy). Bakerbond octadecyl disposable extraction columns were purchased from J. T. Baker (Phillipsburg, NJ, U.S.A.). All solvents used were analytical grade.

High-performance liquid chromatography

The HPLC system consisted of a Model M-45 solvent-delivery system (Waters-Millipore, Bedford, MA, U.S.A.) and a Model 7125 injector (Rheodyne, Berkeley, CA, U.S.A.) equipped with a 100-μl loop. The column was reversed-phase Supelcosil LC-18 DB (15 cm × 4.6 mm I.D., 5 μm particle size) (Supelco, Bellefonte, PA, U.S.A.). The Coulochem 5100 A electrochemical detection system (ESA, Bedford, MA, U.S.A.) had a 5011 A analytical cell. The first electrode was set at +0.20 V and the second electrode at +0.70 V, with a palladium reference electrode. The detector gain was set to 500 for the first electrode and 400 for the second one. Chromatograms were analysed with a Chemresearch chromatographic data management computer (ISCO, Lincoln, NE, U.S.A.) monitoring the second detector signal. The mobile phase was 0.01 *M* potassium phosphate (pH 3.00)–acetonitrile (85:15, v/v). Elution was carried out isocratically at room temperature at a flow-rate of 0.8 ml/min.

Standards and controls

Aqueous and methanolic stock standards solutions of NTX and 6β-NTX-OL (1 mg/ml, 10 μg/ml, 1 μg/ml, 100 ng/ml) were prepared and stored at 4°C. Plasma standards covering the range 5–500 μg/ml were prepared daily by adding known amounts of the aqueous stock standards to drug-free human plasma; these standards were used daily to create a calibration curve as a control.

Procedure for sample preparation

Each sample (1 ml), with 100 μl of naloxone (100 ng/ml) added, was applied to an octadecyl disposable extraction column (1 ml) that had been preconditioned with 2 ml each of methanol and 0.01 *M* potassium phosphate buffer (pH 7). The column was washed with 2 ml of water, and dried under a stream of nitrogen. NTX and 6β-NTX-OL were eluted with 3 ml of benzene–butanol (85:15) into 5-ml glass tubes. The tube contents were evaporated to dryness under nitrogen at room temperature and redissolved in 100 μl of mobile phase, and 20 μl were injected into the HPLC column.

Study in humans

Six healthy subjects, who gave consent to the study, received orally 50 mg of NTX as an Antaxone capsule (Simes). Blood samples (5 ml) were collected before and 0.5, 1, 2, 4, 8 and 24 h after administration. They were immediately centrifuged at 1000 *g* for 5 min. Plasma was collected and stored at −20°C until analysis.

RESULTS AND DISCUSSION

Extraction procedure

The present assay required a sample clean-up using an octadecyl (C_{18}) solid-phase extraction (SPE) column, because liquid–liquid extraction with benzene–butanol (85:15) or with chloroform resulted in the appearance of several interfering peaks in the chromatograms from both blank and spiked plasma samples. The only disadvantage of SPE was an analytical recovery of *ca.* 85% (Table I).

TABLE I

RECOVERY OF NTX AND 6β-NTX-OL FROM SPIKED PLASMA SAMPLES

Concentration added (ng/ml)	*n*	Recovery (mean ± S.D.) (%)	
		NTX	6β-NTX-OL
50	10	86.25 ± 1.9	84.33 ± 1.0
100	10	86.28 ± 1.7	84.38 ± 0.8
500	10	85.0 ± 3.1	84.16 ± 1.2

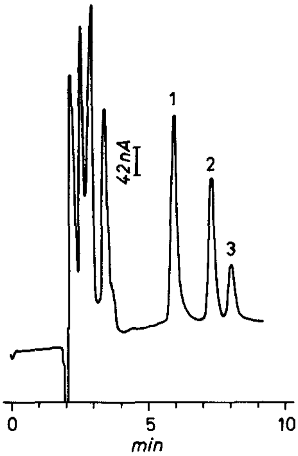

Fig. 1. Chromatogram obtained from a plasma sample containing 50 ng/ml naloxone as the I.S., 27 ng/ml 6β-naltrexol and 16.5 ng/ml naltrexone. Peaks: 1 = naloxone; 2 = 6β-naltrexol; 3 = naltrexone.

High-performance liquid chromatography

Fig. 1 illustrates the chromatogram of plasma sample containing 16.5 ng/ml NTX and 27 ng/ml 6β-NTX-OL. The retention times were: NTX, 7.40 min; 6β-NTX-OL, 6.86 min; the internal standard, 5.85 min.

After some low potentials had been tested for the first electrode, the value of +0.20 V was chosen because it permitted the removal of compounds with a lower oxidation potential than the analytes, which could have interfered with the assay. Furthermore, at this potential no response was observed for NTX and 6β-NTX-OL. The potential of +0.70 V for the second electrode corresponded to the maximum response for the analytes in this type of detector.

The use of a deactivated octadecyldimethylsilyl column, instead of conventional reversed-phase column (Supercosil LC 18, 15 or 25 cm × 4.6 mm I.D., 5 μm particle size) improved the performance and gave good peak shapes. The limit of quantitation observed with this method was 5 ng/ml for NTX (R.D.S. = 7%) and 5 ng/ml for 6β-NTX-OL (R.S.D. = 4%).

Calibration curves

The calibration curves of peak height (mm) *versus* amount of analyte (ng/ml) were prepared and checked daily from spiked plasma carried through the entrire procedure; they were linear over the range 5–500 ng/ml (regression equations: $y = 4.75x + 5.91$ for NTX and $y = 6.76x + 11.65$ for 6β-NTX-OL).

Analytical recoveries from plasma containing 50, 100 and 500 ng/ml added NTX and 6β-NTX-OL were assessed in ten replicate samples (Table I).

TABLE II

WITHIN-DAY PRECISION FOR NTX AND 6β-NTX-OL IN SPIKED PLASMA SAMPLES

Concentration added (ng/ml)	Mean concentration found (ng/ml)		R.S.D. (n = 6) (%)		Relative error (%)	
	NTX	6β-NTX-OL	NTX	6β-NTX-OL	NTX	6β-NTX-OL
50	44.05	42.66	1.0	1.3	−11.9	−14.6
100	87.98	85.01	2.0	1.0	−12.0	−15.0
500	440.11	422.83	5.6	1.5	−12.0	−15.4

TABLE III

BETWEEN-DAY PRECISION FOR NTX AND 6β-NTX-OL IN SPIKED PLASMA SAMPLES

Concentration added (ng/ml)	Mean concentration found (ng/ml)		R.S.D. (n = 6) (%)		Relative error (%)	
	NTX	6β-NTX-OL	NTX	6β-NTX-OL	NTX	6β-NTX-OL
50	43.95	42.55	1.2	1.5	−12.0	−14.9
100	86.60	85.00	1.7	1.2	−13.0	−15.0
500	433.00	422.52	4.1	1.5	−13.0	−15.5

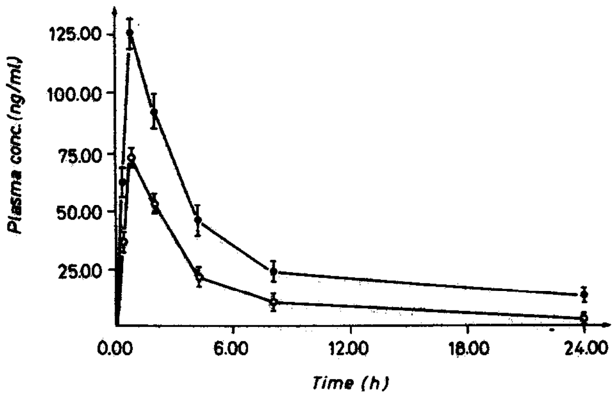

Fig. 2. Mean plasma levels (± S.D.) of naltrexone (○) and 6β-naltrexol (●) in six healthy volunteers after oral administration of 50 mg of NTX.

Within-day precision

The within-day precision was checked by determining six plasma samples spiked with three different concentrations of NTX and 6β-NTX-OL (Table II).

Between-day precision

Three concentrations of NTX and 6β-NTX-OL were determined in duplicate every day for six days (Table III).

CONCLUSION

The present method was applied to the determination of the plasma concentration of NTX and 6β-NTX-OL in six healthy volunteers after oral administration of 50 mg of the drug (Fig. 2). The method was also used to investigate the pharmacokinetic parameters of different pharmaceutical preparations of NTX. Insufficient blood concentrations of the drug have resulted in heroin reassumption in addicts, thus rendering ineffective the treatment with this opioid antagonist.

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