

SIMULTANEOUS DETERMINATION OF NALTREXONE AND 6- β -NALTREXOL IN SERUM BY HPLC

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ABSTRACT: A reliable and simple method for the simultaneous determination of naltrexone and 6- β -naltrexol in human serum by using high-performance liquid chromatograph (HPLC) was developed. Liquid-liquid extraction with butylacetate from basic solutions (pH 9) was chosen as extraction with nalorphine as an internal standard (IS). Analytes were back-extracted from organic solvent into perchloroacid. The acid extract was chromatographed by HPLC with reverse-phase ODS-column and electrochemical detector. The mobile phase was NaH₂PO₄ solution with acetonitrile as an organic modifier and octanesulphonic acid and tetraethylammonium-hydrogensulphate as ion-pair reagents. The recovery of extraction method was 48% for naltrexone and 75% for 6- β -naltrexol. Limit of quantification was 5.0 ng/ml for naltrexone and 1.0 ng/ml for 6- β -naltrexol. Analysed concentrations of naltrexone differed from theoretic concentrations from 0.7 to 2.3% and those of 6- β -naltrexol 2.6%. Relative standard deviation of within-day assay was from 0.9 to 5.7% for naltrexone and from 0.8 to 4.2% for 6- β -naltrexol and between-day 5.7 and 4.2%, respectively. Our results indicate that the developed method is suitable for determination of naltrexone and 6- β -naltrexol in human serum.

KEY WORDS: Naltrexone; Naltrexol; HPLC.

*Problems of Forensic Sciences, vol. XLIII, 2000, 250–256
Received 9 September 1999; accepted 16 May 2000*

INTRODUCTION

Naltrexone is an opioid antagonist, which has been used for treatment of alcoholism [6, 9, 13] and opiate dependence [3]. Naltrexone undergoes rapid and extensive hepatic metabolism by enzymatic reduction of the ketone to major metabolite 6- β -naltrexol (Figure 1) and to other minor metabolites. Mostly naltrexone and its metabolites are present in conjugated forms [12, 14, 15] 6- β -naltrexol is a weaker opioid antagonist than naltrexone, but it may contribute to the clinical effects of the drug as it persists in higher amounts than naltrexone [11]. Lesser reduction of naltrexone to 6- β -naltrexol seems to occur in liver cirrhosis and such alterations appear to be related to the severity of liver disease [1].

Published methods for the simultaneous quantitative determination of naltrexone and 6- β -naltrexol in biological fluids have used gas chromatography with electron cap-

ture detection (GC-ECD), [10, 12] high-performance liquid chromatography with electrochemical detection (HPLC-EC), [4, 16] gas chromatography-mass spectrometry (GC/MS) [5, 7] and gas chromatography-tandem mass spectrometry (GC/MS-MS) [2, 8].

Fig. 1. The metabolism of naltrexone.

MATERIALS AND METHODS

Reagents

Naltrexone was purchased from Dupont Merck, 6- β -naltrexol HCl was from National Institute of Drug Abuse and internal standard nalorphine HBr from National Agency for Medicines. Acetonitrile was HPLC grade and all other solvents and reagents were analytical grade. Butylacetate was purchased from Acros. Acetonitrile, methanol, perchloric acid, dipotassium hydrogenphosphate and potassium dihydrogenphosphate were purchased from Merck. 1-octanesulfonicacid sodium saltmonohydrate and tetraethylammonium hydrogensulphate were from Fluka. All buffers were prepared with deionized water. The naltrexone and 6- β -naltrexol stock solutions were prepared in methanol to give concentrations of 1 mg/ml of free base. These solutions were used to prepare calibration curves covering the range from 0 to 1000 ng/ml. All calibration standards, blanks and quality control samples were prepared in drug free cattle serum.

Extraction

1 ml sample of serum was extracted for all standard, quality control and unknown clinical samples. 1 ml of 0.5 M $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ (pH 9) was added to make sample basic.

Sample was extracted with 5 ml of butylacetate with nalorphine (1 µg/100 ml) as internal standard. After vortex-mixing (30 s) sample was centrifuged for 5 min at 3000 rpm. The upper butylacetate layer was transferred to clean test tube and back-extracted into 150 µl of 0.1 M HClO₄. Sample was vortex-mixed for 30 s. After 5 min centrifugation (at 3000 rpm) upper butylacetate phase was wasted. Acid phase was transferred to the autosampler vial and a 25 µl injection was made for HPLC-analysis.

Chromatographic conditions

Hewlett-Packard 1090 Series II-high-performance liquid chromatography with Esa Coulochem 5100 A electrochemical coulometric detector (Det 1 + 0.2 V; Det 2 + 0.5 V) was used to analyse naltrexone and 6-β-naltrexol. The mobile phase was acetonitrile-potassium dihydrogenphosphate (19 mM) (10:45, v/v) with 1-octanesulphonic acid (5 mM) and tetraethylammonium hydrogensulphate (5 mM) as ion-pair reagents. Chromatographic separation was achieved using a ODS Hypersil-reverse-phase column with a length of 125 mm, an i.d. of 4 mm and a particle size of 5 mm. The flow-rate was 1.2 ml/min with recirculation of the mobile phase. Data acquisition and integration were performed using HP Chemstation Software.

RESULTS AND DISCUSSION

Chromatograms of control sample spiked with naltrexone and 6-β-naltrexol and unknown clinical serum sample are shown in Figure 2. The identification of drugs was based on retention times, which are shown in Table I. Retention times were checked with calibrating standards before each determination. Linear one-point calibration based on peak heights was used for quantification of drugs.

TABLE I. THE RETENTION TIMES OF MONITORED COMPOUNDS

Compound	Retention time [min]
Nalorphine (IS)	5.1
6-β-Naltrexol	6.1
Naltrexone	6.9

The recovery of the extraction method was determined at three concentration levels by analysing two sets of samples. Set 1 included five samples per concentration, naltrexone and 6-β-naltrexol were added before extraction. In set 2 (three samples per concentration) naltrexone and 6-β-naltrexol were added after extraction. The recovery was calculated by dividing the average of results in set 1 by the corresponding average in set 2. The extraction recoveries for naltrexone and 6-β-naltrexol are shown in Table II.

TABLE II. THE EXTRACTION RECOVERIES FOR NALTREXONE AND 6- β -NALTREXOL

Compound	Concentration [ng/ml]	Recovery [%]
Naltrexone	100	46,5
	250	50,7
	500	47,4

Fig. 2. Chromatogram of a control sample piked with 50 ng/ml of naltrexone ($R_t = 7.00$) and 6- β -naltrexol (6.22). Nalophrine ($R_t = 5.14$) was used as internal standard.

6- β -naltrexol	100	71,6
	250	77,4
	500	76,2

Validation

Reliability of the developed determination method was tested with the linearity, accuracy and within-day and between-day assay precision. Two sets of standard samples were prepared at concentration range from 0 to 1000 ng/ml to determine the linearity. The linearity ranges and the mean correlation coefficients (r^2) for naltrexone and 6- β -naltrexol are shown in Table III. The limit of quantification was determined by analysing sets of six samples at low concentration levels. Relative standard deviations (RSD) of these samples were calculated. At the limit of quantification signal-to-noise ratio should be more than three and RSD-value under 20%. The limits of quantification with RSD-values for both compounds are shown in Table III.

TABLE III. THE LINEARITY RANGE, MEAN CORRELATION COEFFICIENT (R^2) AND LIMIT OF QUANTIFICATION WITH RSD-VALUE FOR BOTH COMPOUNDS

Compound	Linearity range [ng/ml]	r^2	Limit of quantification [ng/ml]	RSD [%]
Naltrexone	5–1000	0.9987	5	5.7
6- β -Naltrexol	1–1000	0.992	1	15.4

Accuracy and within-day precision was tested at two concentration levels with six samples. Average of concentrations found, accuracy and within-day assay precision for naltrexone and 6- β -naltrexol are shown in Table IV.

TABLE IV. AVERAGES OF CONCENTRATIONS FOUND, ACCURACY AND WITHIN-DAY ASSAY PRECISION FOR NALTREXONE AND 6- β -NALTREXOL

Compound	Concentration added [ng/ml]	Concentration found [ng/ml]	Accuracy [%]	Precision [%]
Naltrexone	5	4.97	0.7	5.7
	150	146.6	2.3	0.9
6- β -Naltrexol	5	4.87	2.6	4.2
	150	146.1	2.6	0.8

Between-day precision was determined by analysing same serum standard 15 times in 30 days. Serum standard spiked with 100 ng/ml of naltrexone and 6- β -naltrexol was stored at -20°C . Between-day assay precision for both compounds are shown in Table V.

TABLE V. BETWEEN-DAY ASSAY PRECISION FOR BOTH COMPOUNDS

Compound	Concentration added [ng/ml]	Average of Concentration found [ng/ml]	Precision [%]
Naltrexone	100	98.8	5.7
6- β -Naltrexol	100	98.9	4.2

Small changes in laboratory conditions (room temperature, atmospheric moisture) had no effect on results. Retention times seemed to increase slightly because of contamination in chromatographic system. This had however no effect on results because the retention times were checked with calibrating standards before each determination. On basis of validation results the developed method is found suitable for determination of naltrexone and 6- β -naltrexol in human serum. The method met our criteria for accuracy.

The within-day and between-day assay precisions were acceptable for all tested concentration levels.

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