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Journal of Pharmaceutical and Biomedical Analysis 35 (2004) 169–176

JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS

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Determination of naltrexone and 6-β-naltrexol in human plasma following implantation of naltrexone pellets using gas chromatography–mass spectrometry

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Received 4 June 2003; received in revised form 9 January 2004; accepted 9 January 2004

Abstract

An alternative to detoxification by oral therapy with the narcotic antagonist naltrexone is the subcutaneous implantation of naltrexone pellets. From detoxified patients with naltrexone implants (1 g) 26 blood samples were collected up to 73 days after implantation. The assay for naltrexone and 6-β-naltrexol in plasma was developed using automated mixed-mode solid-phase extraction, catalysed trimethylsilylation and gas chromatography–mass spectrometry in single ion monitoring mode with naloxone as internal standard. The analytical method was very sensitive with limits of detection of 0.1 ng/ml and was linear up to 60 ng/ml for naltrexone and 200 ng/ml for naltrexol. Intra-day precision for naltrexone and naltrexol were 24.3 and 22.9%, respectively, at the LLOQ (accuracy 1.4 and 0.4%, respectively) and less than 10% (2.0, 6.0 and 20.0 ng/ml, $n = 6$ each) above. Inter-day precision was 7.9% (accuracy −0.6%) for naltrexone and 10.9% (accuracy 1.6%) for naltrexol (20 ng/ml, $n = 10$). Extraction recoveries were 83% for both analytes (10 ng/ml, $n = 6$). The concentrations of naltrexone and naltrexol in the plasma samples were in the range of 0.7–13.7 and 0.9–17.0 ng/ml, respectively. The simple analytical procedure described provided good sensitivity for the assay of naltrexone and naltrexol in plasma after naltrexone pellet implantation.
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Keywords: Naltrexone; 6-β-Naltrexol; Plasma analysis; Gas chromatography–mass spectrometry

1. Introduction

The competitive narcotic antagonist naltrexone is increasingly considered an important aid in the detoxification of opiate addicts. Naltrexone is available as an oral dosage form, well tolerated and blocks opiate receptors effectively for up to 72 h after the last dose [1,2]. However, a basic handicap is the malcompliance in many patients, because addicts are often ambivalent about treatment. Therefore, since 1975, efforts have continued to develop pellets which can be implanted and provided naltrexone continuously in an

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doi:10.1016/j.jpba.2004.01.013

effective dose for several weeks in animals [3–6] or humans [7–10]. Since effective plasma levels of naltrexone can be 1 ng/ml and less [8–10], very sensitive analytical methods have to be employed to measure those levels. It is also necessary to assay its metabolite 6-β-naltrexol (naltrexol), because it is pharmacologically active [11] and probably important for the long duration of the pharmacological effect [12,13]. Almost all analytical procedures make use of very sensitive detection techniques after chromatographic separation. These include electron-capture gas–liquid chromatography (GC–EC) [14–18], GC with tandem mass spectrometry [19], GC with negative chemical ionisation (NCI) MS [20,21], high pressure liquid chromatography (HPLC) with electrochemical detection [22–26], HPLC with coulometric detection [27], HPLC with amperometric detection [28] and HPLC with mass spectrometric detection [34]. In two reports HPLC with UV detection was used, but these procedures were not sensitive enough for assaying effective concentrations, because the limits of detection were reported to be about 8 ng/ml [29] and 2 ng/ml [26]. Derivatization for gas chromatography–mass spectrometry (GC–MS) was carried out using perfluorated acyl groups such as pentafluoropropionic acid or heptafluorobutyric acid, with acetic anhydride [30], but silylation with trimethylsilyl groups was not successful [21] or reported to be not sensitive enough for analysis of blood samples [17].

The aim of the present work was to develop a sensitive, reliable and labour-saving analytical assay of naltrexone and naltrexol in human plasma using common GC–MS. This method was to be used for drug monitoring in patients who received naltrexone implants in an on-going clinical trial.

2. Experimental

2.1. Chemicals and reference standards

Reference substance of naltrexone and 6-β-naltrexol (naltrexol) were supplied by DuPont Pharmaceuticals Company (Garden City, NY) and the internal standard naloxone (1 mg/ml in methanol) was purchased from Cerilliant (Promochem, Wesel, Germany). The derivatization reagent was prepared as 2% of *N*-trimethylsilyl-imidazole (TSIM) in *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), both were from Macherey & Nagel (Dueren, Germany), all other reagents and organic solvents were of analytical grade and from Merck (Darmstadt, Germany).

2.2. Plasma samples

Blood samples were obtained from eight former opiate addicts who participated in a pilot study with a new formulation of naltrexone implants (containing 1 g drug base, prepared in the hospitals pharmacy) [31] (Partecke et al., Effects of naltrexone implants on relapse in opiate addicts, submitted for publication) up to 73 days after implantation (inguinal region) in the Jüdisches Krankenhaus, Berlin. The study protocol was approved by the local Ethical Committee, and written informed consent has been obtained from the study participants. The samples were collected in EDTA containing tubes, were centrifuged for plasma separation and the supernatant was stored at −20 °C until analysis within 1 year where both analytes were reported to be stable [32].

2.3. Determination of naltrexone and naltrexol in human plasma using gas chromatography–mass spectrometry (GC–MS)

Preparation and extraction of plasma samples was performed using a standard procedure for basic drugs [33]. An aliquot of 1 ml was diluted with 4 ml of 0.1 M phosphate buffer pH 6.0 and 50 μl of internal standard solution (1 ng/μl of naloxone in methanol) was added and vortexed. The diluted samples were extracted using mixed-mode solid-phase extraction cartridges with an extraction robot. After elution and evaporation the dry extracts were dissolved using 40 μl of MSTFA +2% TSIM and were derivatized at 100 °C for 30 min.

GC–MS analyses of the extracts were performed on a Hewlett-Packard (Waldbronn, Germany) GC–MS (HP 6890 Series plus GC, HP 7683 series injector, HP 5973 mass selective detector) using a Chrompack CP-Sil 5 CB lowbleed/MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) from Varian (Darmstadt, Germany) which was shielded by 1.8 m of deactivated (DPTMDS) glass capillary (0.25 mm i.d.) from BGB Analytik AG (Anwil, Switzerland). Helium was used as carrier gas at a flow rate of 1.5 ml/min. Two microlitre of the extracts were

injected into the split/splitless injection port (310 °C) of the GC. The temperature program increased from 100 to 310 °C with 40 °C/min and was held at 310 °C for additional 6 min. The GC–MS transferline was heated to 310 °C, the MS ionisation energy was 70 eV. Quantification of naltrexone and naltrexol was carried out in single ion monitoring (SIM) mode with naloxone as internal standard. The following fragment ions (cf. full scan spectra in Fig. 1) were used in SIM mode (quantifier ion underlined): naloxone 3TMS *m/z* 528, 543, 438, naltrexone 3TMS *m/z* 542, 557, 484 and naltrexol 3TMS *m/z* 544, 559, 372. Data analysis was performed using the HP ChemStation software (Rev. C.03.00).

For validation of the analytical method and in-series calibrations, pooled drug-free plasma was spiked with naltrexone and naltrexol, and analysed as described above. Calibration curves were prepared from analysis of one blank and 10 calibration samples containing naltrexone in a concentration range of 0.1–60.0 and 0.1–200.0 ng/ml naltrexol. The limits of detection (LOD) were determined by serial dilutions of known concentrations of the two analytes in the range of 0.05–5.0 ng/ml. The LOD was established as the lowest concentration of each analyte that provided a signal-to-noise ratio greater than 3 for the least intense fragment ion and greater than 5 for the other two ions. Analytical recoveries for naltrexone and naltrexol were determined at 10.0 ng/ml ($n = 6$), in-series reproducibility (intra-day precision) was determined at the respective lower limit of quantification and at 2.0, 6.0 and 20.0 ng/ml ($n = 6$ each) and inter-day precision and accuracy was determined on 10 days at 20 ng/ml (Table 1).

The specificity of the procedure was tested with drug-free pooled plasma containing amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxyethylamphetamine (MDE), *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB), cocaine, cocaethylene, benzoylecgonine, ecgonine methyl ester, ecgonine ethyl ester, 6-monoacetylmorphine, morphine, codeine, dihydrocodeine, methadone, tilidine, nortilidine, tramadol, dextromethorphan, doxylamine and diphenhydramine at 250 ng/ml each, Δ^9-tetrahydrocannabinol and 11-hydroxy-Δ^9-tetrahydrocannabinol at 12.5 ng/ml, 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid at 50 ng/ml, diazepam and nordiazepam at 1000 ng/ml, temazepam, oxazepam and bromazepam at 200 ng/ml, flunitrazepam, 7-aminoflunitrazepam and norflunitrazepam at 100 ng/ml.

Table 1
Validation of the assay of naltrexone and naltrexol in human plasma using GC–MS

	Naltrexone	6-β-Naltrexol
Limit of detection (LOD, S/N > 5)	0.1 ng/ml	0.1 ng/ml
Range of linearity	0.1–60 ng/ml	0.3–200 ng/ml
Regression equation	$y = 16.19x$	$y = 44.35x$
Regression coefficient	0.999	0.998
Standard error of estimate	0.94	4.55
Standard deviation of slope	0.22	0.88
Extraction recovery (10.0 ng/ml, $n = 6$)	82.6 ± 2.7%	83.1 ± 11.0%
Intra-day precision (accuracy)		
0.15 ng/ml, $n = 6$	24.3% (1.4%)	n.d.
0.5 ng/ml, $n = 6$	n.d.	22.9% (0.4%)
2.0 ng/ml, $n = 6$	11.1% (n.d.)	12.7% (n.d.)
6.0 ng/ml, $n = 6$	4.3% (n.d.)	8.5% (n.d.)
20.0 ng/ml, $n = 6$	5.7% (n.d.)	9.5% (n.d.)
45.0 ng/ml, $n = 6$	2.3% (5.6%)	n.d.
150.0 ng/ml, $n = 6$	n.d.	16.8% (10.6%)
Inter-day precision (accuracy)		
20.0 ng/ml, $n = 10$	6.2% (−0.6%)	10.4% (1.6%)

n.d.: not determined.

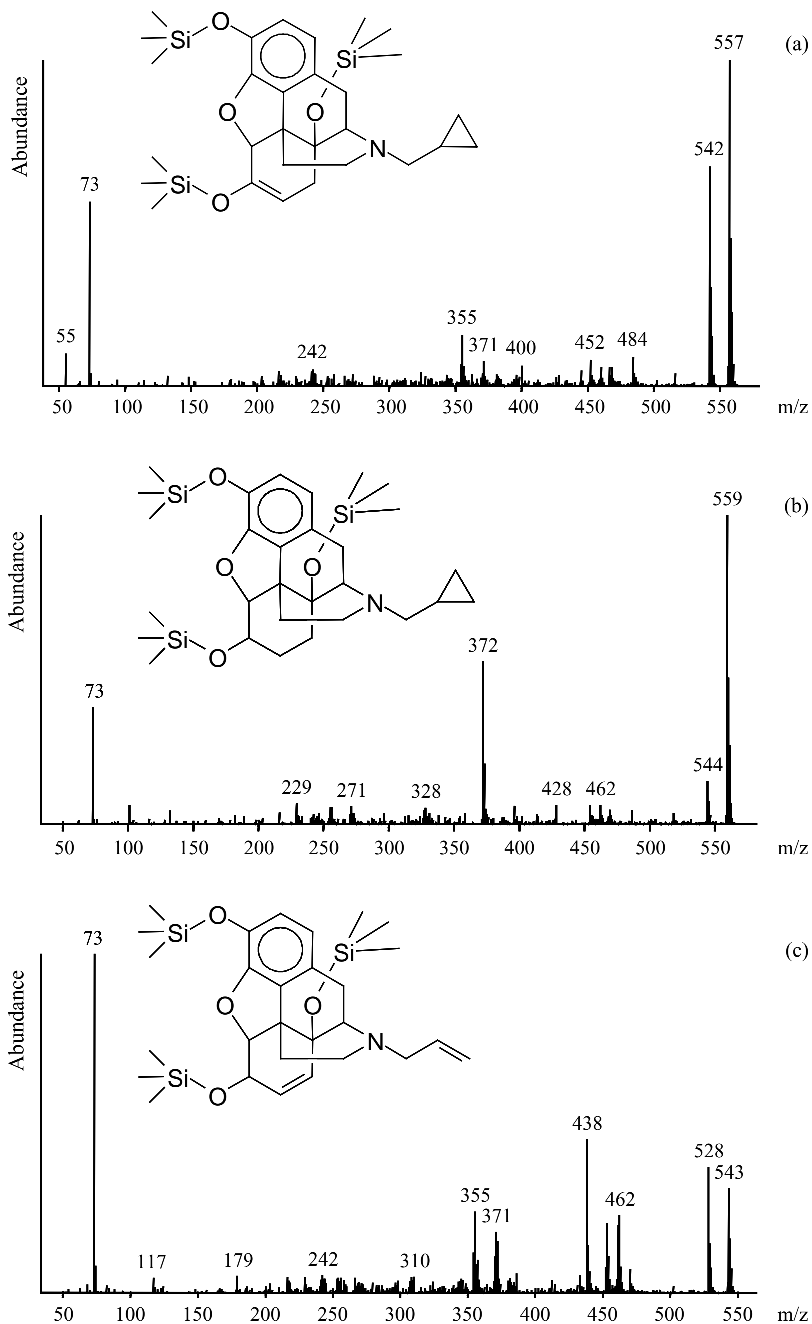

Fig. 1. Mass spectra of the pertrimethylsilylated analytes (a) naltrexone, (b) 6-β-naltrexol and (c) the internal standard naloxone.

3. Results and discussion

3.1. Determination of naltrexone and naltrexol in human plasma by GC–MS

The concentrations of naltrexone and naltrexol in plasma after implantation of sustained-release preparations have been found to be lower than 1 ng/ml [9,10,20]. Therefore, the determination of these low but pharmacologically active concentrations is demanding and requires very sensitive and expensive detection techniques like GC–NCI–MS or HPLC with electrochemical or mass spectrometric detection. For the determination of naltrexone and naltrexol in plasma samples from a clinical study of a new type of implantable naltrexone preparation, a new assay was developed, which makes use of common GC–MS equipment as available in typical toxicological laboratories.

The derivatization of naltrexone for GC–MS is associated with the problem of partial derivatization of the enolic form of the ketone group [19]. Huang et al. [21] tried trimethylsilylation, which works well for steroids, but were unsuccessful in obtaining complete derivatization of naltrexone. Therefore, they developed a simple pentafluoropropionylation procedure for GC–NCI–MS, where only small amounts of mono- or tris-derivatized naltrexone species were produced. In our experiments with acylation techniques we found derivatives with a considerable degree of partially derivatized species (acetylation, pentafluoropropionylation and heptafluorobutyrylation were tested). Recently, a complete derivatization using acetic anhydride was reported [30] but the reaction took 6–14 h and an additional evaporation step was necessary. Furthermore, the sensitivity was not sufficient for the monitoring of the low concentrations expected in patients with naltrexone implants. Therefore, trimethylsilylation using MSTFA was tried. We found that complete derivatization not only of the phenol group but also of the tertiary alcohol functional group and the enolic form of the carbonyl group in naltrexone could be obtained by addition of the catalyst TSIM (2%). The presented derivatization procedure is very simple and time saving, because the derivatization agent (MSTFA +2% TSIM) serves as solvent for the dry extract and needs no evaporation step after the derivatization. The reaction products are volatile and pose no problem for the stationary phase of the capillary column and have no impact on the chromatographic separation. The injection port and transferline temperatures were set to 310 °C because trimethylsilylated naltrexone and naltrexol are high boiling substances and elute both at 310 °C. This modification to standard procedures markedly increased the sensitivity of the method.

Naloxone was chosen as internal standard [14,27,28] due to its chemical similarity to both analytes. Naloxone is an opiate receptor antagonist like naltrexone but it is mainly used for the treatment of acute opiate intoxications and exhibits a short elimination half life. Therefore, it was not expected to be present in blood samples from patients treated with naltrexone implants.

For the two analytes and the internal standard, no interferences with matrix compounds was observed for each of the three selected fragment ions at the respective retention times in plasma from 10 different sources (cf. Fig. 2). The procedure was also tested for interference with several other commonly abused drugs and no coelution was found for amphetamine and derivatives, cocaine and metabolites, Δ^9-tetrahydrocannabinol and metabolites, opiates, several opioids, two common antihistaminics, diazepam and metabolites, flunitrazepam and metabolites and bromazepam.

Though trimethylsilylation was reported to be not sensitive enough 20 years ago [17] using flame ionisation detection and a packed column, the procedure described above provided excellent sensitivity and specificity in plasma. Limits of detection were very low, being 0.1 ng/ml for both analytes (cf. Fig. 2). We recommend to extend the calibration far above 50 ng/ml [21] as we have found concentrations as high as 51 ng/ml naltrexone and 189 ng/ml naltrexol in authentic plasma samples. Therefore, the range of calibrators used was up to 60 ng/ml for naltrexone and up to 200 ng/ml for naltrexol, which was sufficient for all cases analysed so far and should cover typical therapeutic concentrations in acute and chronic treatment as reported by Verebey et al. [2]. Limits of quantification (LOQ) were 0.1 ng/ml for naltrexone and 0.3 ng/ml for naltrexol, where the respective determined values were within a 15% deviation of the calculated values. Further details on validation data for linearity, extraction recovery, intra- and inter-day

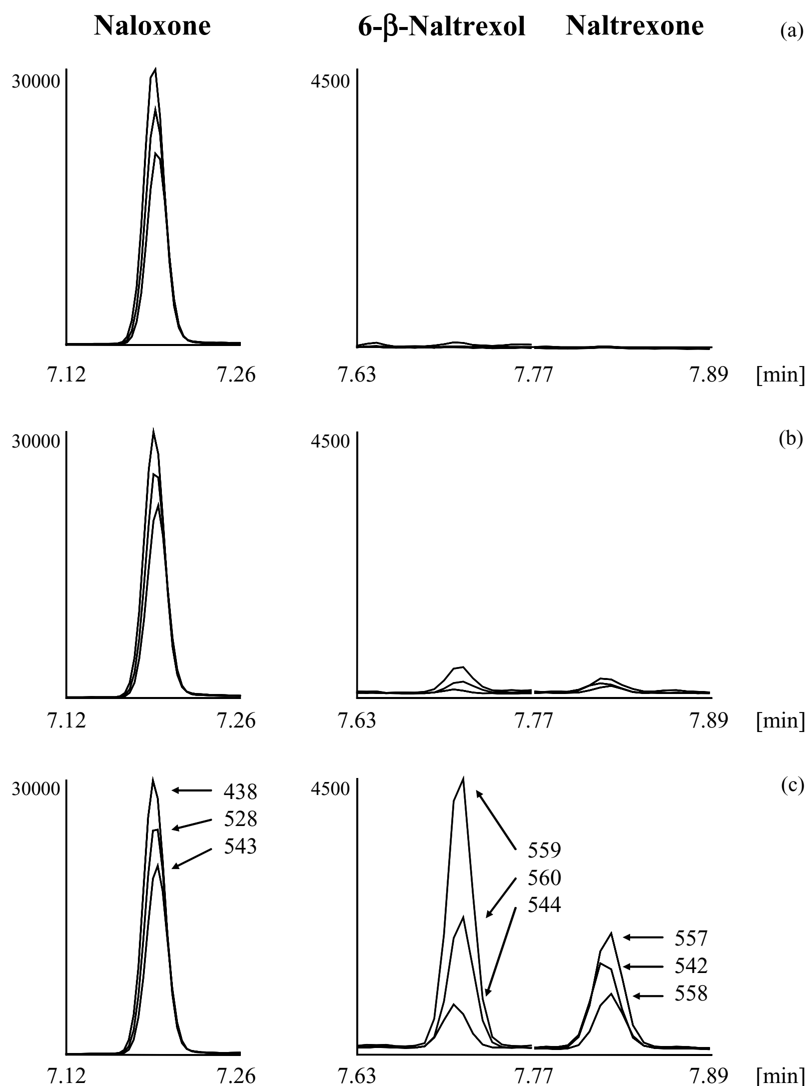

Fig. 2. Extracted ion chromatograms for naloxone (internal standard, 50 ng/ml in all samples), naltrexol and naltrexone in extracts of (a) pooled blank plasma, (b) pooled blank plasma spiked with naltrexone and naltrexol at LOD level (0.1 ng/ml each) and (c) an authentic plasma sample from a patient 59 h after implantation of the naltrexone pellet (concentrations determined were 2.4 ng/ml naltrexone and 2.0 ng/ml naltrexol). For reasons of visualisation the abundances of the naltrexone and naltrexol ion traces are amplified over the abundance of the internal standard.

precision and accuracy are summarised in Table 1. The presented assay is superior to most of the published methods with respect to sensitivity [26,27,29,30], selectivity [23,25] and time effectiveness in sample preparation. It is [19–21,25,30] a robust and efficient alternative to recently published methods using GC–NCI–MS [21], GC–MS–MS [19] or HPLC with electrochemical [25] or mass spectrometric [34] detection and can be performed in every laboratory with common GC–MS equipment. It provides sufficient sensitivity and assay validity and the method proved to be highly specific as no interferences were found during assay validation and serial analyses of authentic samples.

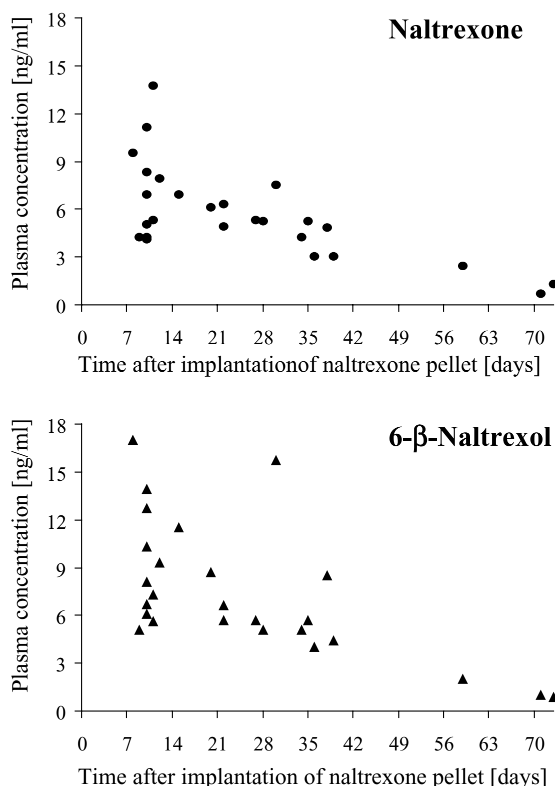

Fig. 3. Concentrations of naltrexone and 6-β-naltrexol in plasma samples from eight subjects after naltrexone pellet implantation.

3.2. Plasma concentrations of naltrexone and naltrexol after implantation of naltrexone pellets

When this method was applied to plasma samples from the eight participants in the pilot study [31] (Partecke et al., Effects of naltrexone implants on relapse in opiate addicts, submitted for publication) up to 73 days after implantation of the naltrexone pellets, the concentrations of naltrexone and naltrexol were in the range of 0.7–13.7 and 0.9–17.0 ng/ml, respectively (Fig. 3). Due to the sort of compliance problems common with this type of patient not all of the scheduled blood samples could be obtained, but all patients gave samples at 8–12 days after the last naltrexone implantation. The concentrations at about 10 days after pellet implantation were 4.1–13.7 ng/ml (median 6.9 ng/ml) for naltrexone and 5.1–17.0 ng/ml (median 8.1 ng/ml) for naltrexol ($n = 11$). All naltrexone and naltrexol concentrations in plasma samples taken during the first 6 weeks after implantation were greater than 3 ng/ml which indicates, that pharmacologically active naltrexone concentrations >2.0 ng/ml [13] can be maintained for more than 39 days following implantation with this type of pellet.

Chiang et al. [9,10] studied in the 1980s the application of only 63 mg naltrexone and found very low plasma concentrations (<1 ng/ml). In the 1990s, Kranzler et al. [8] found plasma levels greater than 1 ng/ml during 21 days after implantation of 206 mg naltrexone. The present concentration–time data match those reported by another workgroup [35] (A.S. Christophersen, L. Olsen, 39th Annual International Meeting of the International Association of Forensic Toxicologists (TIAFT) in Prague, Czech Republic, 2001) using 1 g naltrexone implants from an other source (Wedgewood Pharmacy, NJ, USA) and are also in agreement with the report of 3–5 ng/ml naltrexone found in patients 4–5 weeks after naltrexone implantation [36].

From two patients blood could be obtained 70 days after implantation and the naltrexone and naltrexol concentrations were still above 0.7 ng/ml which Chiang et al. [9,10] believed to be sufficiently effective, as they showed that levels below 1 ng/ml provided an at least partial suppression of the effects of a 15 mg morphine challenge.

The simple method described for the determination of naltrexone and 6-β-naltrexol in human plasma using common GC–MS equipment provided the same sensitivity and reliability as more complicated procedures for the analysis of plasma samples after naltrexone implantation.

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