ELSEVIER

Journal of Chromatography B, 761 (2001) 85–92

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Quantification of naltrexone and 6,β-naltrexol in plasma and milk using gas chromatography–mass spectrometry Application to studies in the lactating sheep

Chooi Fen Chan[a], Gregory M. Chiswell[b], Roberta Bencini[c], L. Peter Hackett[b], Leon J. Dusci[b], Kenneth F. Ilett[a,b,*]

[a]*Department of Pharmacology, The University of Western Australia, Crawley, Western Australia 6009, Australia*
[b]*Clinical Pharmacology & Toxicology Laboratory, The Western Australian Centre for Pathology & Medical Research, Nedlands, Western Australia 6009, Australia*
[c]*Animal Science, Agriculture, The University of Western Australia, Crawley, Western Australia 6009, Australia*

Received 19 December 2000; received in revised form 26 June 2001; accepted 3 July 2001

Abstract

A selective gas chromatography–mass spectrometry method using solid-phase extraction has been developed for the detection and quantification of naltrexone and its metabolite, 6,β-naltrexol in plasma and milk from humans and sheep at pharmacologically relevant concentrations. Di- or tri-acetyl derivatives were formed and quantified by selected-ion monitoring. Recoveries of naltrexone (30 μg/l) and 6,β-naltrexol (250 μg/l) from both human plasma and milk were greater than 70%. Intra-assay and inter-day precision ranged from 3 to 21% for naltrexone and 2–18% for 6,β-naltrexol for all matrices investigated, with an overall mean accuracy of 104% for naltrexone, and 99% for 6,β-naltrexol. Human samples containing these analytes were stable for at least 3 weeks at −20°C or 6 weeks at −80°C. Analysis of the plasma and milk from the lactating sheep showed mean milk-to-plasma ratios of 55 for naltrexone and 3 for 6,β-naltrexol.

Keywords: Naltrexone; 6β-Naltrexol

1. Introduction

Naltrexone is a potent competitive antagonist at the opioid μ-receptor [1,2]. In humans, it has a plasma half-life of 2–14 h [3–5], a volume of distribution of 15 l/kg [6] and it is widely used as a treatment for heroin addiction [7] and alcohol dependence [8]. It is extensively metabolised to its major metabolite, 6,β-naltrexol and to a lesser extent, to 2-hydroxy-3-*O*-methylnaltrexol [4,9]. Animal experiments indicate that 6,β-naltrexol has about one-hundredth the potency of naltrexone at the μ-receptor [10]. Following a 100-mg oral dose naltrexone in humans, plasma concentrations of naltrexone ranged from 2 to 20 μg/l while those of 6,β-naltrexol ranged from 75 to 200 μg/l [11]. Our long-term aim

*Corresponding author. Department of Pharmacology, University of Western Australia, 35 Stirling Hwy, Crawley, 6009 Western Australia, Australia. Tel.: +61-8-9346-2985; fax: +61-8-9346-3469.

E-mail address: kilett@receptor.pharm.uwa.edu.au (K.F. Ilett).

0378-4347/01/$ – see front matter
PII: S0378-4347(01)00309-7

is to study the extent of excretion of naltrexone in human milk and hence to assess safety for the breastfed infant. In the present investigation, we have developed a gas chromatography–mass spectrometry (GC–MS) method for quantification of both naltrexone and 6,β-naltrexol in plasma and milk. We also present preliminary data showing its application to the distribution of naltrexone and 6,β-naltrexol between plasma and milk in sheep.

2. Experimental

2.1. Specimens

The biological matrices used for method validation were plasma and milk from humans and sheep.

2.2. Chemicals and reagents

Naltrexone hydrochloride was purchased from Sigma (St. Louis, MO, USA) and naloxone hydrochloride was purchased from Endo Labs. (Garden City, NY, USA). 6,β-Naltrexol was synthesized by the method of Chatterjie and Inturrisi [10]. Acetic anhydride, pyridine, ammonia solution (specific gravity 0.91) and dibasic potassium phosphate were obtained from BDH Laboratory Supplies (Poole, UK) while trichloroacetic acid was purchased from Chem-Supply (Gillman, Australia). All other reagents were of analytical or high-performance liquid chromatography (HPLC) grade. BondElut Certify LRC (130 mg) solid-phase extraction (SPE) columns were obtained from Varian (Harbor City, CA, USA).

2.3. Sample preparation

The SPE method was developed for both plasma and milk. Sheep milk and plasma (separated from heparinised blood by centrifugation) samples were obtained from lactating sheep held at the University of Western Australia Shenton Park Animal Facility, while human milk samples were obtained from volunteer breastfeeding mothers at the King Edward Memorial Hospital for Women (Subiaco, Western Australia) and human plasma samples (separated from heparinised blood by centrifugation) from volunteer laboratory staff. The procedure was a modification of that reported by Huang et al. [12]. Internal standard (25 ng of naloxone) was added to plasma (1 ml) or milk samples (0.5 ml for sheep or 1 ml for human samples) in 10-ml polypropylene centrifuge tubes. These samples were sonicated for 10 s (Type FX12 Ultrasonic Bath, Unisonics, Australia), and vortex-mixed for 1 min. Distilled water (3 ml) was added and the samples were vortexed for a further 30 s. Cold 10% (w/v) trichloroacetic acid (2 ml) was added to each tube and immediately vortex-mixed for 20 s. The samples were centrifuged at 1500 *g* for 10 min, after which the supernatant was decanted into a clean tube. The, pH of the supernatant was adjusted to 9.4–10 with 1 ml of dibasic potassium phosphate (50%, w/v) and 0.6–0.8 ml of 1 *M* NaOH as required and the samples were then re-centrifuged as above.

BondElut Certify SPE columns were preconditioned with 2 ml of methanol followed by 2 ml of water. The deproteinated plasma or milk samples were applied to the columns and allowed to flow through slowly. The columns were then sequentially washed with 2 ml of water, 1 ml acetate buffer (0.1 *M*, pH 4) and 2 ml of methanol. The columns were dried under vacuum (10 mmHg; 1 mmHg=133.322 Pa; Vacuum Manifold, Alltech, Deerfield, IL, USA) for 1 min and the tips of the Vacuum Manifold delivery needles were wiped. The analytes of interest were eluted with 2 ml methylene chloride–isopropanol–ammonium hydroxide (80:20:2). Eluates were collected in clean 100 mm×16 mm borosilicate glass tubes, dried under a stream of N_2 at 50°C, and stored at 4°C in a desiccator prior to derivatisation.

2.4. Derivatisation

The extracts were derivatised by heating at 80°C for 6–14 h with a mixture of 100 μl acetic anhydride and 100 μl pyridine. Excess reagent was evaporated to dryness at 40°C under dry N_2 prior to reconstitution in 50 μl ethyl acetate. Aliquots (2 μl) were injected onto the GC column.

2.5. GC–MS analysis

GC–MS analysis of naltrexone and 6,β-naltrexol was performed using a Hewlett-Packard 5890 Series II gas chromatograph coupled to a Hewlett-Packard

5971A mass selective detector with electron impact ionisation. The capillary column was 30 m×0.25 mm I.D. coated with 0.25 μm thickness of 5% phenyl methyl siloxone (J&W Scientific Products, Koln, Germany). The gas carrier was helium at a flow-rate of 1 ml/min. Temperatures were 250°C in the injection port and 280°C at the detector. The oven temperature was set at 60°C for 2 min initially, and programmed to 100°C (at 70°C/min) and then to 300°C (at 20°C/min). Quantification was achieved using selected-ion monitoring (SIM). Plots of the ion signal peak area for the analytes of interest to that of naloxone (internal standard) versus known analyte concentrations were used to quantify unknowns. The ions monitored were: naloxone m/z=411 (quantification ion; dwell time 50 ms) and 453; naltrexone m/z=425 (quantification ion; dwell time 30 ms) and 467; 6,β-naltrexol m/z=427 and 469 (quantification ion; dwell time 30 ms).

2.6. Preparation of standards

Stock solutions of naltrexone (1 mg/10 ml), 6,β-naltrexol (10 mg/10 ml) and naloxone (1 mg/10 ml) were prepared in ethanol. Stock solutions were diluted with ethanol to give working standards as follows: naltrexone=10 μg/10 ml; 6,β-naltrexol=100 μg/10 ml; naloxone=10 μg/10 ml. All stock and working solutions were stored at 4°C. Fresh standards were prepared every 2 months.

2.7. Method validation

Five-point standard curves ranging for naltrexone and 6,β-naltrexol were constructed for each batch of samples. Relative standard deviations (RSDs) were determined using five separate aliquots from a pool of drug free plasma or milk (see Section 2.1 for sources) spiked with appropriate concentrations of naltrexone and 6,β-naltrexol. Recoveries were determined by comparing non-extracted analytes with those extracted by SPE at 30 μg/l for naltrexone and 250 μg/l for 6,β-naltrexol. Limits of detection (LODs) for each analyte were defined as the concentration that produced 2.5–3 times the background noise while limits of quantification (LOQs) were defined as the lowest concentration with an RSD of ≤20%.

2.8. Storage stability

Storage stability of naltrexone and 6,β-naltrexol in human plasma and milk was assessed after 0, 3 and 6 weeks at either −20°C or −80°C. Stability was assessed at 10 and 60 μg/l of naltrexone in human plasma, 10 μg/l of naltrexone in human milk, 10 and 500 μg/l of 6,β-naltrexol in human plasma, and 10 and 150 μg/l of 6,β-naltrexol in human milk (n=4 for each drug and concentration). At each time point, samples were assayed in a single run, and analyte concentrations were determined from a standard curve prepared on that day.

2.9. Pharmacokinetic studies in sheep

Four lactating Merino ewes (57.0±0.7 kg) were fitted with indwelling catheters (Venflon 2, 17 ga×45 mm; Ohmeda, Sweden) in each jugular vein. A naltrexone HCl dose (4.5 mg/kg) was administered intravenously via one of the indwelling catheters over a period of 1–2 min. Blood samples (6 ml) were collected in heparinised Vacutainers before the dose and at 0.08, 0.25, 0.42, 0.58, 0.67, 0.83, 1, 2, 3, 4, 6, 8, 10, 23 and 24 h after the dose. Plasma was obtained by centrifugation at 1500 g for 10 min. Milk samples (10 ml) were expressed manually at 0, 2, 4, 6, 8, 10 and 24 h. Plasma and milk samples were stored at −20°C after collection and assayed within 4 weeks.

2.10. Data analysis

Data are summarised as mean±S.E. unless otherwise specified. One-way analysis of variance (ANOVA) (SigmaStat Ver 2.03, SPSS, USA) was used to investigate storage stability of analytes. Plasma pharmacokinetic parameters (terminal half-life, volume of distribution and clearance) in sheep were determined using non-compartmental analysis in Topfit Version 2.0 [13]. Area under the plasma concentration– and milk concentration–time curves were also calculated using Topfit (trapezoidal rule) to facilitate calculation of the ratio of drug between milk and plasma.

3. Results

3.1. GC–MS analysis

Naltrexone, 6,β-naltrexol and the internal standard naloxone all have three functional groups that can be acetylated (positions 3, 6 and 14). Variations of the time and temperature of the derivatisation reaction were therefore investigated to determine conditions that would give complete derivatisation at all three sites. Optimal conditions for the formation of the tri-acetyl derivatives of naloxone, naltrexone and 6,β-naltrexol were achieved at 80°C for 6–14 h. The derivatives were stable when stored at 4°C for at least 48 h. A typical selected-ion chromatogram for derivatised naloxone, naltrexone and 6,β-naltrexol in plasma is shown in Fig. 1 while the mass spectra for tri-acetyl derivatives of naltrexone, 6,β-naltrexol and naloxone are shown in Fig. 2a–c, respectively. There was a good chromatographic separation between tri-acetylated naloxone, naltrexone and 6,β-naltrexol with retention times of 15.45, 16.54 and 16.66 min, respectively. The m/z 467 ion corresponds to the molecular ion of the tri-acetyl derivative of naltrexone while the m/z 469 ion corresponds to the molecular ion of the tri-acetyl derivative of 6,β-naltrexol. For quantification, the molecular ion was used for 6,β-naltrexol, while major fragments were used for naltrexone (m/z 425) and naloxone (m/z 411), respectively because these ions were usually more abundant than the molecular ion. With naloxone at longer derivatisation times (14 h), the m/z 453 ion was absent and hence the m/z 411 ion was used for quantitation.

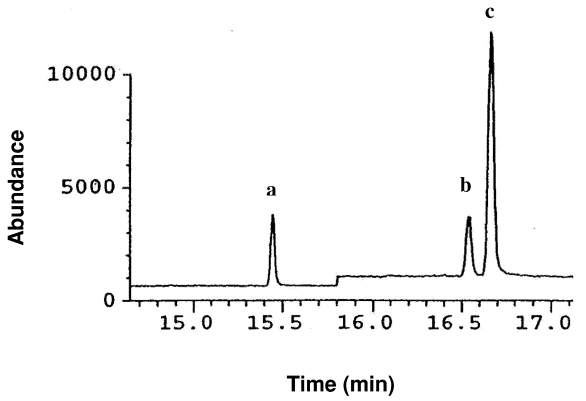

Fig. 1. Selected-ion chromatogram of derivatised extract of plasma that has been spiked with 25 μg/l of naloxone (m/z 410, 411, 453), 30 μg/l of naltrexone (m/z 425, 467) and 250 μg/l of 6,β-naltrexol (m/z 427, 469). Peaks: (a) naloxone=15.45 min; (b) naltrexone=16.54 min; (c) 6,β-naltrexol=16.66 min.

3.2. Validation of the assay

Standard curves for naltrexone and 6,β-naltrexol in the milk and plasma prepared using appropriate aliquots of the working standards, were linear in the following concentration ranges: 2–20 μg/l (naltrexone in human milk), 2–60 μg/l (naltrexone in human plasma), 2–500 μg/l (6,β-naltrexol in human plasma), 2–400 μg/l (6,β-naltrexol in human milk), 10–600 μg/l (naltrexone in sheep milk), 2–150 μg/l (naltrexone in sheep plasma), 10–1000 μg/l (6,β-naltrexol in sheep milk) and 2–2500 μg/l (6,β-naltrexol in sheep plasma). All standard curves had correlation coefficients of 0.99 or better. If necessary, samples were diluted with blank matrix to bring them into the appropriate range.

The LOD for both naltrexone and 6,β-naltrexol in plasma and milk (signal-to-noise ratio=2.5) was 1–2 μg/l. The RSDs (intra-assay and inter-day precision) for naltrexone and 6,β-naltrexol in all sample matrices ranged from 2 to 18% over the relevant concentration ranges (Tables 1 and 2). The LOQs for both naltrexone and 6,β-naltrexol in human and sheep plasma, and in human milk were 2 μg/l, while in sheep milk the LOQ was 10 μg/l. Accuracy was investigated for both intra-assay and inter-day RSD experiments (Tables 1 and 2); mean of 104% (range 94–118%) of the spiked value for naltrexone and mean 99% (range 87–109%) of the spiked value for 6,β-naltrexol. Recoveries for naltrexone (30 μg/l) and 6,β-naltrexol (250 μg/l) were investigated using human plasma and milk and were found to be similar to those reported by Huang et al. [12].

3.3. Storage stability

Stability of naltrexone and 6,β-naltrexol in human plasma and milk was studied over a period of 6 weeks. Using one-way ANOVA, samples stored at −20°C were stable for at least 3 weeks, while samples stored at −80°C were stable for at least 6 weeks.

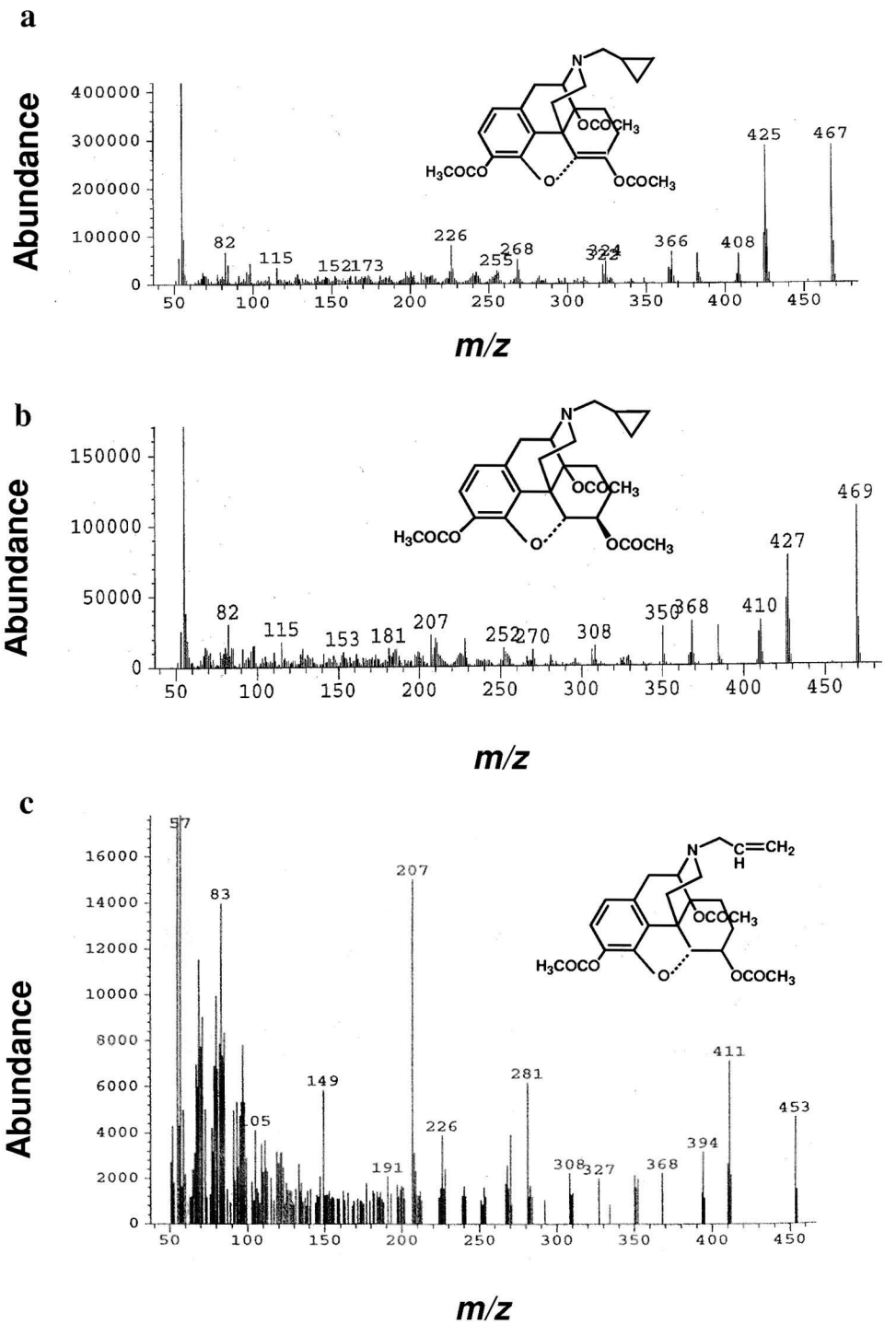

Fig. 2. Electron impact ionisation spectra of tri-acetyl derivatives of naltrexone (a), 6,β-naltrexol (b) and naloxone (c). The structures of the derivatives are also shown.

Table 1
Intra-assay relative standard deviations[a], and accuracy[b] measurements for naltrexone and 6,β-naltrexol in plasma and milk

Biological fluid	Naltrexone (μg/l)	RSD (%)	Accuracy (%)	6,β-Naltrexol (μg/l)	RSD (%)	Accuracy (%)
Human plasma	2	14		2	10	
	5	15	118	5	11	89
	30	3		50	3	
	60	7	101	500	5	94
Human milk	2	18		2	16	
	5	10	94	50	2	102
	50	4	98	300	4	94
Sheep plasma	2	21[c]		2	14[a]	
	5	9	103	5	8	108
	100	3	104	1000	6	101
Sheep milk	10	12[c]	103	10	17[a]	96
	20	14[d]		20	8[b]	
	600	10	107	200	14	97

[a] $n=5$ for each RSD unless otherwise indicated.
[b] Accuracy is % (mean) of spiked value.
[c] $n=4$.
[d] $n=3$.

3.4. Sheep studies

Plasma and milk concentration–time profiles of naltrexone and 6,β-naltrexol in the four lactating sheep are shown in Fig. 3a and b, respectively. Naltrexone was not detectable in plasma after about 2 h following the i.v. dose, while 6,β-naltrexol concentrations in plasma were consistently higher and remained detectable at 24 h. The elimination of naltrexone from plasma was rapid with a mean $t_{1/2}$ of 0.44±0.05 h. The elimination of 6,β-naltrexol from plasma was biphasic with a mean terminal $t_{1/2}$ of 6.8±1.2 h. Total plasma clearance of naltrexone (2.02±0.50 l/min/kg) was more rapid than that for 6,β-naltrexol (0.10±0.01 l/min/kg). Apparent volume of distribution for naltrexone (81±22 l/kg) was

Table 2
Inter-day relative standard deviations[a] and accuracy measurements[b] for naltrexone and 6,β-naltrexol in plasma and milk

Biological fluid	Naltrexone (μg/l)	RSD (%)	Accuracy (%)	6,β-Naltrexol (μg/l)	RSD (%)	Accuracy (%)
Human plasma	5	7.3	116	5	17.8	108
	100	9.3	96	1000	13.0	94
Human milk	5	4.3	104	50	7.5	109
	50	5.9	97	300	11.6	94
Sheep plasma	5	7.0	108	5	12.9	114
	100	16.2	99	1000	17.3	87
Sheep milk	10	10.9	91	10	13.3	97
	500	4.5	97	300	8.3	93

[a] $n=4$ for each RSD that was determined on 3 separate days.
[b] Accuracy is % (mean) of spiked value.

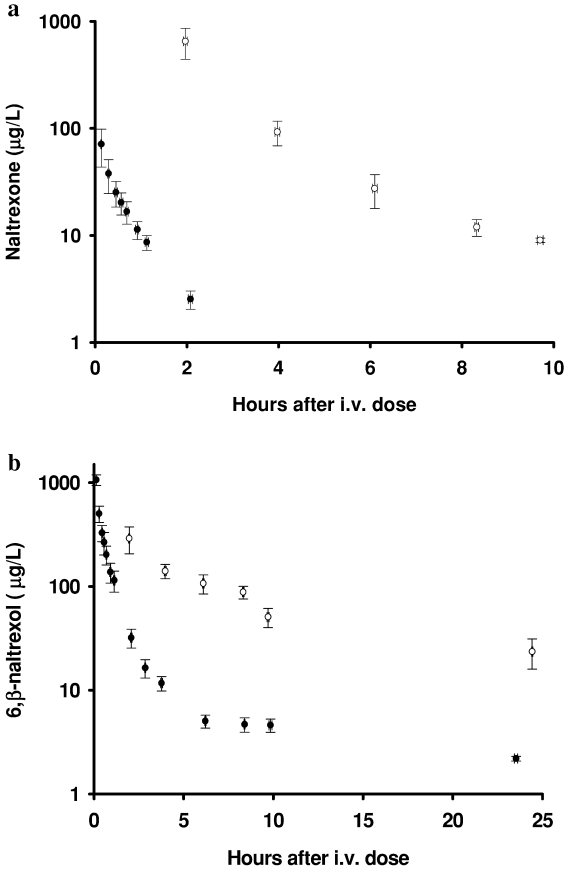

Fig. 3. Mean plasma (●) and milk (○) concentration–time profiles for naltrexone (a) and 6,β-naltrexol (b) in four lactating Merino ewes over a 24 h interval following a 4.5 mg/kg i.v. dose of naltrexone HCl. Data are presented as mean±S.E.

also larger compared to that for 6,β-naltrexol (56±5 l/kg). The distribution of these drugs between mean milk and plasma (M/P ratio) was 55±22 for naltrexone and 2.9±0.4 for 6,β-naltrexol.

4. Discussion

In humans, plasma concentrations of naltrexone and 6,β-naltrexol range from 0.2 to 75 and 18–270 μg/l, respectively, after clinically relevant oral doses of 50–200 mg [11,14,15]. Since our long-term aim was to carry out pharmacokinetic studies of naltrexone in human plasma and milk, we chose to develop a GC–MS assay that could be used to quantify both these analytes at relevant in vivo concentrations.

The extraction and concentration step in the assay proved to be relatively easy as we were able to adapt a plasma SPE procedure previously published by Huang et al. [12] for both plasma and milk matrices from humans and sheep. Recoveries for both analytes from human plasma were in the 70–80% range and similar to previous data (75–80%) [12]. The recoveries from human milk were also high (70–80%) confirming the utility of the SPE cartridge for this matrix [16–18].

Optimal conditions for the formation of the acetyl derivatives of naloxone, naltrexone and 6,β-naltrexol involved heating at 80°C for 6–14 h. Other workers have also recommended acetyl derivatives for GC–MS quantification of codeine and morphine [19]. In our study derivatisation was necessary to improve volatility as the parent hydroxylated molecules had long retention times and poor peak shapes [20], which resulted in lower sensitivity. Although the retention characteristics were changed by derivatisation, the order of elution was the same as for the parent compounds. Additionally, use of a SIM program gave increased sensitivity.

Previously published analytical methods for quantification of naltrexone and 6,β-naltrexol in plasma have utilised thin-layer chromatography [21], high-performance liquid chromatography (HPLC) [4,11,22,23], gas–liquid chromatography [24] or GC–MS [12,25–27]. However, only a few were able to detect and quantify naltrexone and 6,β-naltrexol at sensitivity of ≤2 μg/l in plasma [11,12,22,24,27]. GC–MS with negative chemical ionisation [12,25] or GC–MS–MS [27] have been shown to give sensitivities of ≥0.1 μg/l for both analytes. While our acetyl derivatives only gave sensitivities in 2–5 μg/l range, this is nevertheless suitable for human and animal pharmacokinetic studies and also applicable to a wide range of GC–MS instrumentation.

In all matrices investigated, intra-assay and inter-day precision for naltrexone and 6,β-naltrexol were within acceptable limits as was accuracy. Occasional high RSDs were encountered particularly with milk at low analyte concentrations. However, this was not a practical problem as both analytes were concentrated in milk. The ability to quantify both analytes at acceptable RSDs and LOQs in the low-μg/l range

shows that the assay is suited to human pharmacokinetic studies at usual oral dose rates, as well as to the sheep studies described above. The stability experiment showed that plasma and milk containing these compounds can be stored for at least 3 weeks at −20°C or 6 weeks at −80°C.

Since physicians are hesitant to prescribe naltrexone for lactating women, we undertook a preliminary experiment in lactating sheep to gauge the likely extent of naltrexone transfer into milk. Sheep plasma concentrations of 6,β-naltrexol were consistently higher than naltrexone at all times. These data show that naltrexone is extensively metabolised to 6,β-naltrexol in sheep, as it is in man [3–5,9,11,12,14,15,22,23,28]. The M/P (≈55) for naltrexone in the sheep was greater than that for 6,β-naltrexol (≈3), perhaps reflecting the much greater lipid solubility of the parent compound ($\log_{10} P$ of 0.54 and 0.09 for naltrexone and 6,β-naltrexol, respectively) (Chan et al., unpublished data). The applicability of the sheep data to humans is problematic. If the M/P for sheep is similar in humans, breastfeeding women may need to express and discard milk produced during the first 4 h after their daily oral dose so as to minimise infant exposure (avoids time of peak; [14]). Nevertheless, definitive studies in humans (presently being undertaken in our laboratory) are required to answer this question.

In conclusion, our method assay with SPE and GC–MS has the sensitivity, and precision necessary for the quantification of naltrexone and 6,β-naltrexol in plasma and milk from humans and sheep at relevant in vivo concentrations.

Acknowledgements

We acknowledge funding from Women and Infants Research Foundation for assistance with the assay development.

References

[1] W.R. Martin, D.R. Jasinski, Clin. Pharmacol. Ther. 14 (1973) 142.
[2] A.E. Takemori, P.S. Portoghese, Annu. Rev. Pharmacol. Toxicol. 32 (1992) 239.
[3] K. Verebey, S.J. Mule, Am. J. Drug Alcohol Abuse 2 (1975) 357.
[4] M.E. Wall, M. Perez-Reyes, D.R. Brine, C.E. Cook, Drug Metab. Dispos. 12 (1984) 677.
[5] M. Bertolotti, A. Ferrari, G. Vitale, M. Stefani, T. Trenti, P. Loria, F. Carubbi, N. Carulli, E. Sternieri, J. Hepatol. 27 (1997) 505.
[6] M.J. Kogan, K. Verebey, S.J. Mule, Res. Commun. Chem. Pathol. Pharmacol. 18 (1977) 29.
[7] H.M. Ginzburg, M.G. MacDonald, Med. Toxicol. 2 (1987) 83.
[8] R.M. Swift, New Engl. J. Med. 340 (1999) 1482.
[9] K. Verebely, M.A. Chedekel, S.J. Mule, D. Rosenthal, Res. Commun. Chem. Pathol. Pharmacol. 12 (1975) 67.
[10] N. Chatterjie, C.E. Inturrisi, J. Med. Chem. 18 (1975) 490.
[11] M.C. Meyer, A.B. Straughn, M.W. Lo, W.L. Schary, C.C. Whitney, J. Clin. Psychiatry 45 (1984) 15.
[12] W. Huang, D.E. Moody, R.L. Foltz, S.L. Walsh, J. Anal. Toxicol. 21 (1997) 252.
[13] P. Thomann, in: G. Heinzel, R. Woloszcak, P. Thomann (Eds.), Topfit Version 2, Gustav Fischer Verlag, Stuttgart, 1993, p. 1, Chapter 2.
[14] K. Verebey, J. Volavka, S.J. Mule, R.B. Resnick, Clin. Pharmacol. Ther. 20 (1976) 315.
[15] A. Ferrari, M. Bertolotti, A. Dell'Utri, U. Avico, E. Sternieri, Drug Alcohol Depend. 52 (1998) 211.
[16] R. Shimoyama, T. Ohkubo, K. Sugawara, Ann. Clin. Biochem. 37 (2000) 210.
[17] S. Dodd, A. Buist, G.D. Burrows, K.P. Maguire, T.R. Norman, J. Chromatogr. B 730 (1999) 249.
[18] S.C. Kasper, E.L. Mattiuz, S.P. Swanson, J.A. Chiu, J.T. Johnson, C.O. Garner, J. Chromatogr. B 726 (1999) 203.
[19] B.H. Chen, E.H. Taylor, A.A. Pappas, J. Anal. Toxicol. 14 (1990) 12.
[20] A.C. Moffat, Clarke's Isolation and Identification of Drugs, Pharmaceutical Press, London, 1986.
[21] K. Verebey, J. Alarazi, M. Lehrer, S.J. Mule, J. Chromatogr. 378 (1986) 261.
[22] A.F. Davidson, T.A. Emm, H.J.J. Pieniaszek, J. Pharm. Biomed. Anal. 14 (1996) 1717.
[23] W.J. Hurst, I.S. Zagon, H.Y. Aboul-Enein, Pharmazie 54 (1999) 595.
[24] K. Verebey, A. De Pace, D. Jukofsky, J.V. Volavka, S.J. Mule, J. Anal. Toxicol. 4 (1980) 33.
[25] K.M. Monti, R.L. Foltz, D.M. Chinn, J. Anal. Toxicol. 15 (1991) 136.
[26] R. Meatherall, J. Anal. Toxicol. 23 (1999) 177.
[27] C.C. Nelson, M.D. Fraser, J.K. Wilfahrt, R.L. Foltz, Ther. Drug Monit. 15 (1993) 557.
[28] P. Zuccaro, I. Altieri, P. Betto, R. Pacifici, G. Ricciarello, L.A. Pini, E. Sternieri, S. Pichini, J. Chromatogr. 567 (1991) 485.