Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 42 (2006) 367–371
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

LC–MS Determination of remifentanil in maternal and neonatal plasma

Elena Bossù [a,*], Annalisa Montinaro [a], Raffaele Lecce [a], Anna Farina [a], Ennia Suppa [b], Gaetano Draisci [b], Gianluca Gostoli [a]

[a] *Istituto Superiore di Sanità, Viale Regina Elena n. 299, Rome 00161, Italy*
[b] *Istituto di Anestesiologia e Rianimazione, Policlinico A. Gemelli, Università Cattolica Sacro Cuore (UCSC), Largo A. Gemelli 8, Rome 00168, Italy*

Received 2 February 2006; received in revised form 6 April 2006; accepted 8 April 2006
Available online 30 June 2006

Abstract

An HPLC–MS with electrospray ionization method for the determination of remifentanil in human plasma samples, pre-treated with SPE cartridge, has been developed and validated.

Ionisation was performed by positive-ion electrospray and quadrupole filter mass spectrometer operated in the single ion-recording mode. Pre-treatment was performed using Waters Oasis® SPE cartridges. Chromatographic separation was achieved in isocratic elution using a X-Terra C8 5 μm, 150 mm × 2.1 mm i.d. column. The mobile phase consisted of a mixture of water, methanol and acetonitrile (86:10:4, v/v/v) containing 0.1% of formic acid. The method showed to be linear in the range between 0.5 and 48.0 ng/ml, the estimated LOD is 0.18 ng/ml and the LOQ is 0.5 ng/ml. The method can be used to quantify remifentanil in plasma samples taken from adult and newborn patients in a range suitable for clinical studies.

Keywords: Remifentanil determination; Human plasma; Solid phase extraction; Liquid chromatography–mass spectrometry

1. Introduction

Remifentanil (R), methyl-3-[4-methoxycarbonyl-4-[(1-oxo-propyl)-phenylamino]-1-piperidine]-propanoate (Fig. 1), is an ultra short-acting opioid which binds μ receptors. It belongs to the family of phenyl-piperidine derivatives and it is the only one, among the components of this family, which is not metabolised by liver.

R undergoes extra hepatic metabolism being its N-substituted group hydrolysed by blood and tissue non-specific esterase, resulting in extremely rapid clearance [1,2]. The metabolite resulting from hydrolysis, formerly known as GR90291, is almost completely inactive and excreted in the urine [3,4].

Among available opioids, R may be ideally suited for use in obstetric analgesia, because of its rapid onset, rapid metabolism and short context-sensitive halftime irrespective of the duration of the infusion in both mother and neonate [5]. R was introduced into obstetric analgesia, during labour [6,7] or caesarean section [8–10], towards the end of the last decade but further studies are considered necessary before future routine use [11].

Therefore, in order to investigate placental transfer of R, it was decided to develop and validate a novel analytical method able to quantify R in parturient and newborn plasma samples.

Several methods, which can determine R in blood samples were published: HPLC–UV, GC–N_2 selective detector, GC–MS, HPLC–MS/MS [12–15]. The HPLC–UV method is not enough sensitive to quantify R in samples with expected concentration ranging between 1 and 40 ng/ml after infusion rate of 0.04–0.2 μg/kg/min.

GC–MS methods require time-consuming sample preparation and analytical procedure. HPLC–MS/MS methods have lower detection limits and analytical run times but the disadvantage is the high cost of instrumentation. In order to use a lower cost single quadrupole detector, more available in routine laboratory environment, a simple and sensitive RP-HPLC/MS method, applicable to the analysis of R at therapeutic concentration in human plasma was developed. After validation, the method, based on solid phase extraction (SPE), was applied to determine R in plasma obtained from parturient and newborn patient. The small blood volume of the samples collected from

* Corresponding author.
E-mail address: ebossu@iss.it (E. Bossù).

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.04.029

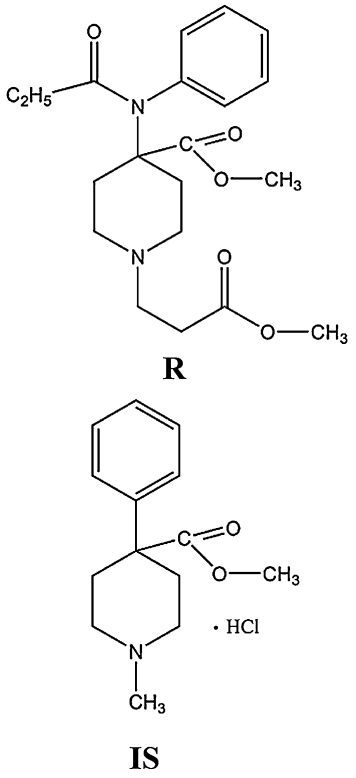

Fig. 1. Chemical structures of remifentanil (R) and pethidine (IS).

umbilical vein and artery, strongly influenced the development of the method.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile, methanol and formic acid (98–100%) were obtained from Riedel-de Haën; water was obtained from BDH (Poole, England). All reagents were of HPLC or analytical grade. Remifentanil (Ultiva® 1) manufactured by GlaxoSmithKline S.p.A. contains 1 mg/vial of R. Pethidine hydrochloride active pharmaceutical ingredient, as internal standard (IS), was supplied from Sigma (St Louis, USA).

X-Terra MS, C8, 5 μm, 150 mm × 2.1 mm i.d. HPLC column and Oasis® HLB (1 cm^3; 30 mg) solid phase extraction (SPE) cartridge were obtained from Waters.

2.2. Instrumentation

HPLC–MS analysis was performed using a Waters Alliance 2695 Separation Module coupled to a Waters Micromass ZQ single quadrupole mass spectrometer (Waters, Milford, MA, USA). WATERS MILLENNIUM^{32} software (version 4.00) was used for HPLC–MS system control and data collection.

2.3. Method

2.3.1. Analytical HPLC-system

Chromatographic separation was achieved using isocratic elution with a mobile phase consisting of water, methanol and acetonitrile (86:10:4, v/v/v) containing 0.1% of formic acid. The flow rate and injection volume were 0.2 ml/min and 20 μl, respectively. Degassing of the mobile phase was carried out continuously with an on-line degasser. The autosampler and the column temperature were set respectively at 5 °C and 32 °C.

2.3.2. MS-system

MS operated in electrospray ionization (ESI) positive ion mode under the following source conditions: source temperature 150 °C, desolvation temperature 300 °C, capillary potential 3.4 kV, sampling cone potential 30 V, extractor 3 V, nitrogen flow rate 500 l/h. LC/MS chromatograms were obtained in the single ion recording mode (SIR) at 377 *m/z* (protonated R) and at 248 *m/z* (protonated IS).

2.4. Stock solutions and spiked samples

2000 ng/ml R stock solution in 0.1% formic acid to prevent R degradation, stored at −20 °C, was used as spiking solution. This solution was prepared using remifentanil Ultiva® 1. Calibration standards were prepared by the addition of appropriate amounts of stock solution to 250 μl of blank human plasma. The final concentration of R in the calibration standards were: 0.96, 2.4, 4.8, 9.6, 24.0 and 48.0 ng/ml. IS stock solution was prepared at a concentration of 50 ng/ml in 0.1% formic acid in water and stored at +4 °C. About 50 μl of this solution were added to each calibration standard, clinical plasma sample and spiked blank plasma sample.

2.5. Clinical samples

Approval was obtained from research ethics committee of Policlinico A. Gemelli, UCSC, Rome and all patients gave written informed consent. Subjects (parturients or patients undergone to gynecological surgery) were administered Ultiva® 1 by intravenous infusion. Blood samples were collected prior and during administration of drug in heparinized tube containing 50% (w/w) citric acid solution (10 μl/ml of blood), these samples were centrifugated at +4 °C for 10 min at 2000 rpm. The obtained plasma was stored in Eppendorf vials at −70 °C until analysis. Umbilical venous (UVP) and umbilical arterial (UAP) cord blood samples, taken from a segment umbilical cord, were collected and treated as above described.

2.6. Extraction procedure

Aliquots of 250 μl of calibration standards, spiked blank plasma samples and clinical plasma samples were acidified with 5 μl of phosphoric acid (85%) to release bound drug from plasma proteins, and diluted to 500 μl with water. Then, the solutions were vortexed and centrifugated at +8 °C for 10 min (3000 rpm). The samples were loaded onto Oasis® HLB SPE cartridges, previously conditioned with 1 ml of methanol followed by 2 ml of water. The column was washed with water containing 2% acetic acid, followed by 1 ml of water containing 5% methanol

and 2% acetic acid. Finally, the analytes were eluted with 1 ml of methanol 60% containing 2% acetic acid. The eluates were evaporated to dryness under nitrogen, reconstituted in 125 μl of water containing 0.1% formic acid, filtered though a 0.2 μm filter and injected onto the HPLC system.

2.7. Validation of the method

2.7.1. Linearity, accuracy and precision assessment

Linearity was tested using six concentration levels of R: 0.96, 2.4, 4.8, 9.6, 24.0 and 48.0 ng/ml. Linear regression analysis was carried out on the standard curve generated by plotting peak areas ratio of R and Internal standard (R_{area}/IS_{area}) versus concentration of R.

Accuracy was assessed by spiking three different levels of R in blank plasma samples 3.6, 14.4 and 36 ng/ml, respectively in order to cover the range of concentrations to be tested (a ±15% from the theoretical value was set as acceptance criteria).

The intra-day precision was assessed by injecting blank plasma samples spiked with three different concentrations of R in triplicate (the same concentrations used for accuracy calculation) and the inter-day precision was assessed by spiking blank plasma samples at the same concentrations used for accuracy study in three different analytical run. During each analytical run spiked blank plasma samples, calibration standards and new eluent were prepared. Different column batch was used during the first analytical run.

2.7.2. Limit of detection and quantitation

Limit of detection (LOD) and limit of quantitation (LOQ) were estimated by extrapolation of a calibration curve by means of the lower concentration standard curve points using the following formulas: $LOD = 3.3\,\sigma/S$; $LOQ = 10\,\sigma/S$, where σ is the residual standard deviation of the regression line [16] and S is the slope of the regression line.

2.7.3. Samples stability

All stability studies were conducted by using spiked blank plasma samples at two concentration levels (3.6 and 36 ng/ml) with three determination for each point.

To assess stability of extracted samples in the autosampler ($T = +5\,^{\circ}C \pm 3\,^{\circ}C$) spiked blank plasma samples were analysed immediately and after 3, 6, 9 and 12 h of residence time.

To assess bench top stability, unextracted spiked blank plasma samples were kept at room temperature for 24 h. Samples were extracted and analysed as described above.

Long-term stability was assessed by storing spiked blank plasma samples at −70 °C for 1 month.

3. Results and discussion

The aim of the study was to set up a reproducible and sensitive method to quantify R in plasma samples in order to obtain data useful to assess its safety and efficacy, before routine use in obstetrics.

3.1. Development of the method

SPE technique was chosen in order to concentrate and purify plasma samples from interfering components considering that SPE methods are easy to perform and reproducible.

Pethidine was used as internal standard due to its similarity to R in chemical structure.

Waters Oasis® HLB 1 cm^3 SPE cartridge were used and a protocol based on that proposed by the manufacturing company was developed. No washings with basic solutions were carried out considering that R is prone to very fast degradation under basic conditions, therefore acidic and neutral solutions were used in development phase. Different percentage of methanol were tested for washing and eluting solutions in order to optimise cleaning and recovery of the samples. The chosen pre-treatment method was tested using several blank plasma samples spiked with R at a final concentration of 10 ng/ml and the recovery was always about 90%. Recovery was calculated by comparing extracted spiked samples with un-extracted sample prepared in water containing 0.1% formic acid.

Chromatographic method was developed with the aim to separate R and IS from other interfering blood components not removed in the pre-treatment step. Isocratic elution conditions using RP-HPLC column was chosen in order to minimise variability of MS response. On-line MS detection technique was chosen due to its high sensitivity and selectivity. Due to the presence of amine moiety in the IS and R chemical structures, positive electrospray MS ionization conditions were chosen.

3.2. Validation of the method

Specificity of the method was tested using blank plasma from 11 different patients, pre-treated and not spiked with R and IS. No interferences at the retention time of R or IS were found (Fig. 2).

The method showed to be linear in the tested range.

The equation of the curve was $(R_{area}/IS_{area}) = (9.04 \times 10^{-3})[R] + 4.55 \times 10^{-4}$.

The slope standard deviation, the intercept standard deviation [16] and R^2 were 3.66×10^{-5}; 1.60×10^{-3} and 0.9999, respectively.

The recovery of standard curve points using curve equation ranged from 98.4% to 112.9%.

The estimated LOD was 0.18 ng/ml and estimated LOQ was 0.50 ng/ml. Analysis of blank plasma samples spiked with 0.5 ng/ml R and 0.18 ng/ml R were carried out and the LOD and LOQ data were confirmed (recovery of 0.5 ng/ml samples was in the same range of the standard curve points).

Intra-day and inter-day precision and accuracy data are summarised in Tables 1 and 2, all the data obtained from analyses of spiked blank plasma samples did not exceed 15% of difference from nominal value.

The calculated concentration of the spiked blank plasma samples stored at different conditions for stability assessment did not exceed the 15% difference from nominal value, thus showing that the samples are stable through the whole analytical process.

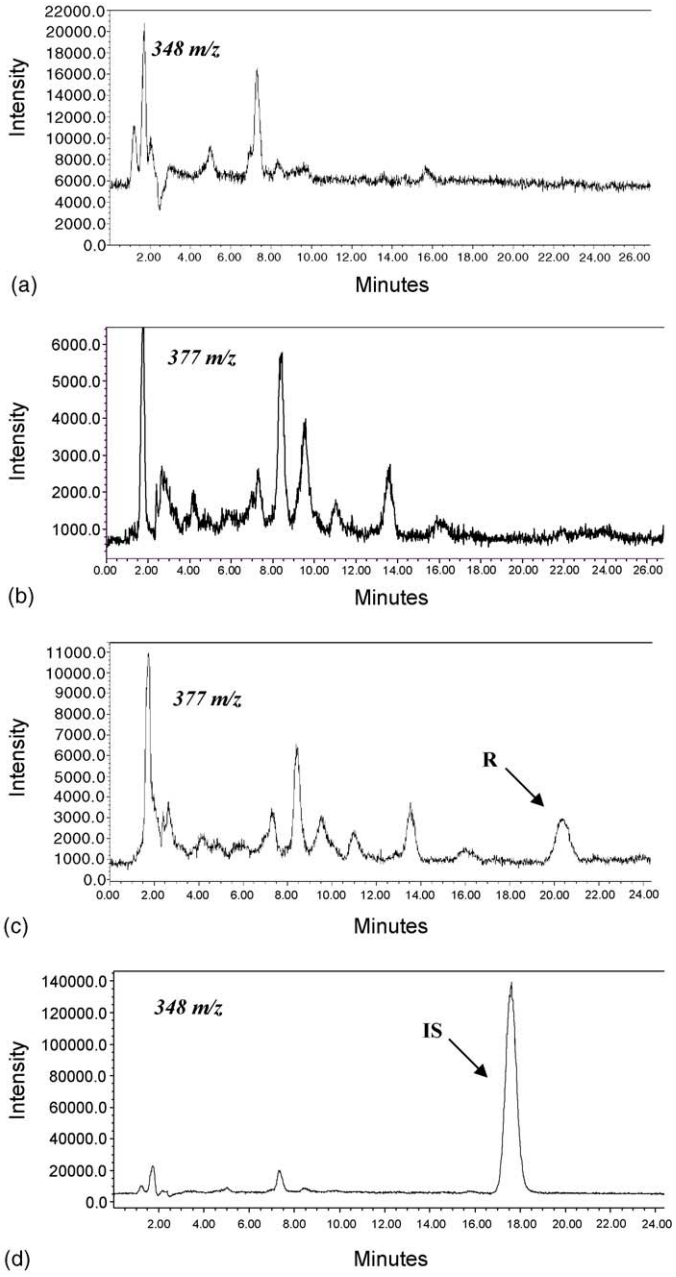

Fig. 2. SIR chromatograms of extracted plasma: (a and c) blank plasma; (b and d) plasma spiked with 1 ng ml^{-1} of R and IS at 10 ng ml^{-1}, respectively.

Table 1
Intra-day and inter-day precision

Nominal concentration (ng/ml)	Intra-day precision $n=3$ (R.S.D.%)	Inter-day precision $n=3$ (R.S.D.%)
3.6	5.1	5.3
14.4	4.1	6.9
36	3.2	3.5

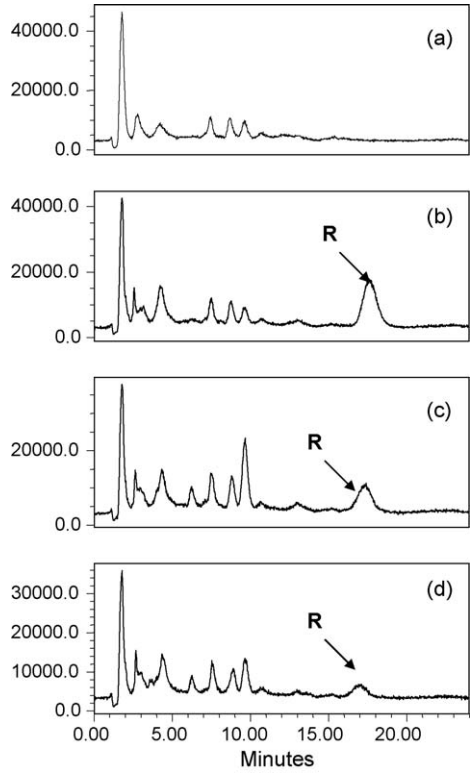

Fig. 3. SIR chromatograms at 377 *m/z* of: parturient extracted plasma collected 30 min before the infusion with Ultiva® 1 (a); parturient extracted plasma collected during the infusion with Ultiva® 1 (b); UVP extracted sample (c) and UAP extracted sample (d).

3.3. Clinical samples

To evaluate the applicability of validated method to real samples, plasma from mother and newborn patients were analysed and exemplary chromatograms are reported in Fig. 3.

Furthermore plasma from two patients (A and B), which underwent gynecological operations were analyzed. An intravenous infusion of Ultiva® 1 administered at a concentration of 50 μg/ml was administered to both patients; the infusion rate

Table 2
Accuracy

Nominal concentration (ng/ml)	Day 1		Day 2		Day 3	
	Mean found concentration ($n=3$)	Recovery%	Mean found concentration ($n=3$)	Recovery%	Mean found concentration ($n=3$)	Recovery%
3.6	3.6	98.7	3.9	109.6	3.7	102.2
14.4	13.4	92.8	14.7	102.2	14.2	98.6
36	34.3	95.4	36.1	100.3	35.2	97.9

Table 3
Clinical samples patients infusion rate

Time (min)	Infusion rate (μg/kg/min)
0–1	1
1–11	0.5
11–21	0.25

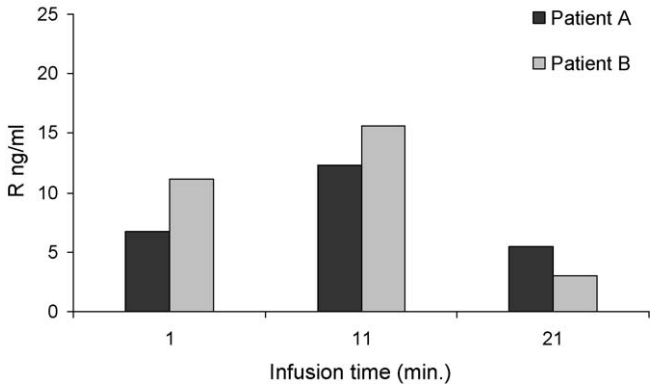

Fig. 4. Remifentanil plasma concentration in patients A and B vs. time (see Table 3 for the rate and dose infusion).

was varied during the administration (see Table 3) and different samples were collected at different time. The R plasma concentration observed in the samples from patients A and B reflect the expected similar fluctuations during time (Fig. 4).

4. Conclusions

Validation data show that LOD, LOQ, accuracy and precision of the developed LC–MS method for determination of R in plasma samples, are adequate to quantify this substance. This method is simple and do not require the use of expensive multiple quadrupole MS detectors.

Analytical data obtained from real clinical samples demonstrated that the developed LC–MS method is suitable to follow the fate of R in biological fluids collected from adult and newborn patients.

References

[1] T.D. Egan, Clin. Pharmacokinet. 29 (1995) 80–92.
[2] L.G. Michelsen, C.C. Hug, J. Clin. Anesth. (1996) 679–682.
[3] M.K. James, Exp. Opin. Invest. Drugs 3 (1994) 331–340.
[4] E.H. Cox, M.W. Langemeijer, J.M. Gubbens-Stibbe, K.T. Muir, M. Danhof, Anesthesiology 90 (1999) 535–544.
[5] R.E. Kan, S.C. Hughes, M.A. Mark, C. Charlize, P.G. Preston, E.P. Errol, Anesthesiology 88 (1998) 1467–1474.
[6] I. Volikas, D. Male, Int. J. Obstet. Anesth. 10 (2001) 86–90.
[7] S.L. Ward, C. Seller, R.G.W. Stacey, Int. J. Obstet. Anesth. 11 (2002) 68.
[8] R. Wadsworth, R. Greer, J.J.M. MacDonald, A. Vohra, Int. J. Obstet. Anesth. 11 (2002) 38–43.
[9] J. Brown, D. McAtamney, Int. J. Obstet. Anesth. 12 (2003) 63–64.
[10] W.D. Ngan Kee, K.S. Khaw, C. Kwok, A.S.Y. Wong, B.B. Lee, F.F. Ng, Anesthesiology 104 (2006) 14–20.
[11] P. Volmanen, S. Alahuhta, Int. J. Obstet. Anesth. 13 (2004) 1–4.
[12] C.M. Grosse, I.M. Davis, R.F. Arrendale, J. Jersey, J. Amin, J. Pharm. Biomed. Anal. 12 (1994) 195–203.
[13] S.H. Haidar, Z. Liang, K. Selinger, L.W. Hamlett, N.D. Eddington, J. Pharm. Biomed. Anal. 14 (1996) 1727–1732.
[14] M. Kabbaj, F. Varin, J. Chromatogr. B 783 (2003) 103–111.
[15] J. Bender, J. van den Elshout, K. Selinger, G. Broeders, J. Dankers, C. van der Heiden, J. Pharm. Biomed. Anal. 21 (1999) 559–567.
[16] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, 3rd ed., Ellis Horwood PTR Prentice Hall, Chichester, England, 1997.