Journal of Chromatography B, 879 (2011) 815–818

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Short communication

Determination of remifentanil in human plasma by liquid chromatography–tandem mass spectrometry utilizing micro extraction in packed syringe (MEPS) as sample preparation

Rana Said[a,*], Anton Pohanka[b], Maria Andersson[b], Olof Beck[a], Mohamed Abdel-Rehim[c,d]

[a] Department of Medicine, Division of Clinical Pharmacology, Karolinska University Hospital, 171 76 Stockholm, Sweden
[b] Department of Laboratory Medicine, Division of Clinical Pharmacology, Karolinska University Hospital, 141 86 Stockholm, Sweden
[c] Department of Chemistry and Biomedical Sciences, Faculty of Technology and Science, Karlstad University, 651 88 Karlstad, Sweden
[d] Department of Clinical Pharmacology & DMPK, AstraZeneca R&D, Södertälje, 151 85 Södertälje, Sweden

ARTICLE INFO

Article history:
Received 6 October 2010
Accepted 1 February 2011
Available online 2 March 2011

Keywords:
Remifentanil
MEPS
LC–MS/MS
Low sample volume

ABSTRACT

Remifentanil is a synthetic short-acting opioid with a short half-life that is being used during anaesthesia of small children. In this work an LC–MS/MS method for remifentanil quantification in 20 μL volume of human plasma was developed and validated in connection with a clinical study on neonatal children. Sample preparation was performed with micro extraction in packed syringe (MEPS), which is a miniaturization of solid phase extraction. For this method a mixed phase sorbent M1 (C8, cation exchange), and a protocol for basic compound extraction was followed. Remifentanil-$^{13}C_6$ was used as internal standard. For chromatographic separation, a C18 analytical column with gradient elution was used with mobile phase consisting of aqueous 0.1% formic acid and methanol. The total analysis time was 5.0 min and the measuring range was between 0.05 and 50 ng/mL. Precision and accuracy were with acceptance criteria of ±15%. Plasma samples were stable for 5 weeks at −20 °C and for 4 h at room temperature while 50% was lost after 24 h. This method was successfully applied for remifentanil determination in clinical samples and results agreed with a reference method. With this method using MEPS, a low limit of quantification and much reduced sample volume was obtained as compared with previous methods.

1. Introduction

Remifentanil is a short-acting synthetic opioid belonging to the family of phenyl-piperidine derivatives. It is used for obstetric analgesia as a supplement to general anaesthesia during induction and as an analgesic during maintenance of anaesthesia [1,2]. Remifentanil is not metabolised in the liver unlike other compounds from the same family [3]. Remifentanil is subjected to hydrolysis catalyzed by tissue and circulating esterases. The major metabolite is remifentanil acid, which is inactive [4,5]. The elimination half-life of remifentanil is measured in minutes, and the recovery from the treatment is also quick [6,7].

Because of these circumstances, measurement of remifentanil concentrations in the circulation is challenging. Blood has been preferred for rapid analysis of remifentanil to avoid time spent on plasma preparation. However, Kabbaj [8] has pointed out that plasma should be used since the plasma levels correlate better with the pharmacological effect.

Several methods have been published for remifentanil determination in whole blood [9–14] and plasma [1,8], using liquid chromatography with UV or mass spectrometry (MS) detection and gas chromatography with NPD or MS detection. Previous methods involve liquid–liquid extraction for sample preparation and need for large sample volumes. Sample volume must be considered especially in studies of children and experimental animals.

The aim of this study was to develop a sensitive bioanalytical method for remifentanil determination in human plasma based on small sample volume utilizing LC–MS/MS. MEPS is a new sample preparation method, which has been applied to analysis of several types of drugs and matrices [15]. The main features of this technique are the possibility to use reduced sample volumes, concentration of the sample and on-line coupling to the analytical instrument.

2. Materials and methods

2.1. Materials

Remifentanil hydrochloride and the internal standard remifentanil-$^{13}C_6$ hydrochloride (IS) were purchased from Toronto Research Chemicals (North York, Canada).

* Corresponding author. Tel.: +46 8 58587897; fax: +46 8 58581050.
E-mail address: rana_f7@yahoo.com (R. Said).

1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2011.02.002

Methanol, 25% ammonium hydroxide, acetonitrile and formic acid of HPLC grade were purchased from Merck KGaA (Darmstadt, Germany). The water was from a reagent grade Milli-Q Plus water purification system (Millipore Co., Billerica, MA).

Human citrated plasma for preparing fortified samples was obtained from the Karolinska University Hospital Blood Bank. Patient samples used for method comparison were the decoded left-over samples from the TDM Laboratory at the Karolinska University Hospital.

2.2. Instrumentation

The Accela liquid chromatography (LC) instrument was from Thermo Scientific (Thermo Scientific, Waltham, MA) and included pump and column oven. The CTC-Pal autoinjector was from CTC Analytics AG (Zwingen, Switzerland). The Kinetex C_{18} column (50 mm × 2.1 mm, 2.6 μm) was obtained from Phenomenex (Torrance, CA, USA) and was used as analytical column. The Hypersil Gold C8 (10 mm × 2.1 mm) guard column was obtained from Thermo Scientific. The loop volume was 20 μL. The CTC-Pal tray temperature was set at 8 °C. The MEPS syringe (250 μL syringe and all used sorbents) was obtained from SGE Analytical (Melbourne, Australia).

All works were conducted using a triple quadrupole mass spectrometer (TSQ Quantum) equipped with electrospray ionization source (ESI) and operated in positive ion mode. The monitoring mode was selected reaction monitoring (SRM) using $[M+1]^+$. The selected transition for remifentanil quantification was 377.2 → 285.1 and 383.3 → 291.2 for IS with collision energies of 19 and 16 eV, respectively.

The spray voltage was 3500 V; the sheath pressure was 60 and capillary temperature was 350 °C. Nitrogen (>99%) was used both as drying and nebulizing gas and argon (ICP 5.0 grade, AGA gas AB, Sundbyberg, Sweden) was used as collision gas. For data handling and quantification, Xcalibur software version (2.07 sp1) was used. For the comparison method a Waters UPLC-system coupled to a Quattro Premiere XE mass spectrometer (all Waters, Milford, MA) was used.

2.3. Mobile phase

A gradient mobile phase system was used with solvent A being aqueous 0.1% formic acid and solvent B being MeOH. The gradient started with 20% of solvent B with a hold of 0.50 min and then increased linearly to 90% solvent B at 3.0 min followed by a hold of 0.67 min. Then the mobile phase B was again set to 20% for 1.33 min before the next injection. The flow rate was 300 μL/min and the injected sample volume was 20 μL.

2.4. Standard solutions

A stock solution of 180 μg/mL remifentanil in MeOH was prepared and stored at −20 °C. Working solutions containing 3000, 300 and 20 ng/mL were prepared freshly in MeOH and used as spiking solutions. Calibration standards and quality control (QC) samples were prepared by the addition of appropriate amounts of stock solution to 5.0 mL plasma (final MeOH content <3%). After aliquoting, the samples were stored at −20 °C.

2.5. Sample preparation by MEPS

The MEPS syringe (250 μL) with M1 sorbent was used. Before using for the first time the sorbent was manually conditioned with 50 μL MeOH followed by 50 μL of pure water.

Aliquots of 20 μL of plasma samples were diluted with 160 μL of 0.1% aqueous formic acid containing IS (0.71 ng/mL). The samples were mixed and centrifuged for 10 min (1200 × *g*) and loaded on the CTC autosampler. The activated syringe was mounted and the diluted plasma sample was loaded onto the sorbent by the CTC with four replicate loadings of 50 μL. The sorbent was then washed one time with 100 μL of 5% methanol in aqueous 0.1% formic acid. The analyte was finally eluted with 50 μL of MeOH/water 90:10 (v/v) containing 3% ammonium hydroxide directly to the injector (loop volume 20 μL). Cleaning of the sorbent between injections was performed using 4× 250 μL of MeOH/water 90:10 (v/v) containing 3% ammonium hydroxide followed by 4× 250 μL of 5% methanol in aqueous 0.1% formic acid. The same sorbent (packing bed) was used for approximately 50 extractions before it was replaced.

2.6. Comparison method

A comparison method was used based on protein precipitation as sample preparation. Aliquots of 50 μL of plasma were treated with 100 μL acetonitrile containing IS (1.0 ng/mL). The samples were mixed for 10 s and centrifuged for 3 min at 1200 × *g*. The resulting supernatants were transferred to new glass tubes and were evaporated to dryness under nitrogen at 40 °C. Finally, the samples were reconstituted with 10 μL of 0.1% aqueous formic acid and transferred into autosampler vials. The entire volume was injected into the system.

The comparison method was linear over the measuring range of 0.05–50 ng/mL. Intra-assay precision was studied at 0.2 ng/mL, 15 ng/mL and 40 ng/mL. The intra-assay coefficients of variation (CV) were 10.2, 0.63 and 1.79% (n = 5). LOD, was estimated to 0.015 ng/mL.

2.7. Validation of the method

Calibration curves were generated from seven concentration levels of remifentanil; 0.05, 0.10, 1.0, 10.0, 25.0, 40.0 and 50.0 ng/mL. Calibration curves were constructed by using the analyte/IS peak area ratio against the concentration. The linear regression calculation used $1/x^2$ as weighting factor.

Accuracy and precision were studied by running three concentration levels of the QC samples with five replicates of each QC concentration during six consecutive days. Each day new calibration standards were prepared.

Lowest limit of quantification (LOQ) was defined as the lowest point in the standard curve with precision better than 20%. Limit of detection (LOD) was estimated at a signal to noise ratio of 3. This concentration was reached by dilution the lowest point of the standard curve with blank plasma.

Stability of diluted samples in the autosampler (8 °C) was studied after 4, 8 and 24 h. The stability of remifentanil in plasma samples at room temperature was studied at 4 and 24 h.

The stability studies were studied using middle and high QC samples in three replicates for each point. Long term storage stability was tested by storing the QC samples at −20 °C for 5 weeks. Stability during three cycles of freeze and thawing was also investigated.

3. Results

3.1. Method validation

The method was linear in the range of 0.05–50 ng/mL with correlation coefficient (r^2) ≥ 0.999 (n = 6). The CV of the slope and the intercept were 1.5% and 18%. Back-calculation of the standard curve points using the equation was between 96% and 105%.

Intra- and inter-assay precision and accuracy data are summarized in Table 1. The CV values for intra-assay precision were in the range of 4.4–6.3% and the values for inter-assay precision between

Table 1
Summary of method validation results.

	Measuring range (ng/mL)	Linearity ($n = 6$)	LOD (ng/mL)	LLOQ (ng/mL)
Remifentanil	0.05–50.0	$r^2 \geq 0.999$	0.02	0.05

QC samples (ng/mL)	Intra-day precision CV (%) ($n = 6$)	Inter-day precision CV (%) ($n = 30$)	Accuracy (%) ($n = 30$)
QCL 0.20	6.3	11.3	102
QCM 15.0	4.4	6.3	97.8
QCH 38.0	5.2	7.6	99.0

6.3% and 11.3%. The deviations of the calculated concentrations from their nominal values were within ±15%, between 97.8% and 102.0%.

The lower limit of quantification was estimated to be 0.05 ng/mL based on a signal to noise ratio of 10. This was experimentally verified by analyzing five samples at a concentration of 0.05 ng/mL, with the intra-assay CV calculated to be 13.5%. The limit of detection was found to be 0.015 ng/mL with a signal to noise ratio of 3.

The relation between number of sample loading and recovery was tested by investigating the number of loadings. The maximum recovery was obtained with four sample loadings (Fig. 1). A post-column infusion experiments was carried out in order to investigate the matrix effects on the ionisation. This was done by infusing a 500 ng/mL solution of remifentanil in aqueous 0.1% formic acid and methanol 30:70% at a rate of 7 μL/min and injecting a prepared blank plasma sample while monitoring the remifentanil product ion. A slight suppression at the retention time of the column void was noticed, which was recovered before 1.5 min (Fig. 2).

Influence from matrix effects was also investigated by comparing the mean peak area of three blank plasma samples spiked post extraction with a concentration of 20 ng/mL to the mean peak area of samples spiked in mobile phase A (0.1% formic acid) at corresponding concentration, $n = 3$. The results were between 99% and 103% with a mean of 101%.

Diluted spiked plasma samples ready for MEPS extraction and analysis were placed on the autosampler for different times (4, 8, and 24 h) before analysis. The calculated average concentration was within ±15% of the nominal concentration at all time points. Fortified plasma samples were stable in room temperature for 4 h, but after 24 h there was a 50% decline in concentration. After long term storage at −20 °C for 5 weeks the average concentration was within ±15% of the nominal concentration.

Carry-over was estimated to be ≤0.03% by running a blank sample after the highest calibration standard (50 ng/mL).

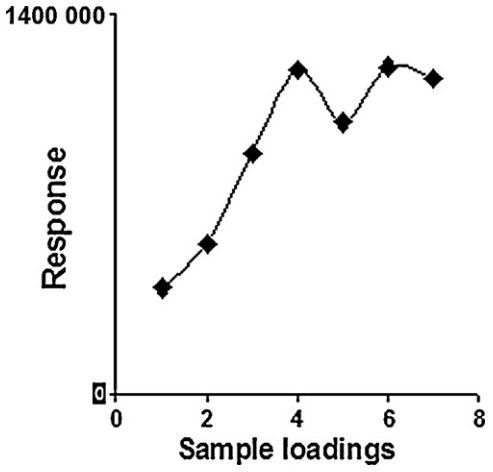

Fig. 1. The remifentanil mass spectrometry response (peak area) as a function of the number of MEPS loading cycles.

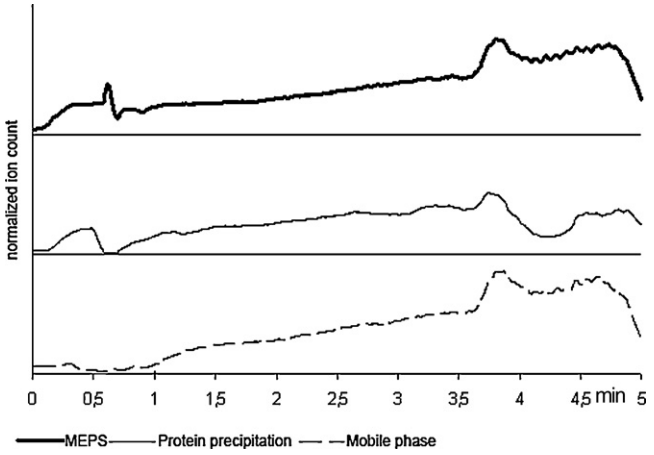

Fig. 2. Results from the study of matrix effects by infusing remifentanil post column and injecting blank plasma extracts and mobile phase.

Interference from other substances was studied by analyzing 20 randomly selected patient samples from our TDM Laboratory sent in for analyses of antiepileptic drugs. In addition, 4 different batches of pooled blank plasma were analyzed. No interfering peaks were observed.

The comparison method was used for analysis of five authentic patient samples. The resulting comparison is shown in Table 2 and a representative chromatogram for patient sample is shown in Fig. 3.

4. Discussion

A sensitive and automated method for quantification of remifentanil in human plasma using MEPS and LC–MS/MS was successfully developed and validated according to FDA guidelines [16]. An LLOQ of 0.05 ng/mL was achieved using only 20 μL of plasma. A small sample volume is of great value in studies on children and experimental animals, and this is was a goal for the method development. The method had a total analysis time of 5 min allowing for high capacity. The only preparation of plasma before loading on the autosampler was dilution with internal standard solution and centrifugation. The diluted plasma was stable for at least 24 h pending instrumental analysis.

Remifentanil contains a methyl ester group, which make it susceptible to enzymatic hydrolysis and to chemical hydrolysis at physiological pH. To overcome this problem, addition of acid to

Table 2
Method comparison using authentic plasma samples.

Patient sample	Concentration (ng/mL) MEPS method	Concentration (ng/mL) Comparison method
1	0.11	0.17
2	0.26	0.25
3	1.65	1.45
4	2.0	1.82
5	11.5	11.9

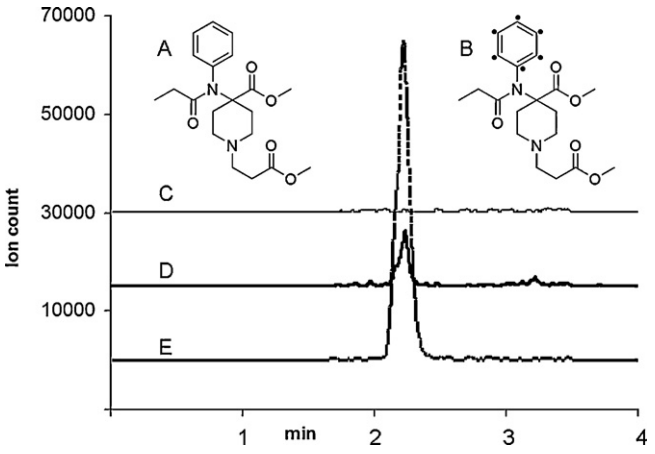

Fig. 3. (A) Remifentanil structure, (B) remifentanil-$^{13}C_6$ (IS) structure with ^{13}C positions marked with dots, (C) ion chromatogram obtained from the analysis of blank, (D) ion chromatogram obtained from the analysis of patient sample determined to contain 0.17 ng/mL using MEPS and (E) ion chromatogram for internal standard from patient sample shown in (D).

the samples is needed since it will prevent both types of hydrolysis. This is will make handling and storage the blood samples much safer [12].

Previously LC-UV and GC–MS have been used for remifentanil determination in dog plasma [8] and rat blood [8,9,12,17]. GC–MS provided good sensitivity with an LLOQ of 0.1 ng/mL. However, these methods require long time for sample preparation and instrumental analysis. The use of LC with MS detection for remifentanil analysis in plasma [1], blood [4] and urine [19] made it possible to decrease the instrumental analysis time but the time for sample preparation was still long.

The extraction of remifentanil and similar compounds from biological fluids has mainly been done using liquid–liquid extraction [4,9,17]. Solid phase extraction has also been used either as a main sample preparation method or after protein precipitation method for remifentanil extraction, but sample volumes were between 250 and 500 μL [1,8,18].

For MEPS optimization different sorbents have been tested. The highest recovery was obtained using mixed mode sorbent (C8 and cation exchange).

In conclusion, the combination of LC–MS/MS with MEPS enabled the development of a method for remifentanil quantification in plasma with good sensitivity, accuracy and precision. The use of MEPS allowed only 20 μL of plasma to be needed; moreover the sensitivity can be enhanced by reducing the dilution. This approach of sample preparation is promising and can solve many analytical problems needing automation and small sample volumes.

References

[1] E. Bossu, A. Montinaro, R. Lecce, A. Farina, E. Suppa, G. Draisci, G. Gostoli, J. Pharm. Biomed. Anal. 42 (2006) 367.
[2] D.J. Duthie, Br. J. Anaesth. 81 (1998) 51.
[3] M. Pitsiu, A. Wilmer, A. Bodenham, D. Breen, V. Bach, J. Bonde, P. Kessler, S. Albrecht, G. Fisher, A. Kirkham, Br. J. Anaesth. 92 (2004) 493.
[4] C. Zhang, L. Zhang, X. Zhang, L. Yang, S. Zhai, L. Duan, Int. J. Clin. Pharmacol. Ther. 46 (2008) 477.
[5] T.D. Egan, Clin. Pharmacokinet. 29 (1995) 80.
[6] L.P. Zhang, L. Yang, S.S. Bi, W. Lu, X.H. Zhang, S.D. Zhai, L.P. Duan, Chin. Med. J. (Engl.) 122 (2009) 1032.
[7] L.G. Michelsen, C.C. Hug Jr., J. Clin. Anesth. 8 (1996) 679.
[8] M. Kabbaj, F. Varin, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 783 (2003) 103.
[9] S.H. Haidar, Z. Liang, K. Selinger, L. Hamlett, N.D. Eddington, J. Pharm. Biomed. Anal. 14 (1996) 1727.
[10] C.M. Grosse, I.M. Davis, R.F. Arrendale, J. Jersey, J. Amin, J. Pharm. Biomed. Anal. 12 (1994) 195.
[11] J. Bender, J. van den Elshout, K. Selinger, G. Broeders, J. Dankers, C. van der Heiden, J. Pharm. Biomed. Anal. 21 (1999) 559.
[12] A.R. Bjorksten, C. Chan, D.P. Crankshaw, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 775 (2002) 97.
[13] C.L. Westmoreland, J.F. Hoke, P.S. Sebel, C.C. Hug Jr., K.T. Muir, Anesthesiology 79 (1993) 893.
[14] A.K. Ross, P.J. Davis, G. Del. Dear, B. Ginsberg, F.X. McGowan, R.D. Stiller, L.G. Henson, C. Huffman, K.T. Muir, Anesth. Analg. 93 (2001) 1393.
[15] M. Abdel-Rehim, J. Chromatogr. A 1217 (2010) 2569.
[16] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman, S. Spector, J. Pharm. Sci. 81 (1992) 309.
[17] S.H. Haidar, J.E. Moreton, Z. Liang, J.F. Hoke, K.T. Muir, N.D. Eddington, J. Pharm. Sci. 86 (1997) 1278.
[18] A.F. Lehner, P. Almeida, J. Jacobs, J.D. Harkins, W. Karpiesiuk, W.E. Woods, L. Dirikolu, J.M. Bosken, W.G. Carter, J. Boyles, C. Holtz, T. Heller, C. Nattrass, M. Fisher, T. Tobin, J. Anal. Toxicol. 24 (2000) 309.
[19] Y.J. Xue, J.B. Akinsanya, J. Liu, S.E. Unger, Rapid Commun. Mass Spectrom. 20 (2006) 2660.