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Specific and sensitive analysis of nefopam and its main metabolite desmethyl-nefopam in human plasma by liquid chromatography–ion trap tandem mass spectrometry

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Abstract

A specific and sensitive liquid chromatography–tandem mass spectrometric (LC–MS–MS) method using an ion trap spectrometer was developed for quantitation of nefopam and desmethyl-nefopam in human plasma. Nefopam, desmethyl-nefopam and the internal standard (ethyl loflazepate) were extracted in a single step with diethyl ether from 1 mL of alkalinized plasma. The mobile phase consisted of acetonitrile with 0.1% formic acid (50:50, v:v). It was delivered at a flow-rate of 0.3 mL/min. The effluent was monitored by MS–MS in positive-ion mode. Ionisation was performed using an electrospray ion source operating at 200 °C. Nefopam and desmethyl-nefopam were identified and quantified in full scan MS–MS mode using a homemade MS–MS library. Calibration curves were linear over the concentration range of 0.78–100 ng/mL with determination coefficients >0.996. This method was fast (total run time < 6 min), accurate (bias < 12.5%), and reproducible (intra- and inter-assay precision < 17.5%) with a quantitation limit of 0.78 ng/mL. The high specificity and sensitivity achieved by this method allowed the determination of nefopam and desmethyl-nefopam plasma levels in patients following either intermittent or continuous intravenous administration of nefopam.

Keywords: Nefopam; Desmethyl-nefopam; Liquid chromatography–mass spectrometry; Analgesia

1. Introduction

Although its mechanism of action is not been fully explained, nefopam [(±)-3,4,5,6-tetrahydro-5methyl-1-phenyl-1*H*-2,5-benzoxazocine, MW = 235.34] is a non-opioid central analgesic widely used in France as postoperative pain killer [1]. Nefopam hydrochloride can be administered either intravenously or orally as a racemic mixture. Its main metabolite is desmethyl-nefopam which contribute in part to its analgesic effects, especially after oral administration associated to important first hepatic pass effect [2,3]. Slow infusion over a period of at least 30 min may improve its tolerance since common side effects such as nausea, tachycardia or sedation, are related to the speed of its administration. However, although the analgesic effect of its intermittent infusion has been well evaluated [4,5], its efficacy when administered as a continuous infusion has, to our knowledge, only been studied in patients undergoing urological surgery [6]. In such indication, in contrast to an intermittent administration, authors showed that continuous intravenous infusion of nefopam did not reduce morphine consumption as rescue. To explain such discrepancy between mode of administration of nefopam, differences in pharmacokinetics and type of surgery were evoked. So, before to draw firm conclusions about the mode of its administration, analgesic effects of nefopam must be studied in a prospective randomized manner in different models of postoperative pain as thyroid, pelvic or gynecological surgery. For studying the pharmacokinetic part of such study, a specific and sensitive assay is required to monitor blood levels of nefopam and of its main metabolite.

Over the last 25 years, several assay methods have been proposed for the determination of nefopam in biological fluid. Most of them involve gas chromatography (GC) coupled to flame

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ionisation [7,8] or nitrogen phosphorus detection [9]. These methods had a limit of quantitation (LOQ) ranging from 5 to 10 ng/mL using 1 mL [8] or 5 mL [7] plasma samples. Another method based on liquid chromatography (LC) with electrochemical detection proved to be highly sensitive (1 ng/mL using 2 mL volume of plasma sample) and was suitable for pharmacokinetic studies. It was somehow hampered by a complex sample pretreatment, including a combination of liquid- and solid-phase extraction [10]. Similarly, Liu et al. proposed a LC method with ultraviolet (UV) detection including a three steps liquid–liquid extraction procedure [11]. Moreover, most of these GC or LC methods did not allow for the quantitation of any metabolite of nefopam. Recently, Aymard et al. [12] published an elegant LC procedure with UV detection for the simultaneous quantitation of nefopam and desmethyl-nefopam in plasma, urine and red blood cells. This method is simple to implement and sensitive enough for pharmacokinetic studies in healthy subjects (LOQ: 1 ng/mL with 1 mL of plasma) [2]. However, it lacks in specificity due to the use of UV detection at a single wavelength, and suffers from a relatively long chromatographic run time (i.e. approximately 20 min per sample) which is poorly compatible with pharmacokinetic studies. Lastly, Chawla et al. [3] developed an interesting sensitive chiral assay using LC with mass spectrometric (MS) detection [3]. This method, which has been used to characterize the pharmacokinetic behavior of enantiomers of nefopam and desmethyl-nefopam after single intravenous or oral administration of the racemate in healthy volunteers [3], also lacks specificity since only one ion per compound for the selected-ion mode detection was used.

In this work, we propose an alternative assay method for the determination of nefopam and of its main metabolite, desmethyl-nefopam levels in human plasma. A particular attention has been paid to optimize the method for both specificity and speed of the analytical run. To this end, a fast, highly specific and sensitive LC technique coupled to ion trap MS operated in full MS–MS scan mode to identify and quantify nefopam and its main metabolite in human plasma. After validation, this method was used in a clinical study monitoring their plasma concentrations after continuous or intermittent intravenous administration of nefopam in patients following surgery.

2. Experimental

2.1. Reagents

Nefopam hydrochloride and desmethyl-nefopam hydrochloride were kindly supplied by Biocodex (Gentilly, France) (chemical structures in Fig. 1). Ethyl loflazepate (internal standard) was purchased from SIGMA (Saint-Quentin Fallavier, France) (chemical structure in Fig. 1). Organic solvents and reagents were all of LC grade. Acetonitrile and diethyl ether were supplied by SDS (Peypin, France). Methanol and formic acid were obtained from Merck (Darmstadt, Germany). Purified water was prepared on a Milli-Q Waters purification system (Millipore, Saint-Quentin en Yvelines, France).

2.2. Biosamples

Blank human plasma samples were supplied from our local blood bank. Authentic blood samples were collected from patients included in a clinical study approved by the local Ethic Committee, University Hospital of Reims, France.

2.3. Standard solutions and calibration curves

Stock standard solutions of nefopam, desmethyl-nefopam and its internal standard (IS) were prepared in methanol at a concentration of 1 mg/mL, and stored at +4 °C. These were further diluted in methanol to give appropriate working solutions used to prepare the calibration solutions. Standard curves were prepared in human plasma (1 mL) to yield final concentrations of 0.781, 1.563, 3.125, 6.25, 12.5, 25, 50 and 100 ng/mL.

2.4. Sample preparation

Plasma sample (1 mL) was extracted with 5.0 mL of diethyl ether after addition of 10 μL IS solution (1 μg/mL ethyl loflazepate in methanol) and 200 μL of carbonate buffer (Na_2CO_3 20%, pH 9.0). The mixture was vortex mixed for 1 min, and then centrifuged at 3000 × *g* for 5 min. The organic layer was transferred into conical glass tubes and evaporated to dryness under a nitrogen stream at 40 °C. The residue was finally dissolved in 150 μL of 0.1% formic acid:acetonitrile (50:50, v:v), and 10 μL were injected into the LC column.

2.5. Liquid chromatography–mass spectrometry

2.5.1. Equipment and chromatographic conditions

The LC–MS–MS system consisted of a Thermofinnigan Surveyor® LC system (Les Ulis, France) equipped with an autosampler. Compounds were screened for, identified, and quantified in plasma using a Thermofinnigan LCQ Advantage® trap ion mass spectrometer, and the Thermofinnigan Xcalibur® data system. Chromatographic separations were carried out by using a 5 μm particle size Hypurity C18 column (150 mm × 2.1 mm i.d., ThermoHypersil-Keystone, Les Ulis, France) whose temperature was maintained at 30 °C. Samples were eluted with a mobile phase consisting of acetonitrile: 0.1% formic acid in purified water (50:50, v:v) delivered at a flow-rate of 0.3 mL/min. The entire flow was directed into the source without splitting. During use, the mobile phase was degassed by an integrated Surveyor® series degasser. In order to optimize the MS–MS parameters and to create a spectra library, infusion experiments were carried out with a 500 μL syringe connected to a pump with a flow-rate of 5 μL/min.

2.5.2. Mass spectrometry conditions

The ionisation technique used was electrospray ionisation (ESI) in the positive-ion mode for both compounds. The spray needle was set at a potential of 4 kV. The heated capillary was set at 200 °C, and the stainless-steel capillary held at a potential of 10 V. Nitrogen was used as drying and nebulising gas. The sheath gas flow-rate of nitrogen was set at 40 (arbitrary units).

The tube lens offset was set at 40 V and the electron multiplier voltage set at 400 V peak-to-peak. Ultra-pure helium (99.995%) was used in the trap as damping and collision gas. The instrument was set to acquire three microscans, and ion injection time into the trap was optimized by using the integrated automatic gain control software.

2.5.3. MS conditions for identification and quantitation

The detection of nefopam, its metabolite, and ethyl loflazepate was performed by LC–MS–MS in full MS–MS scan mode (*m/z* 100–400). Three alternating scan events, generating fragment ions of the molecular ion through CID, were carried out at *m/z* 254, 240 and 361 corresponding to the protonated molecular ions $[M+H]^+$ of nefopam, desmethyl-nefopam and ethyl loflazepate (IS), respectively. Full scan MS–MS spectra were produced by collision induced dissociation (CID) of each molecular ion using a normalized collision energy of 50%.

The reference MS–MS spectra of compounds of interest were previously collected individually using direct injection *via* the integrated syringe pump. Those spectra were obtained by using a normalized collision energy of 50%, and were included in a custom full MS–MS library. Positive peaks were identified by

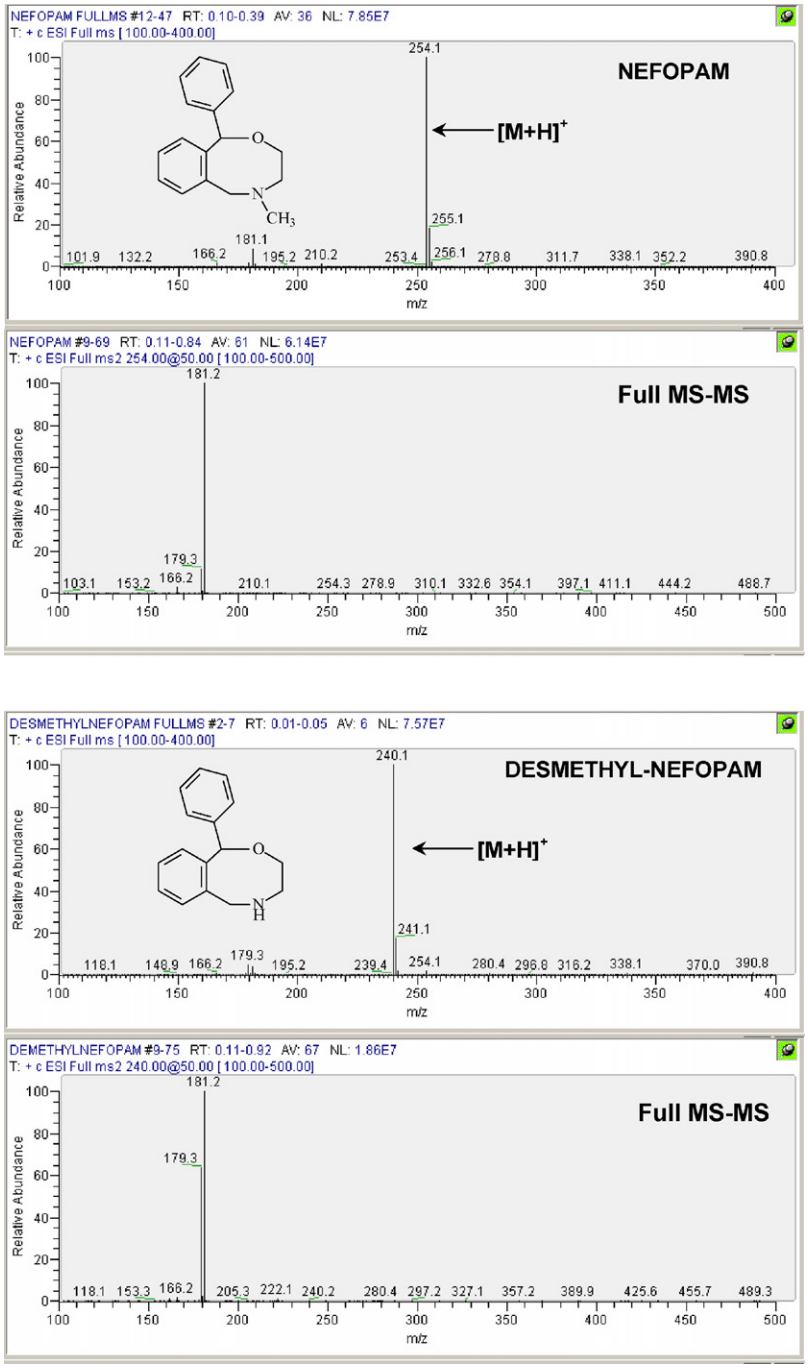

Fig. 1. ESI-MS (top) and ESI-MS–MS (bottom) spectra of nefopam, desmethyl-nefopam and ethyl loflazepate (IS). *x*-Axis: *m/z* values; *y*-axis: relative abundance.

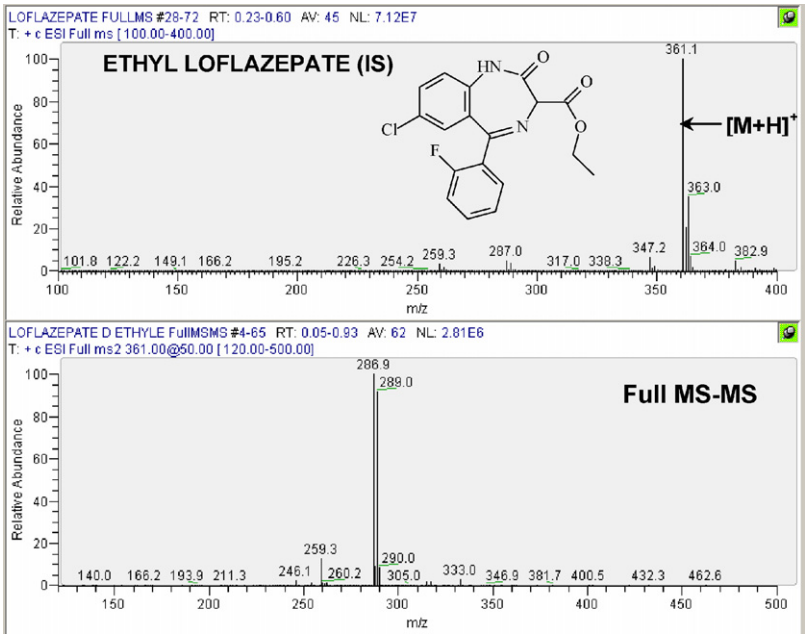

Fig. 1. (*Continued*).

searching and comparing the underlying ESI mass spectra with the reference spectra of our MS–MS library.

Quantitation was also performed in the full scan MS–MS mode. Once full mass spectra of the product ions were generated, post-acquisition data processing was designed to select particular ions for quantitation (usually, fragment ions with the greater intensity). Peak area ratios of the target ions of each compound *versus* that of the IS were compared with calibration curves prepared under the same conditions. If drug concentrations in authentic samples exceeded the calibration range, samples were reanalyzed after appropriate dilution with drug-free plasma.

2.5.4. Calculations

The calibration curves were calculated by weighted least-squares linear regression analysis (weight was 1/concentration) of the concentrations of the analyte *versus* the peak areas ratio of the target ion for quantitation of nefopam (*m/z* 181—parent ion: 254) and desmethyl-nefopam (*m/z* 181—parent ion) to that of the IS (*m/z* 287). Concentrations of unknown samples were determined by applying the linear regression equation of the standard curve to the unknown sample's peak area ratio.

2.6. Method validation

2.6.1. Quality control

Quality controls were prepared from a pool of blank human plasma spiked with three different amounts of bupivacaine corresponding to the low, medium, and high concentrations given in Table 1. Plasma aliquots were stored at −20 °C until assayed and were renewed every 3 months.

2.6.2. Precision and accuracy

Precision and accuracy of the assay were assessed by replicate analysis of quality control samples of nefopam and desmethyl-nefopam. Fifteen and 12 separate samples were assayed for intra- and inter-day evaluations, respectively. Precision expressed as relative standard deviation (% R.S.D.) was expected to be <15% except at the limit of quantitation (LOQ) where 20% was acceptable. Accuracy (bias) was determined as [100 × (mean measured

Table 1
Intra-day ($n = 15$) and inter-day ($n = 12$) precision and accuracy of the LC–MS–MS assay for nefopam and desmethyl-nefopam

Analyte	Spiked low, medium and high (ng/mL)	Mean measured (ng/mL)		R.S.D.[a] (%)		Bias[b] (%)	
		Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
Nefopam	0.781	0.81	0.88	16.3	16.6	4.1	12.2
	6.25	6.34	6.13	10.8	11.8	1.4	−1.9
	50	46.6	48.7	8.9	8.0	−6.8	−2.5
Desmethyl-nefopam	0.781	0.72	0.71	15.8	17.5	−8.3	−8.6
	6.25	5.92	6.15	12.2	9.0	−5.3	−1.6
	50	51.2	48.3	10.2	7.1	2.4	−3.3

[a] Relative standard deviation.
[b] Expressed as [100 × (mean measured concentration/spiked concentration) − 100].

concentration/spiked concentration) − 100]. Bias of ±15% was considered satisfactory, except at the LOQ where ±20% was acceptable.

2.6.3. Limits of detection and quantitation

The limit of detection was determined from the detector response after direct injection of decreasing amounts of nefopam and desmethyl-nefopam. A signal-to-noise ratio of 10 was taken as the limit of detection. A signal-to-noise of at least 20 was taken as the limit of quantitation (LOQ). Criteria for the LOQ were fulfilled by the lowest point of the calibration curve.

2.6.4. Carry-over

The lack of carry-over effect was assessed by alternately analyzing blank plasma samples ($n = 3$) and plasma samples containing concentrations at the upper limit of quantitation of each compounds ($n = 3$). The residual concentration found in the first blank plasma sample following a high concentration sample was used to calculate the rate of carry-over. It was considered minimal if below 0.5% of the LOQ.

2.6.5. Extraction recoveries

Extraction recoveries from human plasma were evaluated at two concentrations levels: 3.125 and 50 ng/mL ($n = 5$). The samples were extracted without IS according to the procedure described above. Ten microliters of IS solution (1 μg/mL ethyl loflazepate in mobile phase) were added to the organic phase, and evaporated to dryness. The residue was dissolved in 150 μL of mobile phase prior to analysis. As controls ($n = 5$), nefopam and desmethyl-nefopam solutions in mobile phase at the two concentrations levels to which were added 10 μL of IS solution were gently evaporated. The residues were then dissolved in 150 μL of mobile phase and analyzed. Recoveries were calculated by comparing peak areas of controls to those of spiked plasma samples.

2.6.6. Specificity and ion-suppression test

The specificity of the method was evaluated by analyzing 10 different plasma samples obtained from healthy volunteers who did not receive nefopam. The ion-suppression effect of the method was also assessed with these plasma samples. After extraction, they were injected in the LC–MS–MS system while continuous post-column infusion of concentrations of 750 ng/mL of nefopam, desmethyl-nefopam and IS (flow-rate of 5 μL/min) was in effect, as described by Müller et al. [13]. Since nefopam and desmethyl-nefopam elute at the same time, additional ion-suppression experiments were carried out to check for a potential mutual ion-suppression effect of these two compounds. Briefly, extracted samples containing a low concentration of either nefopam or desmethyl-nefopam were injected in the LC–MS–MS system with and without an additional HIGH concentration of the corresponding compound ($n = 3$).

2.6.7. Patients' samples

This technique has been applied to measure nefopam and desmethyl-nefopam concentrations in plasma samples collected from two patients enrolled in a clinical study, who received continuous or intermittent intravenous administration of nefopam for pain control following thyroid surgery. Briefly, patients were randomized to receive either 100 mg/day nefopam by constant rate infusion following a 20 mg loading dose over 30 min or 20 mg nefopam over 30 min every 4 h during a day by intravenous bolus injection. For patients randomized to intermittent nefopam, blood samples were collected before dosing and at 5, 10, 15, 30, 60, 120, 240, 360, 480, 510, 570, 720, 750, 840, 1200, 1230 and 1440 min (end of administration), and then at 1470, 1500, 1620, 1800, 1980 and 2880 min after the start of infusion. For patients randomized to continuous nefopam, blood samples were collected before dosing and at 5, 10, 15, 30, 60, 120, 240, 270, 330, 480, 510, 570, 1200 and 1440 min (end of infusion), and then at 1470, 1500, 1560, 1680, 1980 and 2880 min after the start of infusion. After centrifugation, plasma samples were frozen and stored at −20 °C until analysis. Plasma concentrations of nefopam were shown both as observed concentrations and predicted concentrations. The latter were determined by fitting intravenous plasma concentration data to a bi-exponential equation using the WinNonLin software version 4.1 (Pharsight, Palo Alto, CA).

3. Results and discussion

3.1. LC–MS–MS analysis

ESI source was preferred over the atmospheric pressure chemical ionisation source since the latter resulted in bad ionisation of the compounds of interest under our experimental conditions. Similarly, positive-ion mode was chosen in order to obtain the most intense signal of the molecular cation ($[M + H]^+$). Fig. 1 presents the MS–MS spectra data of nefopam, desmethyl-nefopam and IS using a normalized collision energy of 50%. All full MS–MS spectra showed characteristic patterns allowing unambiguous and rapid identification of the compounds *via* our full MS–MS reference library (including approximately 2000 compounds to date). For quantitation purposes, ethyl loflazepate was chosen as an IS because this compound is rarely used in France, is extensively metabolised after oral administration, and was not administered in patients included in the ongoing clinical study. Quantitation of the nefopam and its metabolite were performed in the full scan MS–MS mode. Post-acquisition data processing of full scan MS–MS data permitted the "extraction" of analytes of interest by selecting specific product ions. This mode is known to be the most sensitive MS setup of an ion trap detector, thus permitting the quantitative analysis of analytes in complex matrices with a good sensitivity.

Fig. 2 shows reconstructed ion chromatograms (RIC) of a blank plasma spiked with IS and medium concentrations of nefopam and desmethyl-nefopam. Under our analytical conditions, nefopam is not chromatographically separated from its metabolite, with retention times of 1.75 and 1.69 min for nefopam and desmethyl-nefopam, respectively. However, due to the high selectivity of tandem MS, complete chromatographic separation is theoretically not necessary any more, on condition

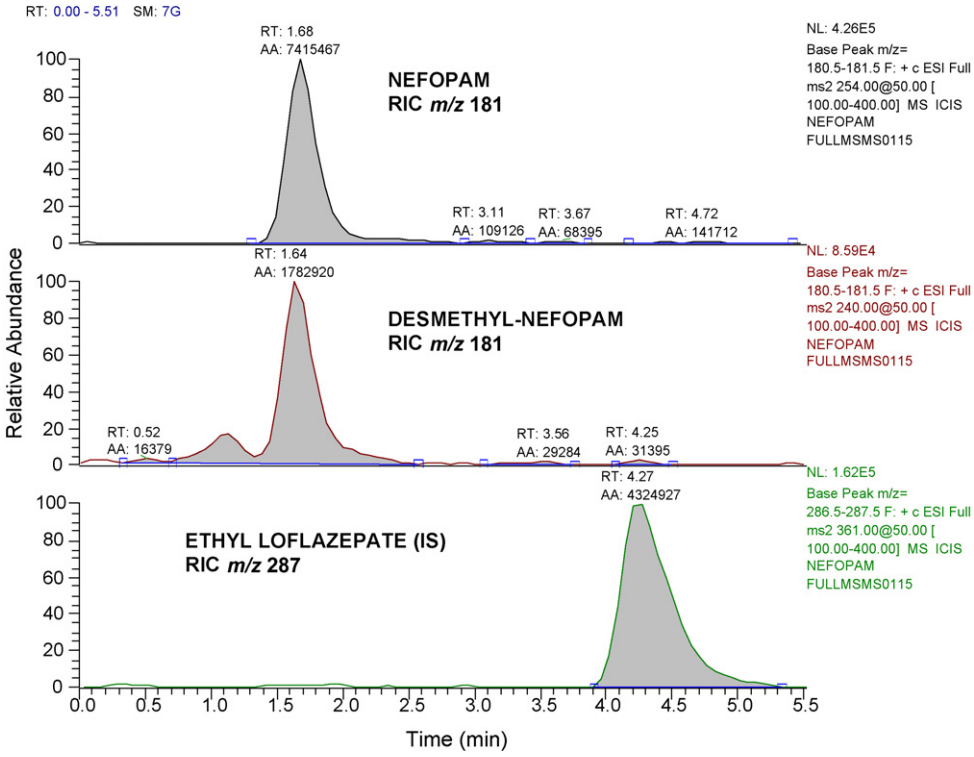

Fig. 2. Reconstructed LC–ESI-MS–MS smoothed ion chromatograms (RIC) of a blank plasma spiked with IS (ethyl loflazepate), nefopam and desmethyl-nefopam at a concentration of 6.25 ng/mL.

to check for the absence of mutual ion-suppression effect (see specific paragraph below). By using an isocratic elution of compounds, total run time was shorter than 6 min per sample. For application to pharmacokinetic studies, the single-step extraction procedure combined to a short chromatographic run time could be considered as an important benefit.

3.2. Validation data

3.2.1. Precision and accuracy

Table 1 summarizes mean values, precision and accuracy of intra- and inter-assay analyses. Precision and accuracy were within ranges acceptable for bio-analytical purposes. Intra-day precision ranged from 8.9 to 16.3%, and accuracy (bias) was less than 10%. Inter-day precision did not exceed 18% over the three quality control samples investigated. The accuracy of the technique was considered satisfactory since between-day bias over the concentration range studied was found to be in the range −3.3 to 12.2%.

3.2.2. Linearity, limits and carry-over

Calibration curves for nefopam and its metabolite in human plasma exhibited good linearity over the concentration range studied (i.e. 0.78–100 ng/mL). Using weighted linear regression analysis, they were best described by the following equations: $Y = 0.0889X + 0.00207$ ($r^2 = 0.998$) and $Y = 0.0195X + 0.0416$ ($r^2 = 0.999$), for nefopam and desmethyl-nefopam, respectively, where Y is the peak area ratio of compound of interest to IS and X is the plasma concentration. Values of the coefficients of determination were all >0.996.

LODs are reported in Table 2. The LOQs for both molecules in plasma were chosen as the lowest calibration standard concentration (0.78 ng/mL) for which the CV and bias did not exceed 20 and ±20%, respectively. Those LOQs are slightly lesser than those described by Aymard et al. (1 ng/mL from 1 mL of plasma sample) [12]. As shown below, this concentration is sufficient for the purpose of pharmacokinetic studies of nefopam in patients following continuous or intermittent intravenous administration of nefopam.

Table 2
LOD, LOQ, linearity and extraction recoveries of nefopam and desmethyl-nefopam in human plasma

Analyte	LOD (ng/mL)	LOQ (ng/mL)	Linearity (ng/mL)	Coefficients of determination (r^2)	Extraction recovery	
					Concentration (ng/mL)	(%)
Nefopam	0.195	0.781	0.781–100	0.998	3.125	65.1
					50	68.2
Desmethyl-nefopam	0.195	0.781	0.781–100	0.998	3.125	67.3
					50	72.1

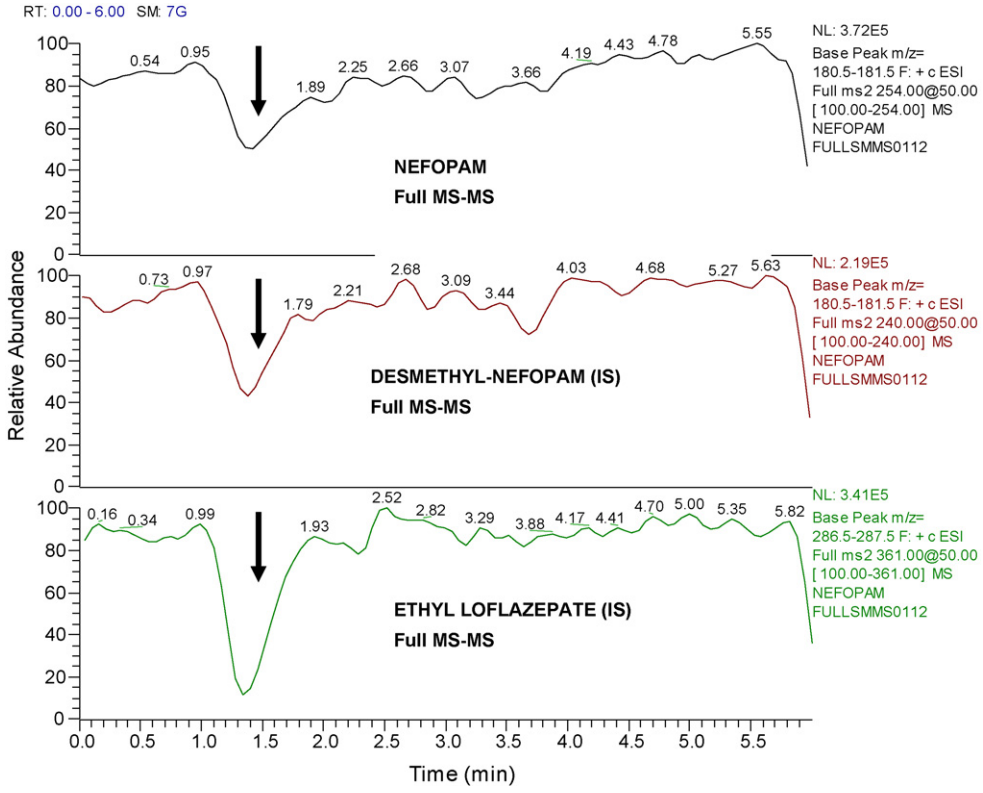

Fig. 3. Results of ion suppression for nefopam, desmethyl-nefopam and IS (ethyl loflazepate) assayed in positive ionisation mode. Continuous post-columns infusion of all the compounds with on-column injection of blank plasma extract. No ion suppression was observed after elution of the LC front (retention time > 1.5 min).

Under our experimental conditions, the carry-over effect was found to be minimal with carry-over rates lower than 0.2% of the LOQ.

3.2.3. Extraction recoveries

Neofpam and desmethyl-nefopam extraction recoveries from human plasma at two levels of concentration are summarized in Table 2. These results indicate that, in contrast to other methods using multiple steps for plasma extraction [10,11], the single-step liquid–liquid extraction procedure used in this assay is sufficient to ensure satisfactory extraction recoveries (i.e. >65%).

3.2.4. Specificity and ion-suppression test

The analysis of 10 blank plasma samples from healthy volunteers showed no interfering peak on the chromatograms. In our assay, no ion-suppression effect has been observed with all blank plasma extracts at the expected retention times of the nefopam, desmethyl-nefopam and IS (Fig. 3). In agreement with Müller et al. [13], ion-suppression effects were detected at the LC front (retention time < 1.5 min, i.e. during the elution of non-retained compounds), but this did not really interfere with the ionisation of compounds assayed in positive ionisation mode. Other ion-suppression experiments have shown that no significant reciprocal ion suppression of nefopam and desmethyl-nefopam occurred during their coelution.

3.2.5. Patients' samples

The mean concentration–time profiles for nefopam and desmethyl-nefopam in plasma obtained from two patients partic-

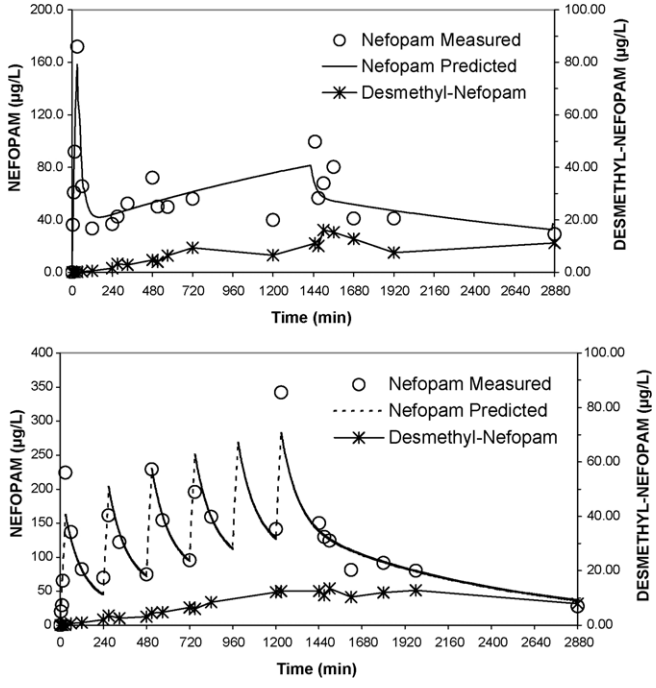

Fig. 4. Observed plasma concentration–time profiles of nefopam (◯) and desmethyl-nefopam (*) after continuous (upper graph, 100 mg/24 h following a 20 mg loading over 30 min) and intermittent (lower graph, 20 mg over 30 min every 4 h during 24 h) intravenous administration of nefopam in two patients (one in each route of administration). The dotted lines show the predicted plasma concentration–time profiles estimated by the software WinNonLin®.

ipating in an ongoing clinical study is shown in Fig. 4. Detailed pharmacokinetic data for all subjects ($n = 120$) enrolled in the clinical study will be reported in a separate article.

In conclusion, we developed a sensitive LC–MS–MS method to quantify simultaneously nefopam and its metabolite in human plasma. In addition to its high specificity, this assay demonstrates acceptable precision and accuracy and has a short analysis run time. It has been successfully applied to determine the concentration–time profiles of both compounds in patients after intermittent or continuous intravenous administration of nefopam.

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References

[1] R.C. Heel, R.N. Brodgen, G.E. Pakes, T.M. Speight, G.S. Avery, Drugs 19 (1980) 249–267.
[2] G. Aymard, D. Warot, P. Demolis, J.F. Giudicelli, P. Lechat, M.E. Guern, C. Alquier, B. Diquet, Pharmacol. Toxicol. 92 (2003) 279–286.
[3] J. Chawla, M.E. Le Guern, C. Alquier, T.F. Kalhorn, R.H. Levy, Ther. Drug Monit. 25 (2003) 203–210.
[4] O. Mimoz, P. Incagnolin, C. Josse, M.C. Gillon, L. Kulhman, A. Mirand, H. Soilleux, D. Fletcher, Anaesthesia 56 (2001) 520–525.
[5] T.T. Mc Lincock, G.N. Kenny, J.C. Howie, C.S. McArdle, S. Lawrie, H. Aitken, Br. J. Surg. 75 (1988) 779–781.
[6] J.C. Merle, D. Vandroux, I. Odin, J.L. Dupuis, Y. Mehaddi, N. Nathan, Ann. Fr. Anesth. Reanim. 24 (2005) 13–18.
[7] D. Schuppan, C.S. Hansen, R.E. Ober, J. Pharm. Sci. 67 (1978) 1720–1723.
[8] H. Ehrsson, S. Eksborg, J. Chromatogr. 136 (1977) 154–158.
[9] S.F. Chang, C.S. Hansen, J.M. Fox, R.E. Ober, J. Chromatogr. 226 (1981) 79–89.
[10] L.C. Burton, N.J. Loftus, D.W. Vere, R. Whelpton, J. Chromatogr. B 526 (1990) 159–168.
[11] D.T. Liu, J.M. Savage, D. Donnell, Br. J. Clin. Pharmacol. 23 (1987) 99–101.
[12] G. Aymard, D. Warot, P. Demolis, I. Laville, B. Diquet, J. Pharm. Biomed. Anal. 30 (2002) 1013–1021.
[13] C. Müller, P. Schäfer, M. Strörtzel, S. Vogt, W. Weinmann, J. Chromatrogr. B 773 (2002) 47–52.