ELSEVIER

Journal of Chromatography B, 748 (2000) 179–188

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Narrow-bore high-performance liquid chromatography in combination with ionspray tandem mass spectrometry for the determination of the substance P receptor antagonist ezlopitant and its two active metabolites in plasma

Amin Kamel*, Chandra Prakash, Claire Saverino, Beth Obach, Hassan Fouda

Department of Drug Metabolism, Global Research and Development, Pfizer Inc., Groton, CT 06340, USA

Received 17 January 2000; received in revised form 28 May 2000; accepted 8 June 2000

Abstract

A simple, but highly sensitive and specific, assay was developed for the quantitative determination of ezlopitant and its two active metabolites in human plasma using narrow-bore reversed-phase high-performance liquid chromatography (HPLC) coupled with electrospray tandem mass spectrometry (ES–MS–MS). Ezlopitant, its two pharmacologically active metabolites, an alkene analogue (CJ-12 458) and a benzyl alcohol analogue (CJ-12 764), and their corresponding trideuterated internal standards (I.S.), were extracted from plasma with methyl *tert*.-butyl ether (MTBE). The dried MTBE extracts were reconstituted and analyzed using a narrow-bore (2.1 mm I.D.) YMC basic HPLC column and a mobile phase of acetonitrile–20 m*M* ammonium acetate, pH 5 (60:40, v/v). Column effluent was monitored by pneumatically assisted electrospray tandem mass spectrometry. Multiple reaction monitoring (MRM) using the parent to product ions was used to quantify ezlopitant and its two active metabolites. The assay exhibited a linear dynamic range of 0.1–100 ng/ml. Average absolute recoveries from plasma were approximately 71, 80 and 99% for ezlopitant and its two active metabolites CJ-12 485 and CJ-12 764, respectively. The precision (RSD %) and accuracy (Deviation %) values for the method were within ±12% and ±15%, respectively, for all analytes. Sample analysis times were less than 5 min from one injection to the next. The assay proved to be suitable for pharmacokinetics studies.

Keywords: Ezlupitant; Substance P

1. Introduction

Ezlopitant, (2S,3S-*cis*)-2-(diphenylmethyl)-*N*-{(2-methoxy - 5 - isopropylphenyl)methyl} - 1 - azabicyclo[2.2.2]octan-3-amine (CJ-11 974, Fig. 1), is a selective antagonist of the NK_1 tachykinin receptor which preferentially mediates the actions of substance P (SP). It is currently being investigated as a potential therapy for disorders in which SP is believed to play a role in the emetic pathway, the transmission of pain signals, and in preventing the cellular responses that are associated with chronic inflammation [1–8].

In addition to the parent compound, an alkene analogue (CJ-12 458, Fig. 1) and a benzyl alcohol analogue (CJ-12 764, Fig. 1) were identified in in vivo and in vitro studies as the main metabolites of ezlopitant. Both metabolites are believed to bind

*Corresponding author.

0378-4347/00/$ – see front matter
PII: S0378-4347(00)00361-3

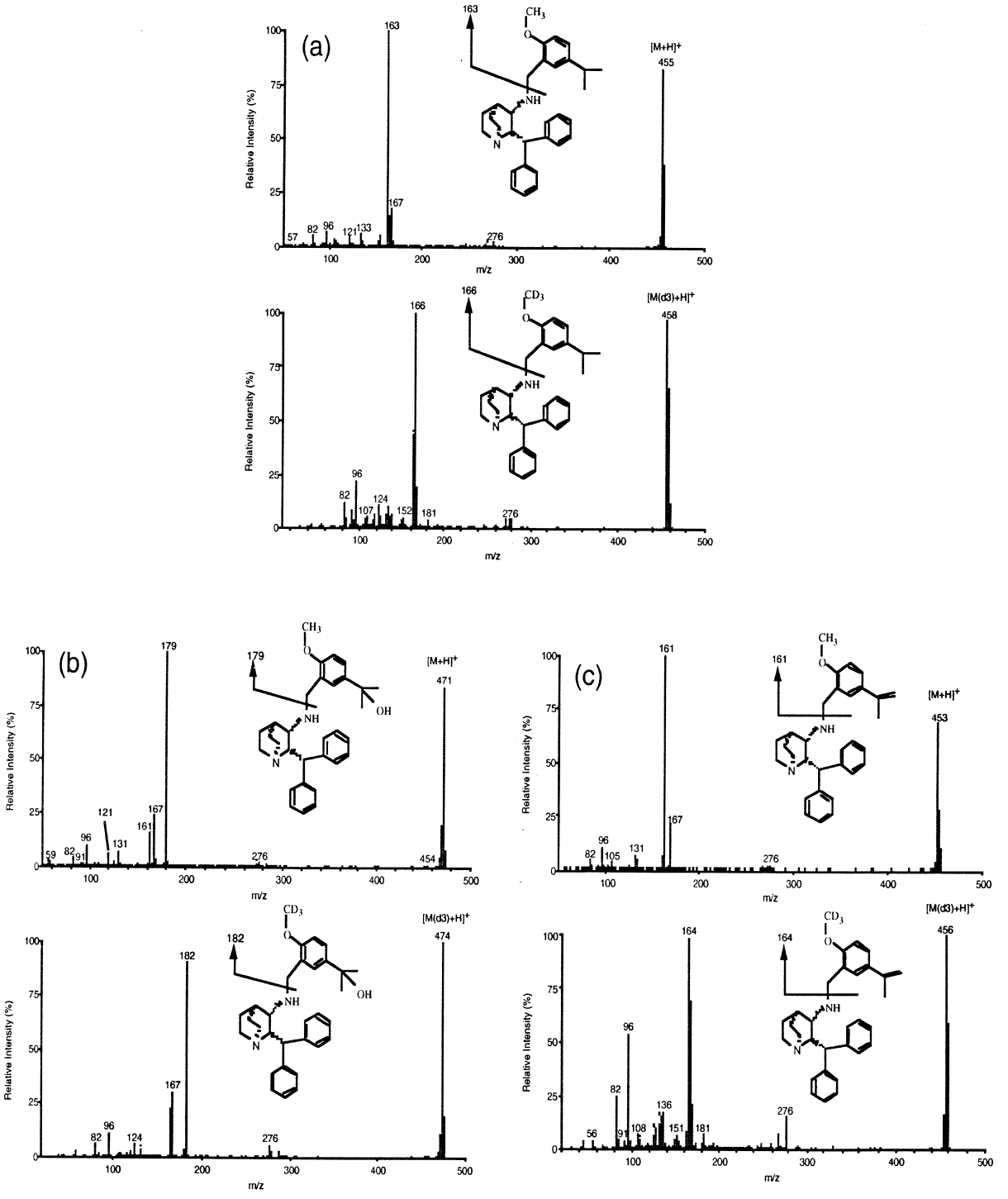

Fig. 1. CID product ion spectra of (a) ezlopitant at m/z 455 and its trideuterated I.S. at m/z 458, (b) benzyl alcohol metabolite (CJ-2764) at m/z 471 and its trideuterated I.S. at m/z 474, and (c) alkene metabolite (CJ-2458) at m/z 453 and its trideuterated I.S. at m/z 456.

with high affinity to the NK_1 tachykinin receptor and thus antagonize responses to SP. In order to evaluate the pharmacokinetics of ezlopitant in humans and preclinical species, and to better understand pharmacokinetic/pharmacodynamic relationships, a sensitive and specific assay for the quantitative determination of ezlopitant and the two active metabolites in the systemic circulation was required.

In early work, an assay for ezlopitant was developed using gas chromatography (GC) with nitrogen-specific detection that was sensitive to 1.0 ng/ml in dog serum. The GC technique was then extended to include the two metabolites in the analysis using electron impact mass selective detection in order to provide better selectivity. The GC method, however, was not suitable for the quantitative determination of ezlopitant and the two metabolites in the low pg/ml range and required a relatively longer (>10 min) GC analysis time. In addition, one problem with the GC assay was the thermal stability of the benzyl alcohol metabolite (CJ-12 764) and its potential to undergo thermally catalyzed spontaneous dehydration to the alkene metabolite (CJ-12 458). Preliminary attempts to utilize atmospheric pressure chemical ionization (APCI) mass spectrometric assay for the quantitative determination of ezlopitant and the two active metabolites were not successful for the same reason. Thus, it can be expected that the simultaneous quantification of the benzyl alcohol metabolite and its corresponding alkene metabolite by GC or APCI will be disconcerted by the unknown extent of spontaneous dehydration that can occur in the heated injector port of the GC or the in the heated nebulizer probe of the mass spectrometer.

Therefore, to avoid any amount of spontaneous dehydration that the benzyl alcohol may undergo under GC or APCI conditions and to fully evaluate the pharmacokinetics of ezlopitant and the two metabolites, a method that both avoids the use of high temperature and provides a much lower limit of detection was required.

Liquid chromatography (LC) coupled with APCI or electrospray ionization (ESI) tandem mass spectrometry (MS–MS) is a proven technique for the rapid, sensitive and specific determination of drugs in biological fluids [9–15]. However, the ionspray process avoids the use of high temperatures, which may degrade thermally-labile metabolites. LC-ion spray MS–MS techniques have been used in our laboratory for the identification of conjugated and non-conjugated drug metabolites of various drugs including ezlopitant [16–19]. We have used this technique for the quantitative determination of CP-122 721 in plasma [20] and have shown very recently the applicability of HPLC–ESI–MS–MS to achieve a very simple but highly sensitive and selective analysis for tetracyclines [21] and antiviral agents [22]. Therefore, it was considered of interest to explore the feasibility of ion spray MS–MS for the determination of ezlopitant and the two metabolites in human plasma.

This report describes the development, validation, and application of a rapid, highly sensitive and specific method utilizing narrow-bore LC–ESI–MS–MS. The assay has an analysis time of less than 5 min and is capable of determining low plasma levels (100 pg/ml) of ezlopitant and its two pharmacologically active metabolites.

2. Experimental

2.1. Materials and reagents

Ezlopitant, (2S,3S-*cis*)-2-(diphenylmethyl)-*N*-{(2-methoxy-5-isopropylphenyl)methyl}-1-azabicyclo-[2.2.2]octan-3-amine (CJ-11 974, Fig. 1), an alkene analogue (CJ-12 458, Fig. 1), and a benzyl alcohol analogue (CJ-12 764, Fig. 1), were synthesized at Pfizer (Nagoya, Japan). Trideuterated internal standards were kindly provided by Doctor S. Obach, Pfizer Central research, Groton, CT, USA. Acetonitrile (HPLC grade) was obtained from J. T. Baker (Phillipsburg, NJ, USA). Glacial acetic acid (GR grade) deionized water, ammonium acetate (HPLC-grades) were obtained from Fisher (Fair Lawn, NJ, USA), and methyl t-butyl ether (MTBE) HPLC-grade was obtained from Burdick and Jackson (Muskegon, MI, USA). Human plasma with EDTA anticoagulant was obtained from Biological Specialties Corporation (Landsdale, PA, USA). Hexamethyldisilazane (HMDS) was obtained from Pierce Chemical Company (Rockfield, IL, USA).

2.2. Instrumentation and chromatographic conditions

LC was performed using a HP-1050 solvent delivery system and a HP-1050 autoinjector. The HPLC was coupled to a Perkin-Elmer Sciex API III$^+$ (Thornhill, Ontario, Canada) triple-quadruple mass spectrometer. The analytical column was a YMC basic (2.1×150 mm, 5 μm, YMC, Greenboro, NC). The mobile phase consisted of acetonitrile–20 m*M* ammonium acetate, pH 5 (60:40, v/v). The HPLC system was operated isocratically at 300 μl/min and at room temperature. The column effluent was split and approximately 30 μl/min was introduced to the ion spray ionization source. The remaining effluent was directed into the waste. The quadruple power supply was set for unit mass resolution. Ion signals were detected with an electron multiplier operated in the pulse-counting mode by counting every tenth pulse. The production efficiency of the protonated molecular ion (MH^+) was optimized by measuring (MH^+) abundance while varying the orifice and ion spray voltages. The ion spray and orifice voltages were set at 5000 V and 60 V, respectively, and a nebulizing gas pressure was set at 60 p.s.i. of N_2. Collisonally-induced dissociation (CID) studies were performed using argon gas at a thickness of 2×10^{15} atoms cm^{-2} and a collision energy of 25 eV. The instrument was programmed for a scan dwell time of 200 ms. These parameters were optimized by systematically varying the argon pressure and collision energy for maximum reproducibility and the abundance of the selected product ion. The responses of ezlopitant, CJ-12 458, CJ-12 764, and their corresponding trideuterated internal standards were measured using the technique of multiple reaction monitoring (MRM); i.e. monitoring selected product ions of specific parent ions. The protonated molecular ions for ezlopitant (*m*/*z* 455), CJ-12 458 (*m*/*z* 453), CJ-12 764 (*m*/*z* 471), and their corresponding trideuterated internal standards at *m*/*z* 458, 456, and 474, respectively, were dissociated by collision in Q2 with argon. The mass spectrometer was adjusted to selectively monitor the transitions *m*/*z* 455→163 for drug ezlopitant and *m*/*z* 458→166 for its I.S. [d_3]ezlopitant, *m*/*z* 453→161 for metabolite CJ-12 458 and *m*/*z* 456→164 for its I.S. [d_3]CJ-12 458, and *m*/*z* 471→179 for metabolite CJ-12 764 and *m*/*z* 474→182 for its I.S. [d_3]CJ-12 764. The area ratios for calibration curves and quantitative analysis were made using the Sciex MacQuan software version 1.3.

2.3. Preparation of standard and quality control samples

All centrifuge tubes were silylated in a vacuum oven following the procedure of Fenimore et al. [23]. Stock solutions of ezlopitant, CJ-12 458, CJ-12 764, and their corresponding trideuterated internal standards (1 mg/ml) were prepared in acetonitrile and were serially diluted with acetonitrile to obtain the desired concentrations. The stock solutions were kept refrigerated and discarded one month after their preparation.

The plasma concentrations of calibration standards for ezlopitant and two metabolites were 0, 0.1, 0.2, 0.5, 2, 10, 30, 50, 80 and 100 ng/ml. Three levels of quality control (QC) samples at 0.5, 30 and 80 ng/ml (low, medium and high), were prepared at the beginning of the validation and were stored at −20°C.

2.4. Extraction procedure

An aliquot of 1 ml human plasma was placed in a centrifuge tube. To this was added 10 ng of each trideuterated I.S.s and the sample was vortex mixed. The plasma was extracted with 5 ml of MTBE for 10 min using a vortex mixer. Following centrifugation (2440 *g*) for 5 min, the organic layer was transferred to a clean centrifuge tube, and the extract was evaporated to dryness in a nitrogen Vortex Evaporator, Zymark Turbo Vap LV, (bath temp = 37°C, ~25 min). The dried residue was reconstituted in 100 μl of HPLC mobile phase, sonicated for 10 min, vortexed for 10 min and centrifuged. Samples were transferred to clean autosampler injection vials and a 15 μl sample volume was injected into the HPLC–MS–MS system.

2.5. Method validation

Human plasma was used throughout this validation assay. In other validation experiments (data not shown), similar results were obtained using rat and

dog plasma. For method validation, duplicate plasma calibration curves and six replicates of the plasma quality controls were analyzed on three different days. The standard curves were constructed by weighted $(1/x^2)$ least-squares linear regression analysis of the peak-area ratios of analyte/IS versus the concentrations of analyte. The equations of the calibration curves were used to calculate the concentration of each analyte in the samples and QCs from their peak area ratios.

The intra-assay precision (RSD%) and accuracy (deviation%) was determined by analyzing a set of QC samples ($n=6$) at each of the three levels, 0.5, 30 and 80 ng/ml. An inter-assay precision and accuracy study was carried out by analyzing QC samples in six replicates on three separate days.

2.6. Recovery

The extraction recoveries of each analyte and its $[d_3]$I.S. from human plasma were calculated by comparing the areas of peaks of the extracted and non-extracted analytes. The recovery study was carried out by spiking control human plasma samples with each analyte at three concentrations (in five replicates), 0.5, 30.0 and 80.0 ng/ml, and with $[d_3]$I.S.s at 10 ng/ml. These samples were extracted as described above. Another set of control human plasma samples was extracted without any prior addition of the analytes. After drying the control extracts, appropriate amounts of each analyte and $[d_3]$I.S. (same level as extracted samples) were added and the final volume was made up to 100 μl with the HPLC mobile phase. Aliquots 15 μl of both extracted and non-extracted samples were analyzed. The areas of the analyte and I.S. were obtained and the percent recovery was calculated by dividing the areas of the extracted analytes over the areas of non-extracted standards.

3. Results and discussion

3.1. Method development

The Full-scan ESI mass spectra of ezlopitant, CJ-12 458, and CJ-12 764 showed protonated molecular ions $(M+H)^+$ at m/z 455, 453, and 471, respectively. The protonated molecular ions of the trideuterated I.S.s $([M(d_3)+H]^+$ were observed at m/z 458, 456, and 474 for $[d_3]$ezlopitant, $[d_3]$CJ-12 458, and $[d_3]$CJ-12 764, respectively.

CID product ion spectra of ezlopitant, CJ-12 458, CJ-12 764, and their corresponding $[d_3]$I.S.s are shown in Fig. 1. The most intense product ion in each CID spectrum resulted from the loss of diphenylmethyl aza-bicyclo[2.2.2]octan-3-amine moiety from the protonated molecular ion and this transition was used to monitor the three analytes and their corresponding $[d_3]$I.S.s.

3.2. Separation and specificity

Narrow-bore column HPLC was used in this work in order to achieve greater sensitivity and increased peak symmetry [24]. A retention time of less than 4 min was achieved for the three analytes (ezlopitant, CJ-12 458, and CJ-12 764). Ion chromatograms for ezlopitant, CJ-12 458 and CJ-12 764 and their corresponding $[d_3]$I.S.s from blank plasma and plasma spiked with the three analytes and I.S.s are shown in Figs. 2 and 3, respectively. For the three analytes and I.S.s, the chromatograms were free of interfering peaks at their respective retention times.

3.3. Linearity, precision and accuracy

Calibration curves were plotted as the peak area ratio (analyte/I.S.) versus analyte concentration. Although the mass spectrometer is notable for its narrow dynamic range due to ESI processes [21,22], this assay has a rather extended dynamic range. The assay was linear in the concentration range of 0.1 to 100 ng/ml. The correlation coefficients were greater than 0.998 for all the curves.

The intra- and inter-assay precisions of the analysis were examined with samples spiked with ezlopitant, CJ-12 458, and CJ-12 764 (QCs). As shown in Table 1, the intra-day variability was determined at three different concentrations in six replicates, which were assayed against a single calibration curve. The intra-assay precisions (% RSD) at these concentrations (0.5, 30 and 80 ng/ml) were within 7, 8, and 11% for ezlopitant, CJ-12 458, and CJ-12 764, respectively, and the accuracy (% deviation) was from −4 to 4% for the three analytes. As shown in Table

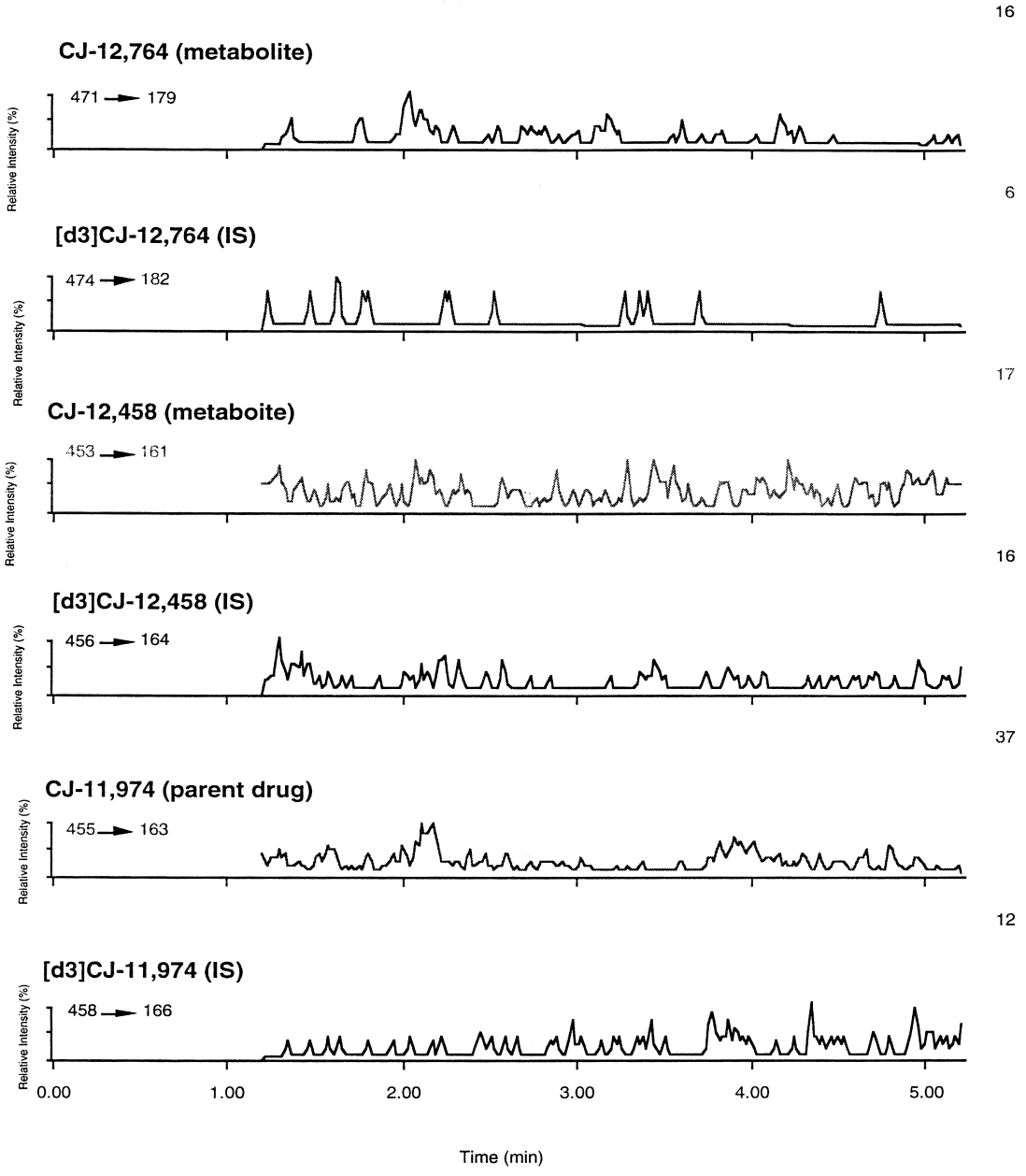

Fig. 2. Representive MRM chromatograms of a processed blank plasma showing the lack of interfering responses.

2, the inter-assay variation was determined by analysis of the spiked plasma (QC samples) on three separate occasions, relative to calibration samples that were freshly prepared each time. The inter-assay precisions at three concentrations (0.5, 30 and 80 ng/ml) were within 2, 5, and 3% for ezlopitant, CJ-12 458, and CJ-12 764, respectively, and the accuracy was from −3 to 4% for the three analytes. These results indicate that the method was reliable within that range and the use of trideuterated internal standards was very effective for reproducibility by LC–MS.

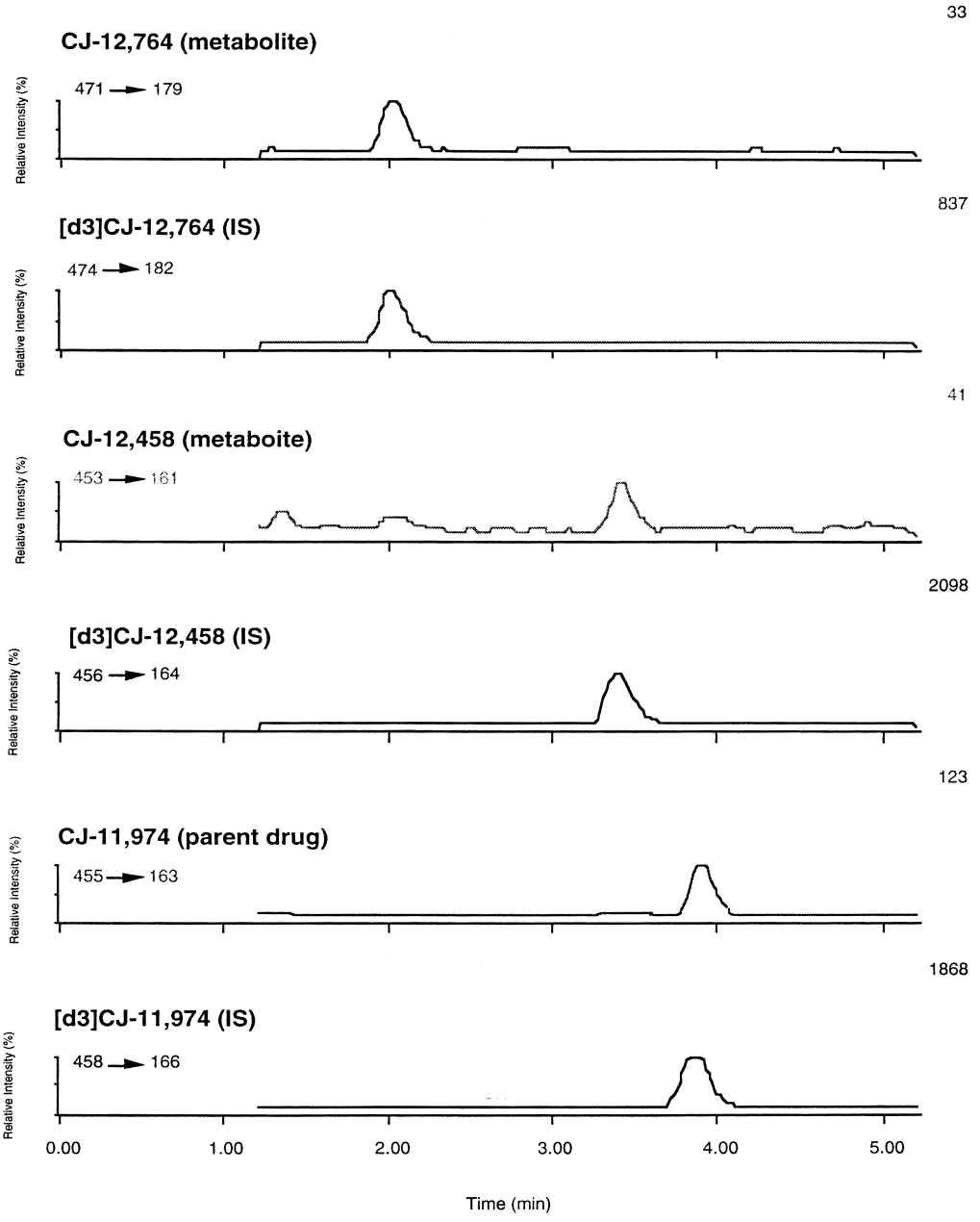

Fig. 3. Representative MRM chromatograms of a processed plasma sample fortified at 0.1 ng/ml with drug and two metabolites and at 10 ng/ml with internal standards.

3.4. Lower and upper limits of quantification

The lower and upper limits of quantification were determined at 0.1 and 100 ng/ml of ezlopitant, CJ-12 458, and CJ-12 764 in human plasma. As shown in Table 3, plasma samples were spiked with 0.1 and 100 ng/ml of each analyte ($n=5$, at each level), extracted and quantified from a standard curve. The precision of the lower and upper limit of quantification for the three analytes was within

Table 1
Intra-assay precision and accuracy for ezlopitant and its two active metabolites in plasma

Analyte	Nominal conc. (ng/ml)	Calculated conc. (mean±SD, $n=6$) (ng/ml)	RSD (%)	Deviation (%)
ezlopitant	0.5	0.5±0.1	7.3	−0.4
(parent drug)	30.0	29.8±0.9	3.1	−0.7
	80.0	79.1±2.1	2.6	−1.2
CJ-12 458	0.5	0.48±0.1	7.6	−4.1
(metabolite)	30.0	31.1±0.4	1.3	3.8
	80.0	79.7±1.6	2.0	−0.3
CJ-12 764	0.5	0.52±0.1	10.8	3.3
(metabolite)	30.0	31.1±1	3.1	3.7
	80.0	81.5±1.7	2.1	1.9

Table 2
Inter-assay precision and accuracy for ezlopitant and its two active metabolites in plasma

Analyte	Analysis sequence	Mean calculated concentration (ng/ml), $n=2$		
		0.5 ng/ml[a]	30 ng/ml[a]	80 ng/ml[a]
ezlopitant	1	0.51	31.2	77.5
(parent drug)	2	0.49	31.5	76.7
	3	0.49	31.0	79.0
Inter-assay				
RSD %		2.3	0.8	1.5
Deviation (%)		−0.7	4.1	−2.8
CJ-12 458	1	0.52	30.3	79.9
(metabolite)	2	0.51	29.9	79.9
	3	0.47	30.7	80.4
Inter-assay				
RSD %		5.3	1.4	0.7
Deviation (%)		0.0	0.9	−0.1
CJ-12 764	1	0.50	31.4	80.5
(metabolite)	2	0.49	30.8	79.3
	3	0.51	29.7.7	78.7
Inter-assay				
RSD %		2.0	2.8	1.2
Deviation (%)		0.0	2.0	−0.6

[a] Nominal concentrations.

Table 3
Precision and accuracy of lower and upper limits of quantification for ezlopitant and its two active metabolites in plasma

Analyte	Nominal conc. (ng/ml)	Calculated conc. (mean±SD, $n=5$) (ng/ml)	RSD (%)	Deviation (%)
exlopitant	0.1	0.11±0.01	8.6	14.7
(parent drug)	100.0	96.9±3.0	3.1	−3.1
CJ-12 458	0.1	0.11±0.01	11.5	8.5
(metabolites)	100.0	100.5±1.2	1.2	0.5
CJ-12 764	0.1	0.11±0.01	11.6	9.0
(metabolites)	110.0	98.0±1.1	1.2	−2.0

±12% and the accuracy was from −3 to 15%. Evidently, the assay permits the simultaneous quantification of ezlopitant and its two active metabolites with adequate accuracy and precision.

3.5. Extraction recovery

A simple liquid–liquid extraction procedure was introduced to extract ezlopitant, its two active metabolites, and their corresponding [d_3]I.S.s from plasma. As shown in Table 4, the recovery of the three analytes was determined at three different concentrations in five replicates. The recovery of [d_3]I.S.s was obtained only at one level (10 ng/ml) which was used during the assay. The average recoveries of ezlopitant, CJ-12 458, and CJ-12 764 were approximately 71, 80, and 99%, respectively. The average recoveries of [d_3]ezlopitant, [d_3]CJ-12 458, and [d_3]CJ-12 764 were very similar, as expected, to their corresponding analytes and were approximately 78, 83, and 100%, respectively.

3.6. Application

The narrow-bore LC–MS–MS method was applied to provide pharmacokinetic data for ezlopitant and its two active metabolites in rat, dog and human plasma following oral administration of [^{14}C]-CJ-11 974. An example of plasma concentration–time curves for ezlopitant and its two active metabolites CJ-12 458, and CJ-12 764 after oral administration of [^{14}C]-CJ-11 974 at 25 mg/kg to male and female rats is shown in Fig. 4. Mean plasma concentrations (average of three animals/gender) of ezlopitant and its two active metabolites CJ-12 764 and CJ-12 458 were measured for 24 h and were rather higher in female rats than in male rats. The plasma concentrations of ezlopitant reached a peak of 97.8 and 258 ng/ml for male and female rats, respectively, and declined in a log-linear manner. Mean C_{max} values for CJ-12 458 were 37.3 ng/ml and 121 ng/ml for male and female rats, respectively and mean C_{max} values for CJ-12 764 were 839 ng/ml in males and 1296 ng/ml in females. These data suggest that the female rats had somewhat higher exposure to parent drug and active metabolites than male rats.

Table 4
Assay recovery for ezlopitant and its two active metabolites in plasma

Nominal conc. (ng/ml)	Percent recovery (mean±SD) (%)	
	ezlopitant	[d_3]ezlopitant (I.S.)
0.5	63.1±9.7	72.5±4.1
30.0	68.3±6.4	75.8±7.6
80.0	82.0±11.5	85.7±12.8
	CJ-12 458	[d_3]CJ-12 458 (I.S.)
0.5	76.0±8.8	78.3±2.9
30.0	76.3±6.5	79.7±7.1
80.0	87.9±7.9	91.1±10.6
	CJ-12 764	[d_3]CJ-12 764 (I.S.)
0.5	118.1±14.0	117.4±9.8
30.0	80.2±11.1	85.80±15.6
80.0	100±15.0	98.8±17.0

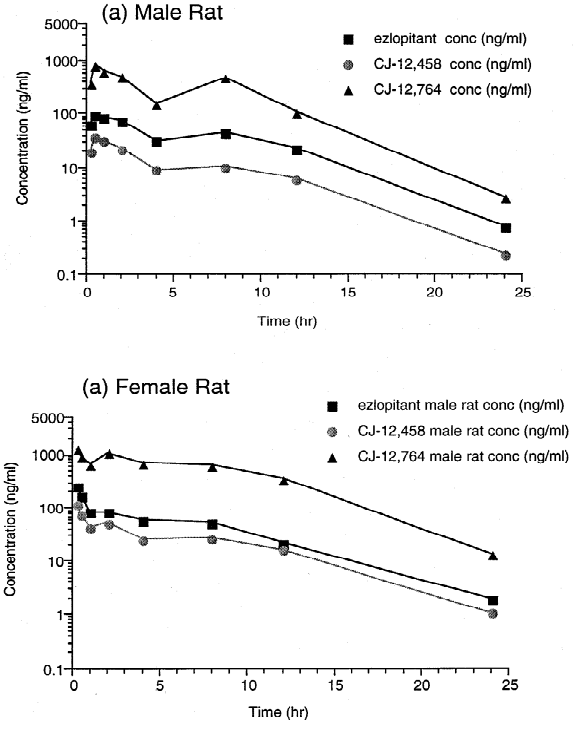

Fig. 4. Plasma concentrations of ezlopitant and its two active metabolites CJ-12 458 and CJ-12 764 in male and female rats.

4. Conclusions

A very simple and sensitive narrow-bore LC electrospray/MS–MS was developed using a liquid–liquid extraction technique. The assay has proven to be fast and rugged, with each sample requiring less than 5 min of analysis time. The method was applied successfully for the determination of ezlopitant and its two active metabolites in rat, dog, and human plasma and eliminated any possibility that the benzyl alcohol metabolite could undergo spontaneous dehydration to the alkene metabolite. The assay has a very wide range of quantification and is capable of determining low plasma level (100 pg/ml) of ezlopitant and its two pharmacologically active metabolites.

Acknowledgements

The authors thank Dr. Scott Obach for the synthesis of trideuterated internal standards, and Dr. Keith McCarthy and Ms. Sandra Miller for the synthesis of radiolabelled CJ-11 974.

References

[1] J. Rudd, M. Ngan, M. Wai, Eur. J. Pharmacol. 366 (1999) 243.
[2] F. Tattersall, W. Rycroft, B. Francis, D. Pearce, K. Merchant, A. Macleod, T. Ladduwahetty, L. Keown, C. Swain, Neuropharmacology 35 (1996) 1121.
[3] C. Bountra, K. Bunce, T. Dale, C. Gardner, C. Jordan, D. Twissell, P. Ward, Eur. J. Pharmacol. 249 (1993) R3.
[4] S. Brunelleschi, Expert Opin. Invest. Drugs 8 (1999) 1873.
[5] S. Gonsalves, J. Watson, C. Ashton, Eur. J. Pharmacol. 305 (1996) 181.
[6] F. Tattersall, W. Rycroft, R. Hill, R. Hargreaves, Neuropharmacology 33 (1994) 259.
[7] J. Watson, S. Gonsalves, A. Fossa, S. McLean, T. Seeger, S. Obach, P. Andews, Br. J. Pharmacol. 115 (1995) 84.
[8] P. Hesketh, R. Gralla, R. Webb, W. Ueno, S. DelPrete, M. Bachinsky, N. Dirlam, C. Stack, S. Silberman, J. Clin. Oncol. 17 (1999) 338.
[9] T.R. Covey, E.D. Lee, J.D. Henion, Anal. Chem. 58 (1986) 2453.
[10] H. Fouda, M. Nocerini, R. Schneider, C. Gedutis, J. Am. Soc. Mass Spectrom. 2 (1991) 164.
[11] J.D. Gilbert, T.V. Olah, A. Barrish, T.F. Greber, Biol. Mass Spectrom. 21 (1992) 341.
[12] A.T. Murphy, P.L. Bonate, S.C. Kasper, T.A. Gillespie, A.F. DeLong, Biol. Mass Spectrom. 23 (1994) 621.
[13] A.E. Mutlib, J.T. Strupczewski, J. Chromatogr. 669 (1995) 237.
[14] Y.Y. Lau, J.M. Selenka, G.D. Henson, R. Talat, N. Ichhpurani, J. Chromatogr. 683 (1996) 209.
[15] J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong, C.M. Whitehouse, Science 246 (1989) 64.
[16] C. Prakash, A. Kamel, J. Gummerus, K. Wilner, Drug Metab. Disp. 25 (1997) 863.
[17] C. Prakash, A. Kamel, W. Anderson, H. Howard, Drug Metab. Disp. 25 (1997) 206.
[18] A. Kamel, C. Prakash, in: Proceedings of the 47th ASMS Conference on Mass Spectrometry and allied topics, Dallas, TX, 1999.
[19] C. Prakash, A. Kamel, K. Johnson, M. Zhuang, K. Colizza, in: Proceedings of the 47th ASMS Conference on Mass Spectrometry and allied topics, Dallas, TX, 1999.
[20] A. Kamel, C. Prakash, J. Chromatogr., B: Biomed. Appl. 700 (1997) 139.
[21] A. Kamel, P. Brown, B. Munson, Anal. Chem. 71 (1999) 968.
[22] A. Kamel, P. Brown, B. Munson, Anal. Chem. 71 (1999) 5481.
[23] D.C. Fenimore, C.M. Davis, J.H. Whitford, C.A. Harrington, Anal. Chem. 48 (1976) 2289.
[24] F. Steiner, in: Application of Narrow-bore Columns in HPLC, Hewlett-Packard, Waldbronn, 1991, p. 89.