Quantitative determination of paroxetine and its 4-hydroxy-3-methoxy metabolite in plasma by high performance liquid chromatography/electrospray ion trap mass spectrometry: application to pharmacokinetic studies

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Abstract

A high performance liquid chromatography method with tandem mass spectrometry detection is described for the determination of paroxetine, an antidepressant drug, and its metabolite (3S, 4R)-4-(4-fluorophenyl)-3-(4-hydroxy-3-methoxyphenoxymethyl) piperidine (HM paroxetine) in human plasma. Plasma samples were hydrolyzed with hydrochloric acid and then analytes were extracted with ethyl acetate at alkaline pH. Extracts were analysed by high performance liquid chromatography coupled to an atmospheric pressure ionisation-electrospray (ESI) interface and an ion trap mass spectrometer. Chromatography was performed on a reversed-phase column using acetonitrile/0.02% formic acid (66:34, v/v) as a mobile phase. The mass spectrometer was operated in the multiple reaction monitoring mode. The method was validated over a concentration range of 0.75-100 µg/L and 5-100 µg/L for paroxetine and HM paroxetine, respectively. Mean recoveries of 77% for paroxetine and 76% for HM paroxetine were found, with precision always better than 15%. The limits of detection and quantification were 0.20 and 0.70 µg/L for paroxetine, and 0.70 and 2.20 µg/L for its metabolite. The method was applied to the analysis of plasma samples obtained from nine healthy male volunteers administered with a single oral dose of 20 mg paroxetine. After the 20 mg-dose, the mean peak plasma concentration was 8.60 µg/L for paroxetine and 92.40 µg/L for HM paroxetine showing a tenfold-ratio between the metabolite and the parent drug along the entire time-concentration curve.

Introduction

Paroxetine, (3S, 4R)-(4-(4-fluorophenyl)-3-(3,4-methylenedioxyphenoxymethyl)piperidine), is an antidepressant which acts as a potent selective serotonine re-uptake inhibitor (SSRI) in the central nervous system.[1] It has been successfully used worldwide for the treatment of a variety of depression, obsessive-compulsive and panic states and other psychiatric disorders.[2,3]

In humans, paroxetine is initially O-demethylenated by CYP2D6, an isoenzyme of cytochrome P450 giving rise to an unstable catechol ((3S, 4R)-4-(4-fluorophenyl)-3-(3,4-dihydroxyphenoxymethyl)piperidine) which is then methylated in the C3 or C4 positions of the benzene ring. A minor metabolic pathway of the catechol metabolite has also been described leading to the formation of the (3S, 4R)-4-(4-fluorophenyl)-3-(hydroxymethyl)piperidine metabolite.[3-6] The methylation of catechol is putatively catalyzed by catechol-O-methyltransferase (COMT) and the predominant metabolite resulting from this O-methylation is (3S, 4R)-4-(4-fluorophenyl)-3-(4-hydroxy-3-methoxyphenoxymethyl)piperidine (HM paroxetine). This major metabolite was isolated from human urine and identified by mass spectrometry as conjugated to glucuronic acid and sulphate.[7]

Paroxetine metabolites have been reported to be pharmacologically inactive *in vitro* and *ex-vivo* and they are not likely to contribute to the clinical effects of paroxetine.[1,2,5,8] Metabolites have been described to appear in plasma simultaneously with paroxetine, suggesting a first-pass metabolism.[3,6] Non-linear pharmacokinetics behaviour has been described for paroxetine.[5,6,9]

Pharmacokinetic properties of paroxetine in healthy volunteers have been characterized following both single and multiple oral doses in several studies.[3,5,6,10]

However, although paroxetine metabolites have been described in preliminary reports and HM paroxetine has been always reported as a major metabolite[6,7], to our knowledge its pharmacokinetic properties in humans have never been reported in peer reviewed scientific journals. Moreover, the only attempt to detect HM paroxetine in plasma samples involved the determination of its free form;[10] in the event, HM paroxetine was only found in one patient over a series of seven after repeated doses.

This paper reports an analytical method to determine paroxetine and its main metabolite HM paroxetine in plasma samples after acid hydrolysis. After a liquid–liquid extraction with ethyl acetate, high performance liquid chromatography-electrospray ion trap mass spectrometry (LC/MS/MS) was employed to quantify the analytes by monitoring their precursor-product ion combinations in the multiple-reaction monitoring (MRM) mode. The method was developed to support pharmacokinetic studies in healthy volunteers following administration of a clinical dose (20 mg) of paroxetine.

EXPERIMENTAL

Materials

Paroxetine, (3S,4R)-(4-(4-fluorophenyl)-3-(3,4-methylenedioxyphenoxymethyl)piperidine), and HM paroxetine, ((3S,4R)-4-(4-fluorophenyl)-3-(4-hydroxy-3-methoxyphenoxymethyl)piperidine), were synthesized in the “Centro de Investigación y Desarrollo of Centro Superior de Investigaciones Científicas” (CID-CSIC, Barcelona, Spain). Details of the preparation of standards are described elsewhere.[11] Pholedrine (4-hidroxy-N,α-dimethylphenethylamine) was kindly donated by the Deutsche Sporthochschule, Biochemistry Department (Cologne,

Germany). Methoxyphenamine (2-methoxy-N,α-dimethylphenethylamine) and sulfatase type H-1 from Helix Pomatia (14,600 units/g) were supplied by Sigma (St. Louis, MO, USA). β-Glucuronidase from E. Coli K12 (200 units/mL) was obtained from Roche (Manheim, Germany). Ultrapure water was obtained using a Milli-Q purification system (Millipore, Molsheim, France). HPLC-grade acetonitrile, ethyl acetate, hydrochloric acid (37%), formic acid (85%), and sodium hydroxide were obtained from Merck (Darmstadt, Germany). A pool of blank plasma samples was supplied by the blood bank of Hospital del Mar, Barcelona, Spain.

Human subjects, clinical protocol, and blood and urine sampling

Samples were obtained from 9 healthy male subjects (mean [SD] age: 23.1 [2.0] years; mean [SD] weight: 67.2 [9.3] kg; mean [SD] height: 173.3 [3.7] cm), who were given a single 20 mg oral dose of paroxetine (Seroxat, GSK, Spain). The study protocol was approved by the local ethical committee (CEIC-IMAS) and by the Spanish Ministry of Health.

The drug was administered at 9.00 a.m. to the subjects in a fasting state. Blood samples were obtained through a catheter inserted into a peripheral vein before (0 h) and at 1, 3, 5, 8 and 24 h after drug administration. Blood was collected in heparinized tubes. Within 30 minutes after drawing, samples were centrifuged at 1100 *g* and 4ºC for 10 minutes, and plasma was stored at –20ºC until analysis. Urine was also collected at different time periods from all volunteers: 0-3 h, 3-6 h, 6-9h, 9-12h, 12-24 h.

Subjects were phenotyped for CYP2D6 activity using dextromethorphan as drug probe. The dextromethorphan/dextrorphan urinary metabolic ratio[12] was used to classify subjects as extensive or poor metabolizers. All participants were extensive metabolizers.

Instrumentation

Liquid chromatography. Analyses were performed using a HP 1050 liquid chromatograph (Agilent, Palo Alto, CA, USA). The column used was a Synergi 4u MAX-RP 80A (150 x 2 mm x 4µm; Phenomenex, Aschaffenburg, Germany). Isocratic chromatography was conducted at room temperature with a mobile phase consisting of acetonitrile/0.02% formic acid (66:34, v/v) at a flow rate of 0.25 mL/min. All chromatographic solvents were degassed with helium before use. The injection volume was 10 µL. The dead time of the column, t_0, was determined by injection of methanol.

Mass spectrometry. All experiments were performed using an Esquire 3000 ion trap mass spectrometer (Bruker Daltonics, Bremen ,Germany) equipped with an atmospheric pressure ionisation-electrospray (ESI) interface. Experiments were run using positive electrospray ionisation mode. The following ESI conditions were applied: drying gas (nitrogen) heated to 325°C at flow rate of 8 L/min; the pressure of nebulizer gas (nitrogen) was 30 psi. The Esquire LC/MS/MS ion trap mass spectrometer was operated at unit resolution in the multiple reaction monitoring (MRM) mode. The instrument parameters were individually optimised to maximize the signal for the transition of the selected precursor ion to the most abundant product ion for each compound by infusing a constant flow of a solution of each drug dissolved in mobile phase. The fragmentation channels monitored for $[M+H]^+$ to product ions were m/z 330.1→191.8 for paroxetine, m/z 179.8→148.8 for methoxyphenamine (I.S.), m/z 332.1→191.8 for HM paroxetine, and m/z 165.8→134.8 for pholedrine (I.S.). Taking into account the differences of ion trap parameters optimized for each compound, samples were processed with two different ion trap methods in order to obtain the best sensitivity. Maximum MRM dwell times were 70 and 100 ms for paroxetine and HM paroxetine, respectively. Fragmentation was induced with resonant excitation amplitude

of 0.85 V for paroxetine, 0.65 V for methoxyphenamine (I.S.), 0.9 V for HM paroxetine and 0.7 V for pholedrine (I.S.), following isolation of the ion over a selected mass window.

Working standards. Stock standard solutions (1g/L) of paroxetine, HM paroxetine, methoxyphenamine and pholedrine were prepared in methanol. Working solutions at concentrations of 10, 1, 0.1 mg/L were prepared by dilution of the stock standard solutions with methanol, and were stored at –20ºC until analysis.

Preparation of calibration and quality control samples. Calibration standards containing 0.75, 3, 10, 25, 50, 100 µg/L of paroxetine and 5, 10, 25, 50, 100 µg/L of HM paroxetine were prepared in duplicate daily for each analytical batch by adding suitable amounts of methanol working solutions to 1 mL of a pre-checked drug-free plasma pool. At the beginning of the study, quality control samples of 80 µg/L (high control), 40 µg/L (medium control) and 4 µg/L (low control) for paroxetine and 6 µg/L (low control) for HM paroxetine were prepared once, aliquoted and stored at –20ºC. They were included in each analytical batch to control the daily quality of the analytical process and to check the stability of samples under storage conditions. No calibration standards or quality control samples were prepared for urine samples as only qualitative analysis was performed to investigate HM paroxetine conjugation.

Protocol for preparation of plasma samples. One mL of plasma was transferred into 15-mL screw-capped glass tubes and 30 µL of methoxyphenamine (1 mg/L) and 30 µL of pholedrine (1 mg/L) in methanol were added. Acidic hydrolysis was performed by adding 1 mL of 0.5 M hydrochloric acid. Samples were incubated for 30 min at 100°C and then cooled at room temperature. After hydrolysis, plasma samples were adjusted to pH 12 with NaOH (10 M) and extracted with 5 mL ethyl acetate. Samples

were centrifuged 10 min at 3500 rpm and the organic phase was evaporated to dryness. The dried extracts were reconstituted in 200 µL of mobile phase by vigorous vortex mixing and transferred into 200 µL injection vials. Volumes of 10 µL were injected into the chromatographic system.

Protocol for urine hydrolysis and sample preparation. The 0-3 h urine sample from one volunteer was used to perform several experiments of acid and enzymatic hydrolysis. Four aliquots of one ml of urine (diluted 1:5 with ultrapure water) were transferred into 15-mL screw-capped glass tubes and 30 µL of methoxyphenamine (1 mg/L) and 30 µL of pholedrine (1 mg/L) in methanol were added. The following procedures were applied:

- one aliquot of urine was extracted without applying any hydrolysis procedure;
- one aliquot of urine underwent an acidic hydrolysis as described for plasma samples;
- one aliquot of urine underwent an enzymatic hydrolysis with sulfatase (1 mg/mL) in one mL 0.1 M sodium acetate buffer, pH 5.2, 3h at 55ºC;
- one aliquot of urine underwent an enzymatic hydrolysis with glucuronidase (30 µL β-glucuronidase in one mL 0.2M sodium phosphate buffer, pH 7.0) for 3h at 55ºC.

After incubation, samples were cooled at room temperature, adjusted to pH 12 with NaOH (10 M) and extracted as described for plasma samples. Extracted urine samples were analyzed by LC/MS/MS with the same conditions as those used for plasma samples. Aliquots of drug-free urine underwent the same hydrolysis treatments and were used to verify absence of chromatographic interferences. All the experiments were carried out in duplicate.

Validation procedure. Prior to the application to real plasma samples, the method was tested following a 3-day validation protocol. Selectivity, recovery, linearity, stability, precision, accuracy, and limits of detection and quantification were assayed.

The selectivity of the method was studied by analysing several plasma samples (n=7) and checking for the presence of interfering substances at the appropriate MRM transition. Calibration curves were tested over the ranges 0.75-100 µg/L paroxetine and 5-100 µg/L HM paroxetine. Peak area ratios between compounds and I.S. were used for calculations. A weighted (1/concentration) least-squares regression analysis was used (SPSS, version 9.0.2 for Windows). Four replicates were analysed at the following concentrations: 0.75, 3, 10, 25, 50 and 100 µg/L for paroxetine, and 5, 10, 25, 50 and 100 µg/L for HM paroxetine.

Signal-to-noise ratios of 3 and 10 were used for estimating the limits of detection and quantification, respectively. The quantification limits were verified by the analysis of five samples prepared at the respective quantification limits estimated as described. Analytical recoveries were calculated by comparing the peak areas obtained when calibration samples were analysed by adding the reference substances and the internal standards prior to and after the extraction procedure in the drug-free plasma and in the extract from drug-free plasma, respectively. The recoveries were assessed at three concentration levels using four replicates at each level (3, 25 and 100 µg/L) for paroxetine and (5, 25 and 100 µg/L) for HM paroxetine and twelve replicates for both I.S. at a concentration of 30 µg/L.

Three replicates of quality control samples at three different concentrations of paroxetine (4, 40 and 80 µg/L) and HM paroxetine (6, 40 and 80 µg/L) added to drug-

free plasma samples were extracted as reported above and analysed for the determination of intra-assay precision and accuracy. The inter-assay precision and accuracy were determined for three independent experimental assays of the aforementioned replicates. Inter and intra-assay precision were expressed as the relative SD (RSD) of concentrations calculated for quality control samples. Inter and intra-assay accuracy were expressed as the mean of the absolute value of the relative error of the calculated concentrations.

The stability of paroxetine and its metabolite in plasma was evaluated under three freeze/thaw cycles. The test involved a comparison of replicate stability samples, which had been frozen and thawed three times with a fresh plasma sample that had been thawed only once.

Data Analysis. Pharmacokinetic parameters, C_{max} (µg/L), T_{max} (h), K_e (h^{-1}), $T_{1/2}$ (h), and $AUC_{(0\text{-}24h)}$ (µg/L.h) were determined using the PK Functions for Microsoft Excel computer program.[13]

RESULTS AND DISCUSSION

Validation results

The full scan MS/MS spectra as well as the proposed fragmentation patterns for paroxetine, HM paroxetine, methoxyphenamine and pholedrine, are shown in Figure 1. Selected reaction monitoring (SRM) chromatograms are depicted in Figures 2 and 3. Dead time (t_0) of the column was 0.5 min and the compounds showed similar retention times (about 1.5 min) but could be identified without interference due to the different SRM channels for each. The studied compounds as well as the internal standards formed protonated molecules ($[M+H^+]$) in the ion source, and the selectivity of the

MRM approach led to clean chromatograms free of background interference (see Figures 2A and 3A for data for blank plasma).

The recoveries (mean ± SD) obtained were 77.3 ± 3.7 % for paroxetine, 76.1 ± 11.5 % for HM paroxetine, 88.6 ± 10.9% for methoxyphenamine and 67.4 ± 8.1% for pholedrine. Calibration curves (area ratio to the respective I.S.) were linear in the concentration range tested for paroxetine and HM paroxetine, with coefficients of determination (r^2) higher than 0.99 in all cases.

Tables 1 and 2 show the results obtained for intra-assay and inter-assay precision and accuracy for paroxetine and HM paroxetine. These results satisfactorily met internationally established acceptance criteria.[14] Estimated limits of detection and quantification were 0.20 and 0.70 µg/L for paroxetine, and 0.70 and 2.20 µg/L for HM paroxetine. In the case of analysis, in three samples out of nine at 1h after administration, the drug was detected but could not be quantified. On the other hand, samples which had a concentration higher than 100 µg/L of HM paroxetine were diluted with ultrapure water to fit within the working range, and were reanalysed using the same sample preparation procedure described above.

The freeze-thaw stability test showed that paroxetine and its metabolite (HM paroxetine) in human plasma were stable for at least three freeze-thaw cycles (data not shown). Moreover, the stability of HM paroxetine under the hydrolysis conditions was established when analytical recoveries were calculated by comparing the peak areas obtained when calibration samples were analysed by adding the reference substance and the internal standards both prior to and after the extraction procedure. HM paroxetine was stable under acidic hydrolysis conditions, as good responses were obtained from

calibration samples when HM paroxetine and the internal standard had been added prior to the extraction procedure.

Because deuterated analogues of paroxetine were not commercially available, it was necessary to find an alternative internal standard. Methoxyphenamine has proven to be a good choice because it shares several physicochemical properties with paroxetine, making it suitable for this type of analysis. Our previous experience with the analysis of drugs bearing a 4-hydroxy-3-methoxy grouping like HMMA (4-hydroxy-3-methoxy-methamphetamine)[15], using pholedrine as internal standard, showed good results and therefore it was selected for the analysis of HM paroxetine in the present study.

The analytical method presented here for the simultaneous determination of paroxetine and HM paroxetine from plasma samples has demonstrated enough sensitivity, specificity and selectivity for the purposes of the present study. High-performance liquid chromatography (HPLC) with ultaviolet (UV), fluorescence or coulometric detection was utilized in previously published analytical methods.[16-24] However, these detectors were not sufficiently specific and sensitive for the determination of paroxetine, and required laborious and time-consuming sample pre-treatment to remove interfering substances and/or the formation of derivates before HPLC analysis. Recently a LC/MS/MS method for paroxetine determination in plasma has been reported.[25] However, this method does not include the analysis of its main metabolite.

Despite the extensive number of assay procedures for the determination of paroxetine published up to now[16-27], only two studies describe the simultaneous determination of paroxetine and its metabolite HM paroxetine in plasma.[10,24] Kristoffersen et al.[24] provided detection limits around 0.025 µmol/L (8 µg/L) for

paroxetine and 0.12 µmol/L (40 µg/L) for HM paroxetine, which are of use in cases of acute intoxication but are not suitable for the purposes of a pharmacokinetic study in which a single dose of 20 mg is administered and lower concentrations are expected for the parent drug and its metabolite. However, results obtained for real cases of acute paroxetine intoxications were not reported, and it is unknown if the method, as it was described[24], is able to detect HM paroxetine in clinical samples. On the other hand, Härtter et al.[10] described a method for paroxetine and its main metabolite for application in pharmacokinetic studies; HM paroxetine was detected in some samples but could not be quantified. Our previous experience with drugs bearing a methylene-dioxy grouping like MDMA (3,4-methylendioxymethamphetamine)[28] showed that the corresponding hydroxy-methoxy metabolite, HMMA (3-hydroxy-4-methoxy-methamphetamine), is extensively conjugated and that despite very sensitive assays this metabolite in its free form was not quantifiable. This observation oriented the method development towards the introduction of a hydrolysis step in the sample preparation procedure. The kinetics of HM paroxetine in plasma and urine samples has never been reported, but some studies from the pharmaceutical company that developed the drug suggest that this metabolite is mainly excreted as a conjugate.[6,7]

Application to pharmacokinetic study

Figure 4 represents time courses of paroxetine and HM paroxetine plasma concentrations, following a single oral dose of 20 mg paroxetine to each of 9 healthy volunteers. The median T_{max} of paroxetine (5 hours) was slightly shifted with respect to that of HM paroxetine (3 hours). HM paroxetine concentrations were one order of magnitude higher than those observed for the parent drug along the 24 hour time course.

Inter-subject variabilities of 1:10 and 1:5 were observed in the cases of paroxetine (range 1.6-15.7 µg/L at C_{max}) and HM paroxetine (range 33.8-145.7 µg/L at C_{max}) concentrations along the time course of the experimental session. The pharmacokinetic parameters calculated for paroxetine and HM paroxetine are reported in Table 3.

Paroxetine was identified in both hydrolysed and non-hydrolysed urine samples. In contrast, HM paroxetine could not be identified in non-hydrolysed samples, while it was always present in hydrolysed urine, independently from the hydrolysis procedure applied. This observation confirms early results that HM paroxetine is presented in biological fluids mainly as the sulfate and the glucuronoconjugate.[6,7]

Pharmacokinetic parameters obtained here for paroxetine are in agreement with previously published data.[2,6,29] Concerning HM paroxetine, this is the first time that this metabolite has been determined. The fact that the T_{max} of HM paroxetine precedes marginally that corresponding to paroxetine (3 h vs. 5 h), combined with the high HM paroxetine plasma concentrations, support a strong first-pass metabolism and may explian the inter-subject variabilities observed among the 9 healthy volunteers participating in the study (Figure 4).

It is worth noting that, when Härtter et al.[10] analysed HM paroxetine without any hydrolysis step, this metabolite was hardly found in plasma samples of volunteers also administered 20 mg of paroxetine, and the reported limit of detection for HM paroxetine was 5 µg/L. If this figure is compared with the encountered concentrations of HM paroxetine (sum of its conjugated and unconjugated form) (C_{max} 92.40 µg/L), it can be estimated that HM paroxetine is conjugated in plasma to an extent most probably higher than 95%. This hypothesis is confirmed from results observed in urine samples. Indeed

HM paroxetine could not be found in non-hydrolysed urine, while it was observed after acidic and enzymatic hydrolysis with glucuronidase and sulfatase.

The simultaneous determination of paroxetine and HM paroxetine is expected to provide some insight into the non-linear kinetics reported for paroxetine after repeated doses.[5,6,9]

During metabolism, the methylation of the unstable catechol metabolite (3S, 4R)-4-(4-fluorophenyl)-3-(3,4-dihydroxyphenoxymethyl)piperidine, putative intermediate of HM paroxetine, by catechol O-methyltransferase (COMT) may give rise to two metabolites (3S, 4R)-4-(4-fluorophenyl)-3-(4-hydroxy-3-methoxyphenoxymethyl)piperidine (HM paroxetine) and (3S, 4R)-4-(4-fluorophenyl)-3-(3-hydroxy-4-methoxyphenoxymethyl)piperidine. These differ only in the methylation position but share the same fragmentation pattern with the same most intense MS/MS transition (m/z 332.0→191.8). The possibility of a co-analysis of both metabolites should be considered. However, many reports[5,7,10] suggest that methylation at the C3 position (HM paroxetine) is the predominant paroxetine pathway and, moreover, it is known that COMT prefentially alkylates compounds containing phenol moieties at the 3 position, and to a much lesser extent at position 4.[30] If a co-analysis method were available, it is highly likely that concentrations of the metabolite methylated at C4 would be much lower in comparison with that of HM paroxetine.

CONCLUSIONS

In conclusion, the LC/MS/MS method presented here for the simultaneous determination of paroxetine and HM paroxetine requires only a one-step extraction with ethyl acetate for plasma samples prior to isocratic chromatography, which enables a

rapid and simple assay when compared to previous published methods. In addition, MS/MS detection gives adequate specificity for routine monitoring of therapeutic drug administration. With this new method the levels of paroxetine and its metabolite could be measured for up to 24 hours in plasma samples of 9 volunteers administered 20 mg paroxetine. HM paroxetine plasma concentrations are one order of magnitude higher than those observed for the parent drug for the entire 24 hour time course. This metabolite is present in biological specimens as the glucuronoconjugate and sulfate conjugate.

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Figure legends:

Figure 1. Full scan MS/MS spectra and the proposed patterns of fragmentation for (A) paroxetine, (B) HM paroxetine, (C) methoxyphenamine and (D) pholedrine.

Figure 2. LC/MS/MS chromatograms of (A) blank human plasma, (B) human plasma spiked with 3 µg/L of paroxetine and 30 µg/L of I.S., (C) plasma sample from a dosed volunteer containing 9 µg/L of paroxetine.

Figure 3. LC/MS/MS chromatograms of (A) blank human plasma, (B) human plasma spiked with 5 µg/L of HM paroxetine and 30 µg/L of I.S., (C) plasma sample from a dosed volunteer containing 28 µg/L of HM paroxetine.

Figure 4. Plasma concentration versus time for (A) paroxetine, (B) HM paroxetine and (C) the mean profile from 9 healthy volunteers following the administration of 20 mg paroxetine.

Table 1

Intra-day precision and accuracy obtained for paroxetine and HM paroxetine in plasma.

Compound	Concentration (µg/L)	Number of observations	Estimated mean ± SD (µg/L)	Precision (R.S.D)	Accuracy (Error %)
Paroxetine	4	3	3.50 ± 0.10	0.6	12.9
	40	3	36.30 ± 2.40	6.5	9.2
	80	3	76.10 ± 8.50	11.1	8.4
HM paroxetine	6	3	5.2 ± 0.60	11.0	13.8
	40	3	43.90 ± 3.40	7.7	9.8
	80	3	91.40 ± 11.10	12.2	14.2

Table 2

Intermediate precision and accuracy obtained for paroxetine and HM paroxetine in plasma.

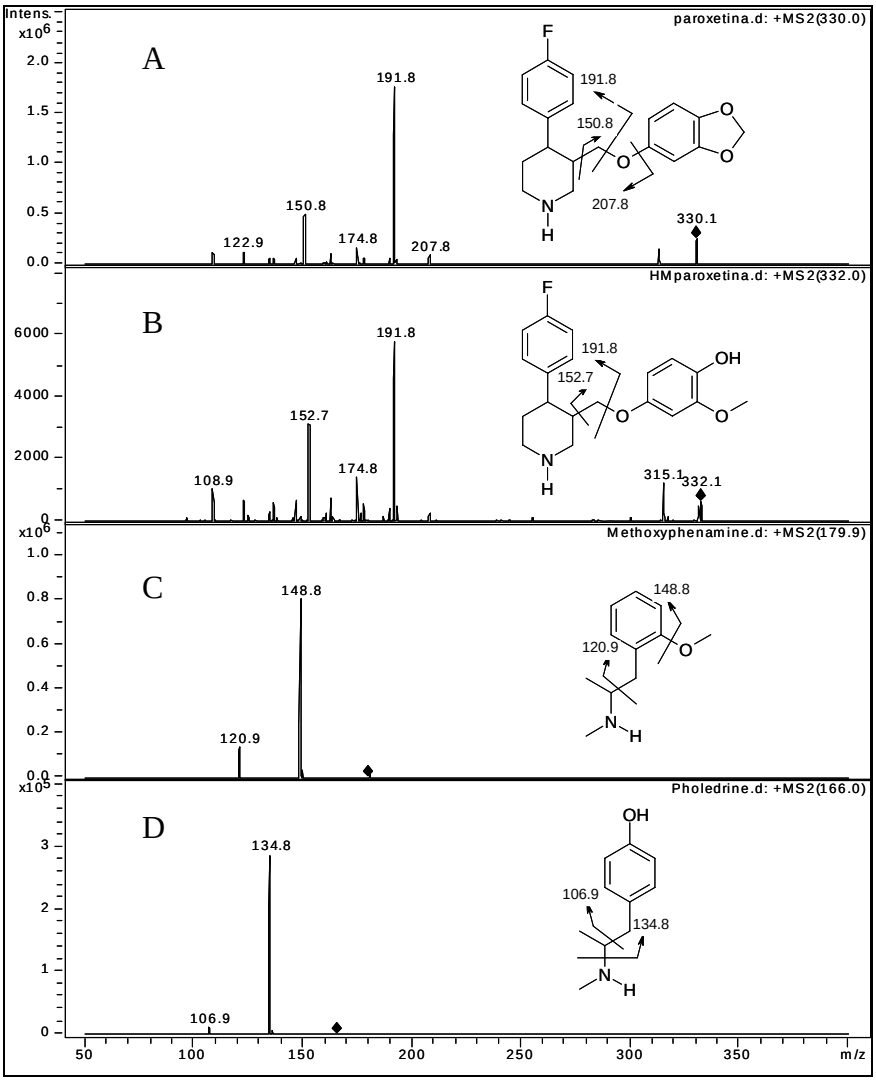
Compound	Concentration (µg/L)	Number of observations	Estimated mean ± SD (µg/L)	Precision (R.S.D)	Accuracy (Error %)
Paroxetine	4	9	3.70 ± 0.50	14.9	13.3
	40	9	40.10 ± 4.90	12.1	10.1
	80	9	82.90 ± 7.70	9.3	8.5
HM paroxetine	6	9	5.90 ± 0.90	14.6	11.7
	40	9	43.80 ± 5.00	11.4	13.6
	80	9	90.20 ± 10.60	11.8	13.1

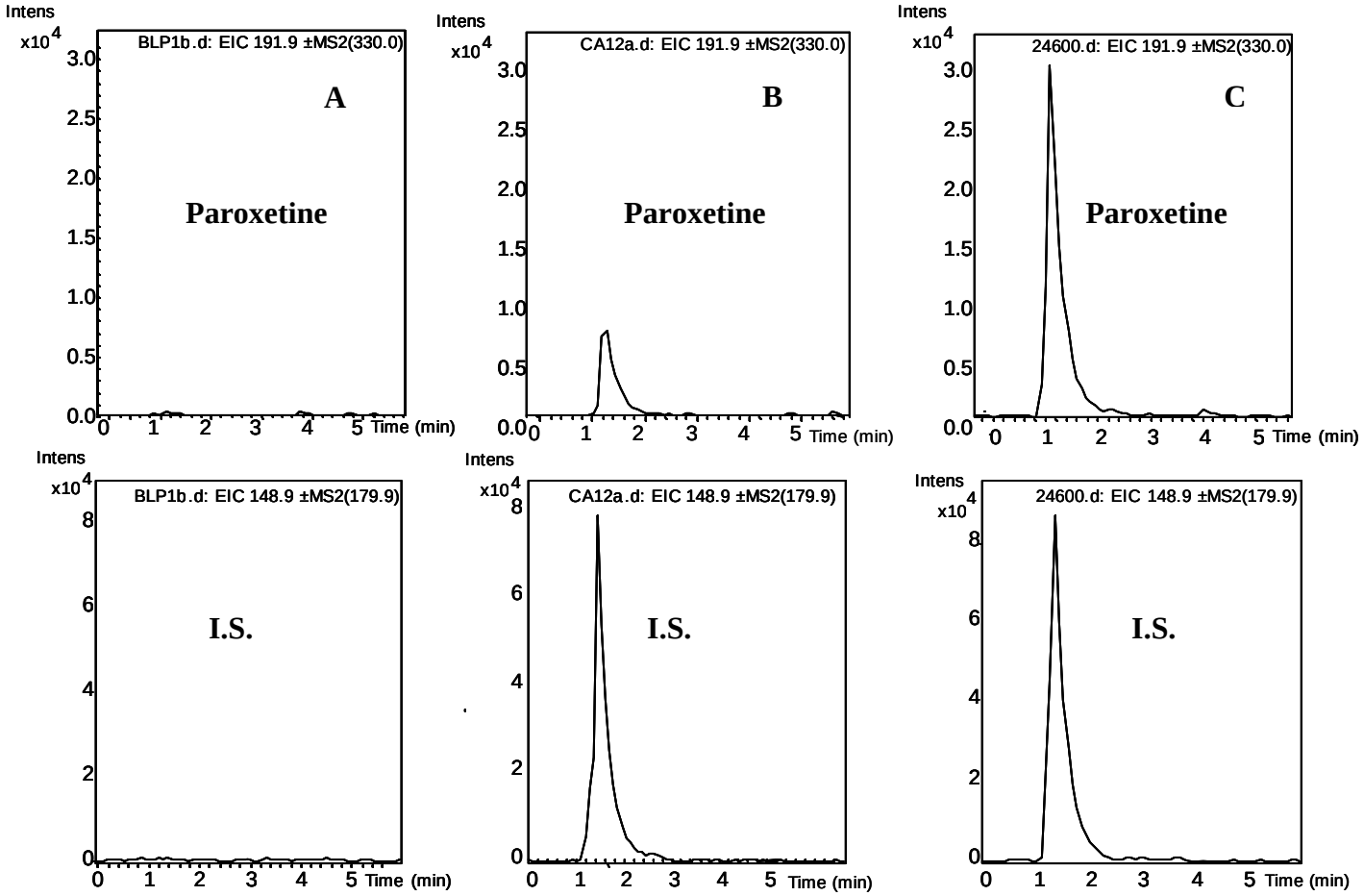
Table 3

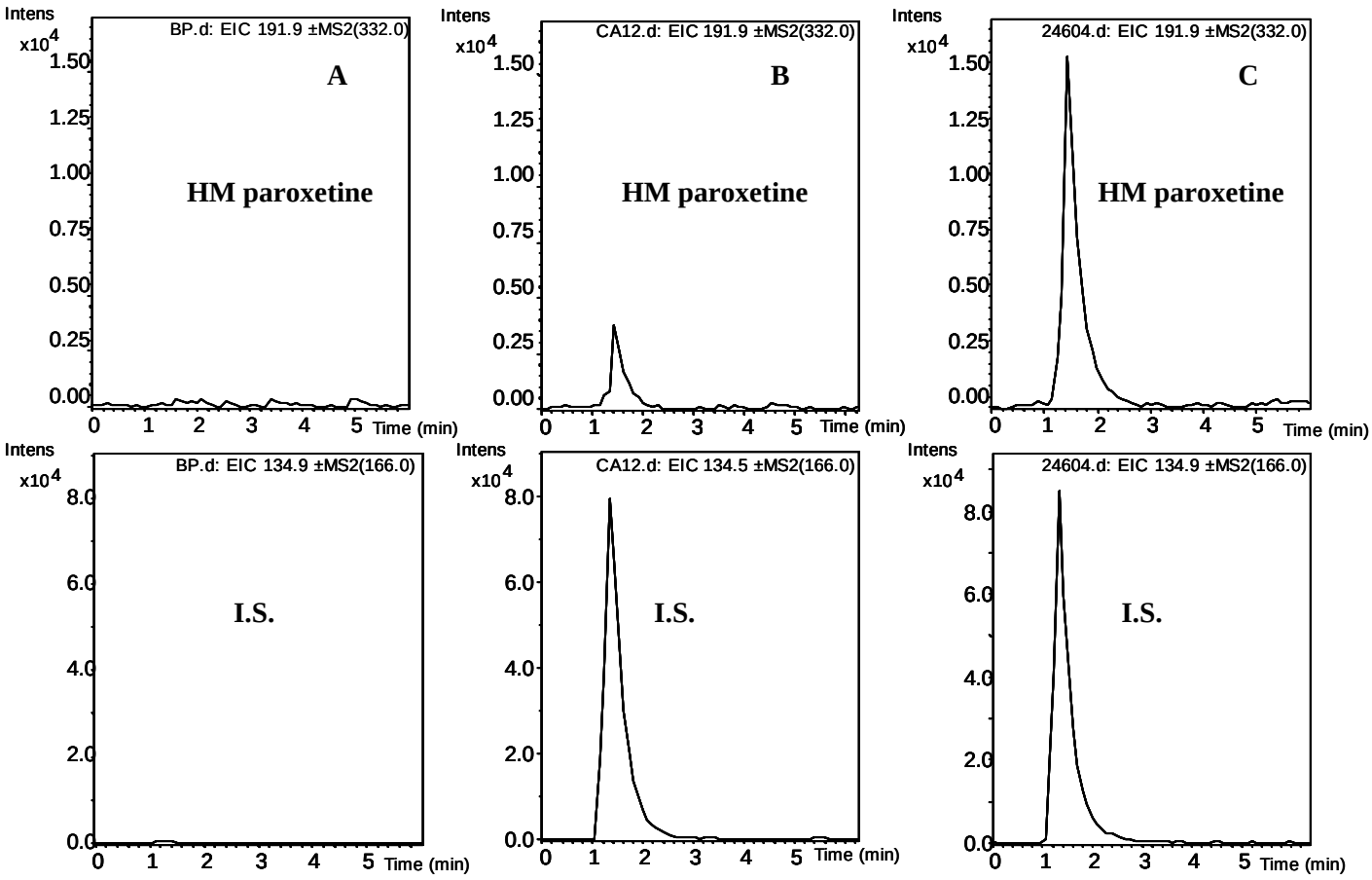
Pharmacokinetic parameters for paroxetine and HM paroxetine (mean ± SD, n=9)

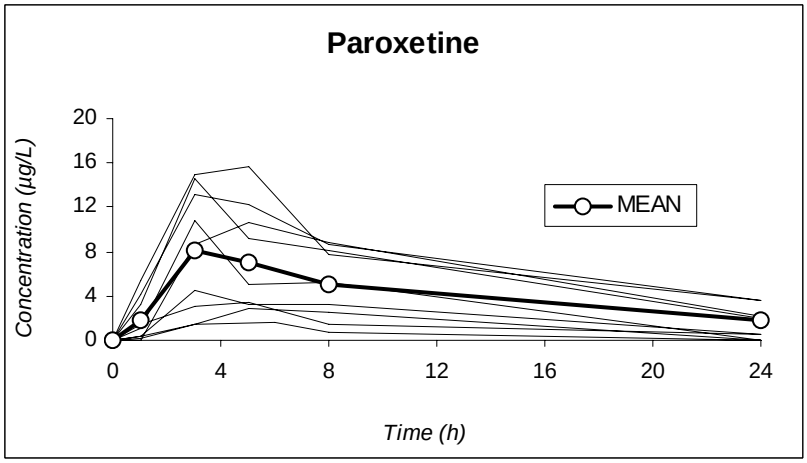
Compound	Paroxetine	HM paroxetine
C_{max} (µg/L)	8.60 ± 5.50	92.40 ± 39.60
T_{max} (h)[a]	5 (3-5)	3 (3-5)
K_e (h^{-1})	0.080 ± 0.013	0.096 ± 0.026
$T_{1/2}$ (h)	8.80 ± 1.50	7.80 ± 2.40
AUC $_{(0\text{-}24h)}$ (µg/L.h)	96.50 ± 65.90	988.10 ± 467.80

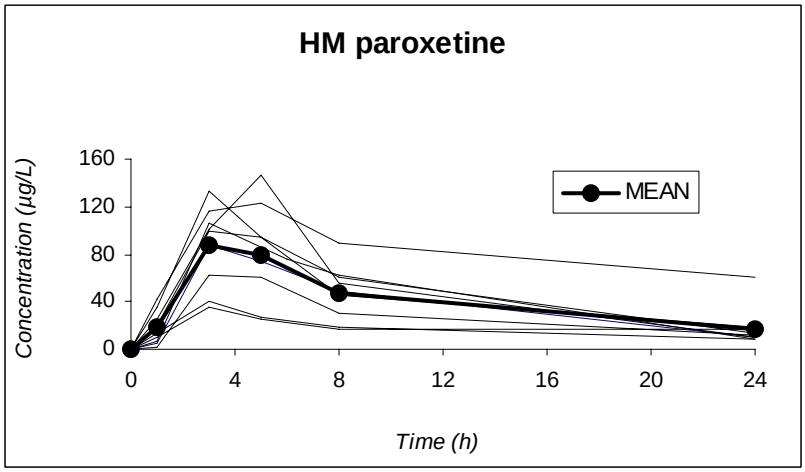
[a] Expressed as a median (range).

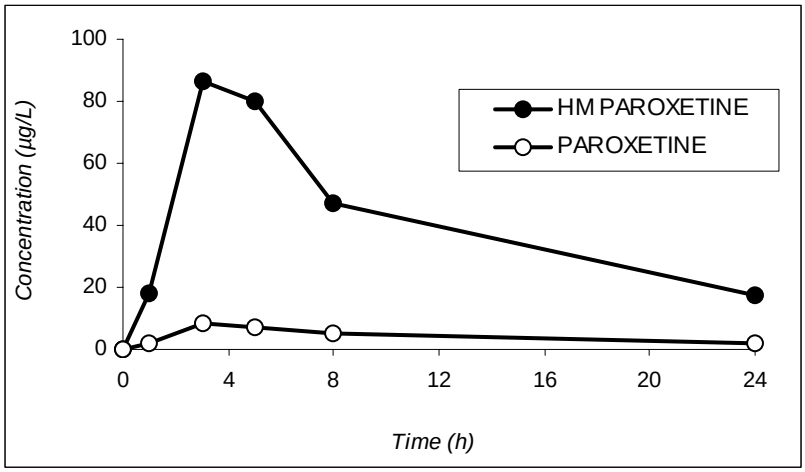

Intens.
x10^6
paroxetina.d: +MS2(330.0)
A
191.8
150.8
122.9
174.8
207.8
330.1
F
191.8
150.8
O
O
O
207.8
N
H
HMparoxetina.d: +MS2(332.0)
B
191.8
152.7
108.9
174.8
315.1
332.1
F
191.8
OH
152.7
O
O
N
H
Methoxyphenamine.d: +MS2(179.9)
C
148.8
120.9
148.8
120.9
O
N
H
x10^5
Pholedrine.d: +MS2(166.0)
D
134.8
106.9
OH
106.9
134.8
N
H
50
100
150
200
250
300
350
m/z


Intens
x10 4
BLP1b.d: EIC 191.9 ±MS2(330.0)
A
Paroxetine
Time (min)
Intens
x10 4
CA12a.d: EIC 191.9 ±MS2(330.0)
B
Paroxetine
Time (min)
Intens
x10 4
24600.d: EIC 191.9 ±MS2(330.0)
C
Paroxetine
Time (min)
Intens
x10 4
BLP1b.d: EIC 148.9 ±MS2(179.9)
I.S.
Time (min)
Intens
x10 4
CA12a.d: EIC 148.9 ±MS2(179.9)
I.S.
Time (min)
Intens
x10 4
24600.d: EIC 148.9 ±MS2(179.9)
I.S.
Time (min)


Intens
x10^4
BP.d: EIC 191.9 ±MS2(332.0)
A
HM paroxetine
CA12.d: EIC 191.9 ±MS2(332.0)
B
HM paroxetine
24604.d: EIC 191.9 ±MS2(332.0)
C
HM paroxetine
BP.d: EIC 134.9 ±MS2(166.0)
I.S.
CA12.d: EIC 134.5 ±MS2(166.0)
I.S.
24604.d: EIC 134.9 ±MS2(166.0)
I.S.
Time (min)


Paroxetine
Concentration (µg/L)
20
16
12
8
4
0
0
4
8
12
16
20
24
Time (h)
MEAN


HM paroxetine
Concentration (µg/L)
160
120
80
40
0
0
4
8
12
16
20
24
Time (h)
MEAN


Concentration (µg/L)
100
80
60
40
20
0
0
4
8
12
16
20
24
Time (h)
HM PAROXETINE
PAROXETINE