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Bioanalysis of mosapride by a novel LC–MS/MS method by using solid phase extraction technique: a pharmacokinetic application in Indian subjects

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ABSTRACT

In this paper the authors proposed a simple, sensitive and selective liquid chromatography–tandem mass spectrometry (LC–MS/MS) assay method for the determination of mosapride in human plasma. This method employed mosapride–d5 as an internal standard (IS). Analyte and the IS were extracted form 100 µL of human plasma using solid–phase extraction with no drying, evaporation and reconstitution steps. The chromatographic separation was achieved on a C_{18} column by using a mixture of methanol and 5mM ammonium acetate (80:20, v/v) as the mobile phase at a flow rate of 1.0 mL/min. The linearity of the method was established in the concentration range 0.18–60.0 ng/mL with $r^2 \geq 0.99$. The intra–day and inter–day precision (%CV) and accuracy results in five validation batches across five concentration levels were well within the acceptance limits. The validated method was found to be applicable to clinical studies.

Keywords: Mosapride, Human plasma, Solid–phase extraction (SPE), LC–MS/MS, Pharmacokinetics

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INTRODUCTION

Mosapride is a selective 5–HT4 receptor agonist which is used as a gastroprokinetic agent [1, 2]. In general the drugs in recommended to take on an empty stomach [3], which accelerates the gastric emptying rate and is clinically used in the treatment of gastritis, dyspepsia [4], irritable bowel syndrome [5] and gastro–oesophageal reflux.

As per the literature, few analytical methods have been reported for the determination of mosapride, including liquid chromatography–mass spectrometric methods [1, 2, 6–9] and a high performance liquid chromatographic (HPLC) method [10]. Most of the analytical methods reported so far were only for investigation of pharmacokinetics of the drug and/or for preclinical application. Of all the above, only two methods are comparable with the present report. Ramakrishna *et al.*, [8] reported a LC–MS/MS method for the determination of mosapride in human plasma samples with an LLOQ of 0.5 ng/mL. This method is not sensitive enough and utilizes 0.5 mL plasma sample volume which may not be favorable for routine drug analysis for a pharmacokinetic/bioequivalence study. One more author, Qin *et al.*, [9] reported a LC–MS/MS method for the determination of mosapride in human plasma with the concentration range of 0.17–68.0 ng/mL. Both of the methods [8, 9] employ multi step liquid–liquid extraction (LLE) procedure for the sample preparation and utilized tamsulosin as internal standard. Moreover, the method proposed by Qin *et al.*, [9] involving complexity like gradient elution and longer chromatographic run time (>3 min).

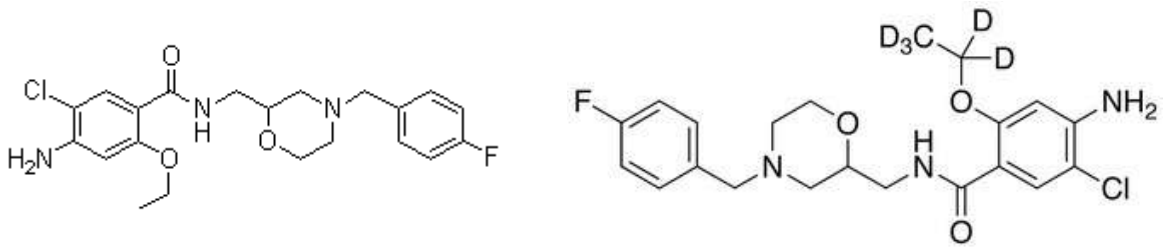
The present paper describes a simple, rapid and sensitive liquid chromatography coupled with electrospray ionization (ESI) tandem mass spectrometry method for the determination of mosapride in 100 µL of human plasma with a chromatographic run time 2.0 min. This method employs solid–phase extraction (SPE) technique for sample preparation. We have also employed mosapride–d5 as an internal standard to avoid the potential matrix effect related problems and variability in recovery between analyte and IS. The application of this assay for a clinical pharmacokinetic study following oral administration of mosapride is described.

MATERIALS AND METHODS

Standards and chemicals

Mosapride reference sample (99.8%) was obtained from SimSon Pharma (Hyderabad, India). Mosapride–d5 (98.2%) was employed as an internal standard and was obtained from Vivan Life Sciences Limited (Mumbai, India). Their chemical structures are shown in Figure. 1. HPLC grade methanol was purchased from J.T. Baker (Phillipsburg, USA). Analytical grade

ammonium acetate was purchased from Merck Ltd (Mumbai, India). Water used for the LC–MS/MS analysis was prepared by using Milli Q water purification system procured from Millipore (Bangalore, India). The control human plasma sample was procured from Deccan's Pathological Lab's (Hyderabad, India).



Mosapride **Mosapride–d5**

Figure 1. Chemical structures of mosapride and mosapride–d5 (IS).

LC–MS/MS instrument and conditions

An HPLC system (Shimadzu, Kyoto, Japan) consisting of a Zorbax SB–C_{18} column (50 mm × 4.6 mm, 3.5 µm), a binary LC–20AD prominence pump, an auto sampler (SIL–HTc) and a solvent degasser (DGU–$20A_3$) was used for the study. Aliquot of 10 µL of the processed samples were injected into the column, which was kept at room temperature (20±5°C). An isocratic mobile phase consisting of a mixture of methanol and 5mM ammonium acetate (80:20, v/v) was used to separate the analyte from the endogenous components. The mobile phase was pumped at a flow rate of 1.0 mL/min into the electrospray ionization chamber of the mass spectrometer. MS–MS quantification was achieved in positive ion mode for the analyte and the IS using an MDS Sciex API–4000 mass spectrometer (Foster City, CA, USA) equipped with a Turboionspray ™ interface at 550 °C. The ion spray voltage was set at 5500 V. The source parameters viz. the nebulizer gas (GS1), auxiliary gas (GS2), curtain gas and collision gas were set at 35, 40, 20, and 10 psi, respectively. The compound parameters viz. the declustering potential (DP), collision energy (CE), entrance potential (EP) and collision cell exit potential (CXP) were 106, 30, 10, 11 V for mosapride and 106, 30, 10, 11 V for the IS. Detection of the ions was carried out in the multiple–reaction monitoring mode (MRM), by monitoring the transition pairs of *m/z* 422.2 precursor ion to the *m/z* 198.0 for mosapride and *m/z* 427.4 precursor ion to the *m/z* 203.0 product ion for the IS. Both the quadrupoles (Q1 and Q3) were set on unit resolution. The analysis data obtained were processed by Analyst Software™ (version 1.4.2).

Preparation of stock and working solutions

Primary stock solutions of mosapride were prepared separately in HPLC grade methanol (1 mg/mL) for the preparation of calibration standards and quality control samples. Working solutions of mosapride were prepared in a mixture of methanol and water (70:30, v/v; diluent). A 1 mg/mL of mosapride–d5 stock solution was prepared by dissolving the compound in HPLC grade methanol. The working concentration of mosapride–d5 (503 ng/mL) was prepared form the above stock solution using the diluent.

Preparation of calibration curve standards and quality control samples in human plasma

Calibration samples were prepared by spiking control K_2 EDTA human plasma with the working solution of the analyte as a bulk, to obtain mosapride concentration levels of 0.18, 0.36, 1.02, 2.56, 6.39, 12.8, 25.5, 36.5, 48.0 and 60.0 ng/mL as a single batch at each concentration. Similarly, quality control (QC) samples were also prepared as a bulk based on an independent weighing of standard drug, at concentrations of 0.18 (LLOQ), 0.52 (low; LQC), 7.28 (middle; MQC–1), 30.3 (MQC–2) and 52.1 ng/mL (high; HQC) as a single batch at each concentration. All the plasma samples were stored in freezer at -70 ± 10^0C until analyses.

Sample processing

A 100 µL aliquot of human plasma sample was mixed with 10 µL of the internal standard working solution (503 ng/mL of mosapride–d5). To this, 100 µL of HPLC grade water was added after vortex mixing for 10 s. The sample mixture was loaded onto an Oasis HLB cartridge (30 mg/1 mL) that was pre–conditioned with 1.0 mL of methanol followed by 1.0 mL of water. The extraction cartridge was washed with 1.0 mL of 50mM ammonium acetate buffer followed by 1.0 mL of water. Analyte and the IS were eluted with 0.5 mL of mobile phase. Aliquot of 10 µL of the extract was injected into the chromatographic system.

Bioanalytical method validation

A thorough and complete method validation of mosapride in human plasma was carried out as per US FDA guidelines [11]. The parameters determined were selectivity, specificity, sensitivity, matrix effect, linearity, precision, accuracy, recovery, dilution integrity and stability.

Pharmacokinetic study design

A pharmacokinetic study was performed in healthy male subjects (n = 6). All the volunteers provided with written informed consent and were fasted for 12 h before the drug formulation administration. Blood samples were collected following oral administration of mosapride citrate (5 mg tablet) at pre–dose and 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16 and 24 h and collected in K2 EDTA vacutainer (5 mL) collection tubes (BD, Franklin, NJ, USA). The

tubes were centrifuged at 3200 rpm for 10 min and the supernatant plasma was collected. The collected plasma samples were stored at –70 ± 10 °C till their use. These samples were spiked with the IS and processed as per the extraction procedure described earlier. WinNonlin Version 5.2 was used to calculate the pharmacokinetic parameters of mosapride by a non–compartmental model. An ISR was also performed by selecting the 2 samples from each subject (12 samples in total) near C_{max} and the elimination phase (3*LLOQ) in the pharmacokinetic profile of the drug. The ISR values were compared with the initial values. The values are considered to be stable when the percent change should not be more than ±20% [12, 13].

RESULTS AND DISCUSSION

Mass spectrometry

Electrospray ionization source (ESI) was used to develop the present method. Initially, analyte and the IS were tuned in positive and negative ionization modes using tuning solution (100 ng/mL), but the response obtained in positive mode much higher than the negative mode. Protonated form of analyte and IS, $[M+H]^+$ ion was the parent ion in the Q_1 spectrum and was used as the precursor ion to obtain Q_3 product ion spectra. The most sensitive mass transition was observed from m/z 422.2 to 198.0 for mosapride and from m/z 427.4 to 203.0 for the IS. The dwell time for each transition was set at 200 ms.

Method development

Various mobile phase compositions with methanol and acetonitrile were tried in different volume ratio in combination with buffers like ammonium formate, ammonium acetate (2mM–10mM), as well as acid additives acetic acid and formic acid (0.01–0.1%). A mobile phase composition with ammonium acetate buffer helped in achieving the symmetric peak shape and improved the analyte detection.

The response obtained with acetonitrile and ammonium acetate as a mobile phase was satisfactory; but not reproducible. Additionally, a variety of chromatographic columns like C_8 and C_{18} of different makes (Zorbax SB C_{18}, 50 × 4.6, 3.5µm; Zorbax XDB–phenyl 75 × 4.6, 3.5µm; Kromasil 100–5C_{18}, 100 × 4.6, 5µm; Ace 3 C_{18} 50 × 4.6, 3µm; Alltima HP C_{18} 50 × 4.6, 3 µm; Hypurity advance 100 × 4.6, 5 µm; Supelco HS C_{18} 100 × 4.6, 5µm) were tested to achieve adequate retention time with short run time, better separation form endogenous components, symmetric peak shape and satisfactory response for the analyte. The best chromatographic conditions were achieved with methanol and 5mM ammonium acetate (80:20, v/v) as a mobile phase under isocratic conditions as it most appropriate to give best sensitivity,

efficiency and peak shape. Zorbax SB C_{18}, (50 × 4.6, 3.5µm) column gave good peak shape and adequate response even at lowest concentration (0.18 ng/mL) level for the analyte. The retention time of analyte and the IS obtained with the above optimized chromatographic conditions were low enough (0.80 and 0.80 min) allowing short run time of 2.0 min.

The earlier reports have employed liquid–liquid extraction (LLE) procedure [8, 9] to extract mosapride from human plasma. Hence, protein precipitation (PP) was tested with methanol/acetonitrile under acidic and basic conditions. But, the response was inconsistent with un–acceptable chromatography especially at the LQC level due to ion suppression and matrix effect. Clean samples are essential for minimizing ion suppression and matrix effect for LC–MS/MS analysis. Solid phase extraction (SPE) is helpful in producing a clean sample with no or minimal matrix effect. Therefore, SPE was tried with Oasis HLB, Starata–X and Orpheus C_{18} extraction cartridges with/without acidic buffer addition to obtain the clean sample and to remove the endogenous interferences. But, the promising results were obtained with Oasis HLB extraction cartridges, which can produce a clean chromatogram for a blank sample and yields the maximum recovery for the analyte from the plasma. Use of mobile phase as an eluting solvent helped in achieving reproducible and quantitative recovery for the analyte devoid of drying and reconstitution steps. Mosapride stable labeled isotope, mosapride–d5 was used as an IS to increase assay precision and limit variable recovery between analyte and the IS.

Selectivity and sensitivity

The selectivity of the method was examined by analyzing blank human plasma extract (Fig. 2A) and a blank plasma extract spiked only with the IS (Fig. 2B). As shown in Fig. 2A, no significant direct interference in the blank plasma traces was observed from endogenous substances in drug–free human plasma at the retention time of the analyte and the IS. Similarly, Fig. 2B shows the absence of direct interference from the IS to the MRM channel of the analyte. Fig. 2C depicts a representative ion–chromatogram for the LLOQ sample (0.18 ng/mL). Fig. 3 depict a representative chromatograms resulting from the analysis of subject blank plasma sample and 0.5 h subject plasma sample after the administration of a 5 mg oral single dose of mosapride tablet.

The lowest limit of reliable quantification for the analyte was set at the concentration of the LLOQ (0.18 ng/mL). The precision and accuracy at LLOQ concentration were found to be 6.03% and 113%, respectively.

Matrix effect

Matrix effect assessment was done with the aim to check the effect of different lots of plasma on the back calculated value of QC's nominal concentration. The precision and accuracy for

mosapride at LQC concentration were found to be 0.81% and 101%, and at HQC level they were 0.31% and 103%, respectively. No momentous matrix effect was observed in all the six batches of human plasma.

Linearity, precision and accuracy

The analyte showed good linearity in the concentration rage of 0.18–60.0 ng/mL. Both the regression models (1/x and $1/x^2$) were compared and best fit for the concentration–detector response relationship was obtained with a weighting factor of $1/x^2$. The mean correlation coefficient values were >0.99 for all the analytical runs generated during entire course of validation.

The intra–day and inter–day precision and accuracy results in plasma QC samples are summarized in Table 1. The precision (% CV) and accuracy values of mosapride for intra– and inter–day ranged from 0.97–3.97% and 99.4–106%, and 2.05–4.14% and 98.5–106%, respectively. The results revealed good precision and accuracy.

Table 1 Precision and accuracy data for mosapride

Quality control	Run	Concentration found	% RSD	Accuracy(%)
Intra–day variations (12 replicates at each concentration)				
LLOQ QC		0.19 ± 0.01	3.97	104
LQC		0.55 ± 0.02	3.68	106
MQC1		7.62 ± 0.10	1.31	105
MQC2		30.2 ± 0.29	0.97	99.4
HQC		55.1 ± 0.85	1.54	106
Inter–day variations (30 replicates at each concentration)				
LLOQ QC		0.19 ± 0.01	3.06	105
LQC		0.56 ± 0.02	2.87	106
MQC1		7.41 ± 0.31	4.14	102
MQC2		29.9 ± 0.61	2.05	98.5
HQC		53.9 ± 2.13	3.95	103

Spiked concentrations of LLOQ QC, LQC, MQC1, MQC2 and HQC are 0.18, 0.52, 7.28, 30.34 and 52.13 ng/mL, respectively.

Recovery and dilution integrity

The mean overall recovery of mosapride was 89.0±1.81% with the precision range of 1.36–4.31% and the recovery of IS was 90.4% with the precision range of 1.71–3.76%. The upper concentration limit of mosapride can be extended to 100 ng/mL for by 1/2 and 1/4 dilutions with screened human blank plasma. The precision (%CV) for dilution integrity of 1/2 and 1/4 dilution was found to be 2.46% and 0.56%, while the accuracy results were found to be 110% and 100%, respectively.

Stability studies

The drug stability at various conditions was evaluated. In the different stability experiments carried out viz. bench top stability (13 h), autosampler stability (45 h), wet extract stability (42 h), repeated freeze–thaw cycles (4 cycles), reinjection stability (28 h) and long term stability at –70 °C for 120 days the mean % nominal values of the analyte were found to be within ±15% of the predicted concentrations for the analyte at their LQC and HQC levels (Table 2). Therefore, the results were found to be within the acceptable limits during the entire validation.

Table 2 Stability data for mosapride in plasma (*n*=6)

Stability test	QC spiked concentration (ng/mL)	Mean ± SD (ng/mL)	Precision (%)	Accuracy/ Stability (%)
Process[a]	0.52	0.56 ± 0.01	2.66	107
	52.1	57.2 ± 0.37	0.64	110
Process[b]	0.52	0.57 ± 0.02	3.78	109
	52.1	58.1 ± 1.66	2.85	111
Bench top[c]	0.52	0.56 ± 0.01	1.40	107
	52.1	57.0 ± 0.14	0.25	109
FT[d]	0.52	0.57 ± 0.01	1.40	109
	52.1	57.8 ± 0.40	0.70	111
Reinjection[e]	0.52	0.57 ± 0.01	2.09	108
	52.1	56.2 ± 1.59	2.84	108
Long–term[f]	0.52	0.56 ± 0.01	1.77	108
	52.1	52.2 ± 0.18	0.34	100
Long–term[g]	0.52	0.56 ± 0.01	2.03	106
	52.1	52.2 ± 0.29	0.56	100

[a] after 45 h in autosampler at 10°C; [b] after 42 h at 2–8°C; [c] after 13 h at room temperature; [d] after 4 freeze and thaw cycles; [e] after 32 h of Reinjection; [f] at –70°C for 120 days; [g] at –20°C for 120 days

Pharmacokinetic study and incurred samples reanalysis

The present method was successfully used to quantify mosapride plasma concentrations for a pharmacokinetic study in healthy South Indian adult male subjects (*n*=6). Fig. 4 depicts the mean plasma concentration *vs* time profile of mosapride after administration of a single 5 mg oral dose of mosapride under fasting condition. The maximum concentration (C_{max}) in plasma (31.8±2.76 ng/mL) for mosapride was attained at 0.67±0.20 h (t_{max}). The area under the plasma concentration–time curve from time zero to last measurable time point (AUC_{0-t}) and area under the plasma concentration time curve from time zero to infinity time point (AUC_{0-inf}) for

mosapride were 72.3±11.27 and 74.5±11.67 ng*h/mL, respectively. The terminal half–life (*t*½) was found to be 6.31±1.32 h.

The reproducibility of the present method was established by reanalysis of incurred samples (ISR). The differences in concentrations between the ISR and the initial values for all the tested samples were less than 20% (Table 3), indicating good reproducibility of the present method.

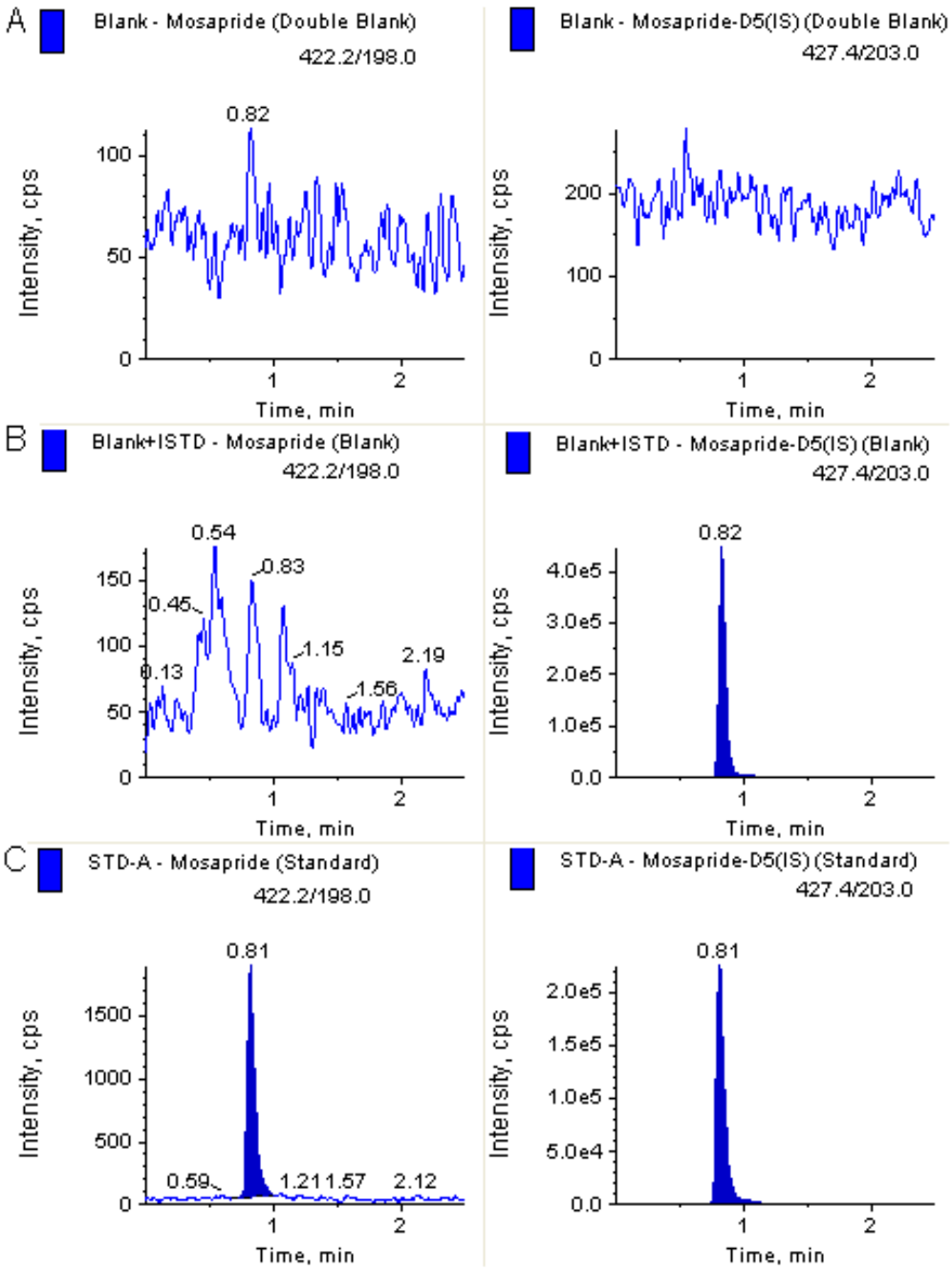


Figure 2. Typical MRM chromatograms of mosapride (left panel) and IS (right panel) in human blank plasma (A), and human plasma spiked with IS (B), a LLOQ sample along with IS (C).

Table 3 Incurred samples re–analysis data of mosapride

Sample	Initial conc. (ng/mL)	Re–assay conc. (ng/mL)	Difference [a] (%)
1	20.4	22.6	–10.5
2	0.58	0.68	–16.5
3	28.6	25.1	13.2
4	0.79	0.72	9.26
5	34.2	32.9	3.74
6	1.02	1.11	–8.10
7	23.2	20.9	10.3
8	0.96	0.90	6.58
9	30.5	32.0	–4.93
10	0.74	0.80	–7.94
11	28.9	33.2	–14.0
12	0.72	0.70	3.80

[a] Expressed as [(initial conc.−re–assay conc.)/average]×100%.

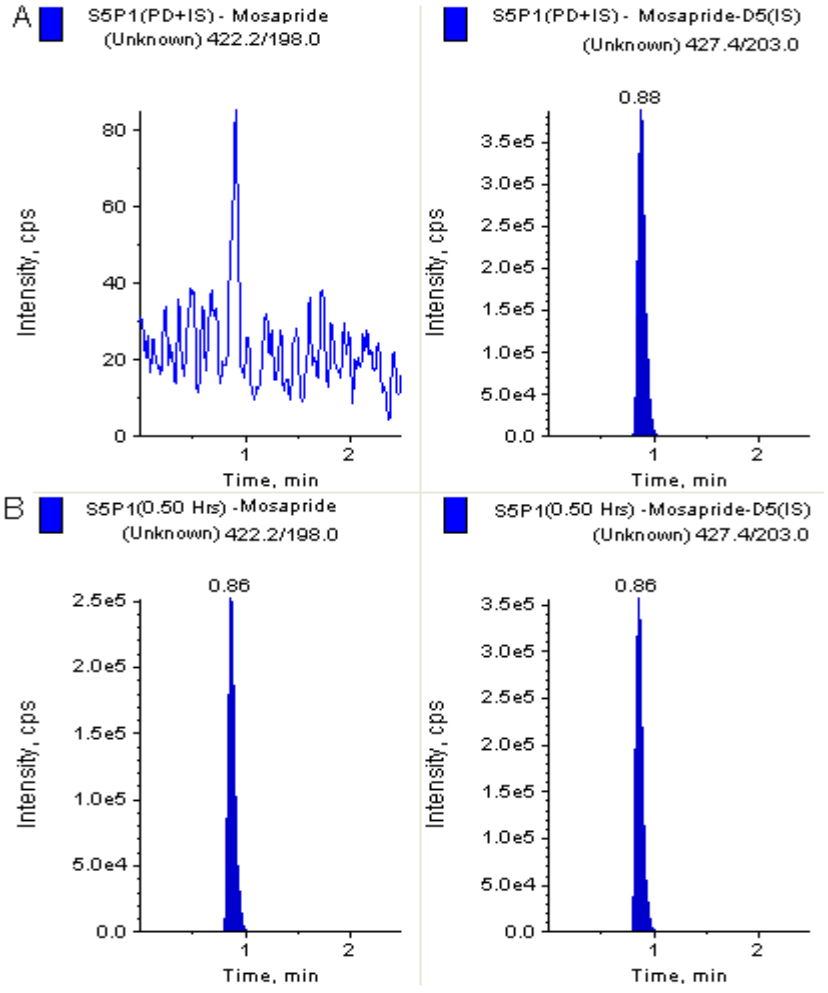


Figure 3. MRM chromatograms resulting from the analysis of subject blank plasma sample along with the IS (A) and 0.5 h subject plasma sample (B), after the administration of a 5 mg oral single dose of mosapride tablet. The sample concentration was determined to be 30.5 ng/mL.

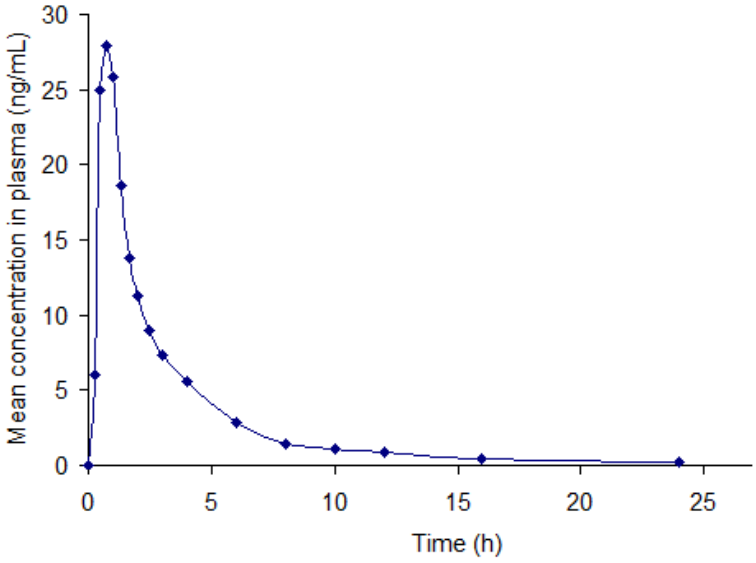


Figure 4. Mean plasma concentration–time profile of mosapride in human plasma following oral dosing of mosapride (5 mg tablet) to healthy volunteers (*n*=6).

CONCLUSIONS

The proposed LC–MS/MS method is sensitive, specific and simple for the quantitative determination of mosapride in human plasma. This method is wholly validated as per the US FDA guidelines. The method presented has the better sensitivity (0.18 ng/mL) and employs low plasma volume (100 µL) for processing compared to other reported procedures. Moreover, the total analysis time (chromatography and extraction) is the shortest. Thus, the advantage of this method is that a relatively more number of samples can be analyzed in short time, thus increasing the output. A simple SPE technique with direct injection (avoids drying, evaporation and reconstitution steps) for sample preparation, thereby significantly reduces the sample processing time. From the results of all the validation parameters, we can conclude that the developed method can be useful for bioavailability and bioequivalence (BA/BE) studies and routine therapeutic drug monitoring with the desired precision and accuracy.

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