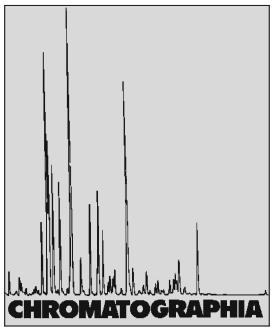
LC–MS Simultaneous Determination of Itopride Hydrochloride and Domperidone in Human Plasma



Anirbandeep Bose, Uttam Bhaumik, Animesh Ghosh, Bappaditya Chatterjee, Uday Sankar Chakrabarty, Amlan Kanti Sarkar, Tapan Kumar Pal✉

Department of Pharmaceutical Technology, Bioequivalence Study Centre, Jadavpur University, Kolkata 700 032, India; E-Mail: tkpal12@gmail.com

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Abstract

A rapid, simple, sensitive and specific liquid chromatography–tandem mass spectrometry method was developed and validated for simultaneous quantification of itopride hydrochloride and domperidone in human plasma. Both drugs were extracted by liquid–liquid extraction with ethyl acetate and saturated borax solution. The chromatographic separation was performed on a reversed-phase C18 column with a mobile phase of water–methanol (2:98, *v*/*v*) containing 0.5% formic acid. The protonated analyte was quantitated in positive ionization by multiple reaction monitoring with a mass spectrometer. The assay exhibited linearity over the concentration range of 3.33–500 ng mL^{-1} for itopride hydrochloride and 3.33–100 ng mL^{-1} for domperidone in human plasma. The precursor to product ion transitions of *m*/*z* 359.1–72.3 and 426.0–147.2 were used to measure itopride hydrochloride and domperidone respectively. The method was found suitable for the analysis of plasma samples collected during phase 1 pharmacokinetics study of itopride HCl 50 mg and domperidone 20 mg in 12 healthy volunteers after single oral doses of the combination drug.

Keywords

Column liquid chromatography
Tandem mass spectrometry
Validation
Itopride hydrochloride
Domperidone

Introduction

Gastro-esophageal reflux disease (GERD) is one of the most prevalent upper gastrointestinal disorders [1]. Several major advances have been made in the medical management of GERD. Acid suppression is the main-stay therapy for GERD and proton pump inhibitors (PPIs) provide the rapid symptomatic relief and heals esophagitis in a high proportion of patients [2]. The novel gastroprokinetic agent, itopride hydrochloride (*N*-[4-[2-(dimethylamino)-ethoxy]benzyl]-3,4-dimethoxybenzamide hydrochloride), stimulates gastrointestinal motor activity through the synergistic blockade of dopamine D_2 receptor and inhibition of acetylcholine esterase [3, 4]. Itopride is a newly developed prokinetic drug. It is mostly used in gastroesophegal reflux disorder disease, which is most prevalent upper gastrointestineal disorder. Itopride hydrochloride is prescribed for the gastrointestinal symptoms caused by reduced gastrointestinal mobility i.e., a feeling of gastric fullness, upper abdominal pain,

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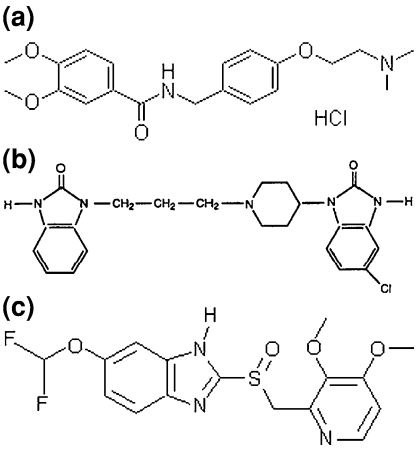

Fig. 1. a Chemical structure of itopride HCl. **b** Chemical structure of domperidone. **c** Chemical structure of pantoprazole

anorexia, heartburn, nausea and vomiting caused by conditions like non-ulcer dyspepsia or chronic gastritis [5–7]. Domperidone is a dopamine-2 receptor antagonist. It acts as an antiemetic and a prokinetic agent through its effects on the chemoreceptor trigger zone (CTZ) and motor function of the stomach and small intestine. Unlike metoclopramide, it does not cause any adverse neurological symptoms as it has minimal penetration through the blood-brain barrier. It thus provides an excellent safety profile for long-term administration orally in the recommended doses. Domperidone is widely used in many countries and can now be officially prescribed to patients in the United States by an investigational new drug application for the treatment of gastroparesis and any condition causing chronic nausea and vomiting. In view of this additional clinical exposure of domperidone to a new generation of gastroenterologists and other specialists, the purpose of this timely review is to revisit the pharmacological, clinical application and safety profile of this beneficial medication. The combination of itopride and domperidone perform better in GERD due to a blockade of dopamine D_2 receptor and inhibition of acetylcholine esterase. In one of our studies, we had formulated a bilayer tablet containing itopride hydrochloride and domperidone as a fixed dose combination product. So an analytical method for the simultaneous determination of both the analytes in plasma was necessary. Figure 1 shows the chemical structures of itopride hydrochloride, domperidone and pantoprazole (IS) used in this study. The literature survey revealed the main pharmacokinetic parameters of domperidone 20 mg after oral administration: $T_{max} = (0.8 \pm 0.7)$ h, $C_{max} = (50 \pm 32)$ ng mL^{-1}, $T_{1/2} = (7.8 \pm 1.6)$ h [8]. The pharmacokinetic parameters obtained after oral administration of itopride HCl dose of 50 mg are: $T_{max} = (0.81 \pm 0.37)$ h, $C_{max} = (230.05 \pm 101.84)$ ng mL^{-1}, $T_{1/2} = (3.42 \pm 0.54)$ h [9]. Determination of itopride hydrochloride viz LC–MS has been reported in the literature [8]. An ideal method should have simple sample preparation, fast on-column separation, sensitive and specific detection.

Literature survey revealed a few analytical methods for the determination of domperidone by LC–FLD (fluorescence detection), LC–MS–MS, LC–UV [9–11].

Liquid chromatography coupled with mass spectrometry (LC–MS–MS) is such an efficient analytical tool, which meets most of the above needs particularly in the simultaneous analysis of a fixed dose combination dosage form [12]. LC–MS–MS facilitates analyzing large samples in a very short period of time. Previously reported methods either had long retention times (10–15 min) or suffered from low sensitivity and in some cases required large sample injection volumes (100 µL). There are various methods reported for the determination of itopride hydrochloride and domperidone separately. To the best of our knowledge, there is no method reported in the literature for the simultaneous determination of these analytes. Hence the main objective of this work was to develop a simple, sensitive, rapid and reliable mass spectrometry (LC–MS–MS) method for the simultaneous quantification of itopride hydrochloride and domperidone in human plasma.

Experimental

Chemicals and Reagents

Itopride hydrochloride was obtained from Burgeon Life Science, Chennai, India; Domperidone was supplied by Kusum Healthcare, Punjab, India, as gift samples. LC grade ethyl acetate and methanol were purchased from Merck (Mumbai). LC grade water generated from Milli Q water purification system was used throughout the analysis.

Apparatus

The LC system consisting of solvent delivery LC 10ADVP, controller LC10ADVP and column oven CTO10ASVP from Shimadzu (Kyoto, Japan). Sample injection was using SIL HTC autosampler from Shimadzu (Kyoto, Japan). The mass spectrometer system used was a API 2000 triple quadrupole mass spectrometer (MDS Sciex, Canada) equipped with an electrospray ionization (ESI) source. Data acquisition was performed with Analyst 1.4.1. software. Chromatographic separation was achieved on a C8 column, 50 mm × 3 mm, 3 µm i.d (Phenomenex, USA).

Chromatographic Conditions

All the chromatographic analysis was carried out at ambient temperature. The composition of mobile phase used for separation of the analytes was water–methanol (2:98, *v*/*v*) containing 0.5% formic acid. The flow rate was set at 1 mL min^{-1}. The injection volume was 20 µL and the total run time was 5 min. The column was maintained at ambient temperature (23 °C) while the auto sampler temperature was set at 10 °C.

Mass Spectrometry

Electrospray ionization (ESI) with multiple reaction monitoring (MRM) was used to acquire the mass spectra of the compounds. All the ions were measured in positive ionization mode. The tuning parameters were optimized by injecting 100 ng mL^{-1} of standard solution containing all three drugs at 20 µL min^{-1} by means of an external syringe pump directly connected to the mass spectrometer. The turbo ion spray source

temperature was set at 500 °C and the turbo ion spray voltage was set at 5,500 V. The nebulizer gas (GS1), the turbo ionspray gas (GS2) and the curtain gas values were set at 30, 60 and 10 units respectively. The collision associated dissociation (CAD) gas value was fixed at 6 (arbitrary units).

Standard Solutions

Separate solutions containing 1 mg mL^{-1} of itopride, domperidone and IS were prepared using water, water–methanol (50:50 *v*/*v*), and methanol for the three drugs respectively. These solutions were further diluted with the mobile phase to obtain a stock solution of 10 μg mL^{-1}. The stock solutions prepared for the drugs were diluted further to obtain seven working solutions for calibration standards. All solutions were stored at 2–8 °C.

Calibration Curves

A standard calibration solution of itopride and domperidone was prepared by spiking blank plasma with appropriate amounts of analytes and IS (100 ng mL^{-1}). Standard curves were prepared in human plasma to yield final concentrations of 3.33, 10, 25, 50, 100, 250 and 500 ng mL^{-1} for the analytes of itopride HCl and 3.33, 10, 25, 50, 100 for the analytes of domperidone. Three quality control (QC) samples were prepared at three concentration levels of 5, 250 and 400 ng mL^{-1} for itopride HCl and 5, 50 and 80 ng mL^{-1} for domperidone. Calibration curves were plotted with peak area ratio of drug and IS on *Y*-axis and concentration on *X*-axis.

Sample Preparation and Extraction

Liquid–liquid extraction (LLE) was used as sample preparation procedure because it provides extracts with low levels of co-extracted matrix components, therefore resulting in minimal ion suppression for analyte quantitation. The protein precipitation is probably the fastest and easiest method of sample preparation, but not for ion suppression. Both technique have been tried in our experiments. The matrix effect found for itopride HCl, domperidone and internal standard, in case of LLE was 112.25, 92.75 and 94.50% respectively. In case of protein precipitation technique the same was found 125.26, 88.56, and 89.98% respectively. The major concern when using protein precipitation by means of organic solvent addition deals with sample dilution (the volumetric ratio plasma to organic solvent is usually 1/2). For improving detection limits, in such a case, the increase of the injection volume is mandatory. Once the injection volume was increased, the risk of column loading with residual matrix also increased. This may reduce column life-time but also affects ionization stage in terms of reproducibility, due to suppression/enhancement effects.

Liquid–liquid extraction procedure was used for the extraction of the drug from the plasma. Calibration standards, quality control samples were treated with a mixture containing 6 mL ethyl acetate and saturated borax (90:10). 100 μL of internal standard (100 ng mL^{-1}) were added to each plasma sample (0.50 mL) and vortex mixed for 2 min followed by centrifugation for another 10 min. The organic layer containing the analytes was separated, transferred to a separate test tube and evaporated to dryness under a stream of N_2 at 40 °C. The residue obtained on drying was reconstituted with 250 μL of mobile phase. The reconstituted sample was transferred to an autosampler vial and injected into the LC–MS–MS system.

Validation

The accuracy, sensitivity, precision, stability, recovery, reproducibility and reliability of the analytical method were confirmed by validation in accordance with the USFDA guidelines [13].

Linearity and LLOQ

To establish linearity, a series of calibration standards were prepared by adding a known concentration of itopride HCl, domperidone and IS to drug-free human plasma and analyzed. The lowest concentration on the standard curve with a detector response five times greater than the drug-free (blank) human plasma was considered as the LLOQ. The analyte peak in the LLOQ sample should be identifiable, discrete and reproducible with a precision of 20% and an accuracy of 80–120%.

Specificity

Specificity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. The specificity of the method was evaluated by screening six lots of blank plasma. These lots were spiked with known concentration of analytes along with IS at low, medium and high concentrations. The spiked samples were analyzed after extraction to confirm lack of interference and absence of batch-to-batch variation.

Accuracy and Precision

Intra-day precision and accuracy of the assay was evaluated by running three validation batches on three separate days. Each batch consisted of one set of calibration standards and five replicates of quality control (QC) samples at low, medium and high concentration. The inter-day precision and accuracy was also assessed in similar manner. A comparison was made between the obtained values and the experimental values. Precision was expressed as percentage of relative standard deviation (% RSD). The mean value of accuracy should be within 15% of the actual value except at LLOQ, where it should not deviate by more than 20%. The precision determined at each concentration level should not exceed 15% of RSD except for the LLOQ, where it should not exceed 20% of RSD.

Extraction Recovery and Matrix Effect

The extraction recovery of the analytes from the plasma was evaluated by comparing the mean detector responses of three replicates of processed QC samples

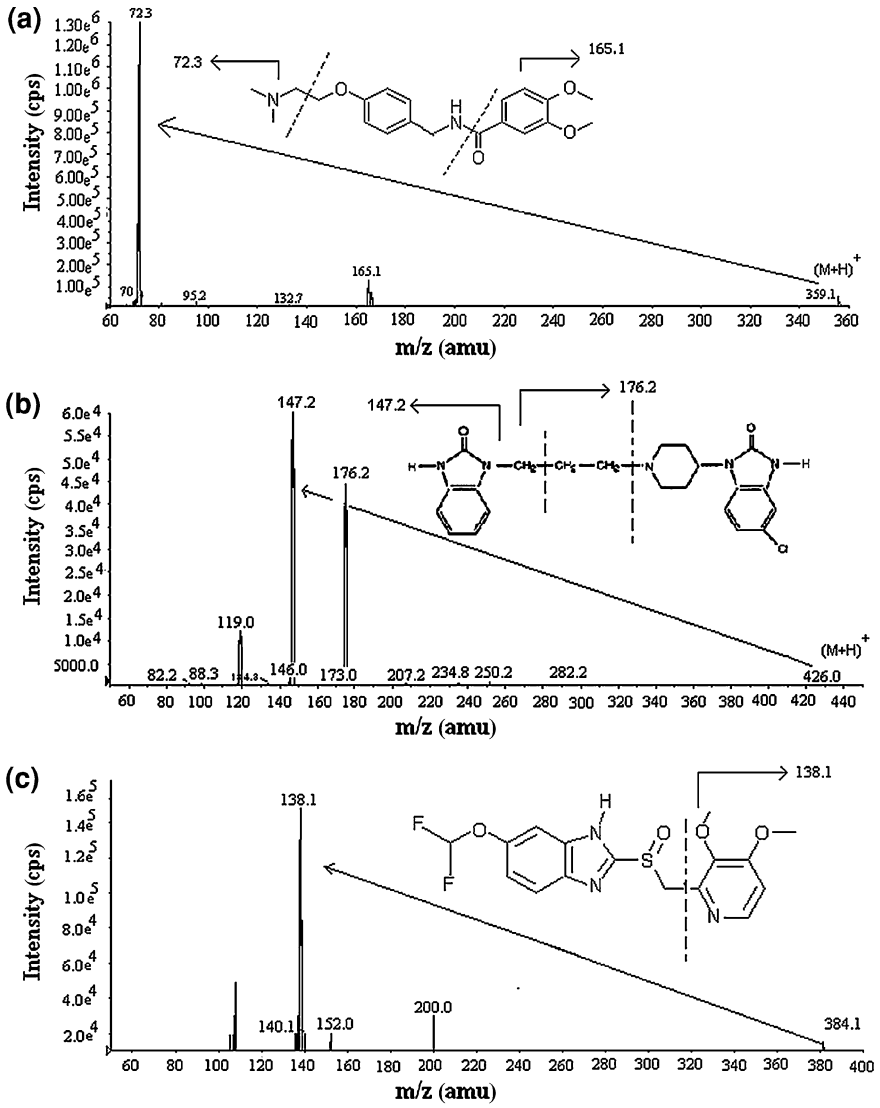

Fig. 2. **a** Product ion mass spectrum of itopride HCl (500 ng mL^{-1}). **b** Product ion mass spectrum of domperidone (500 ng mL^{-1}). **c** Product ion mass spectrum of pantoprazole (IS) (100 ng mL^{-1})

at low, medium high concentrations to the detector responses of standard solutions of same concentration. Recovery of an analyte need not be 100%, but the extent of recovery of analyte and the IS should be consistent, precise and reproducible [14].

Endogenous matrix components may change the efficiency of droplet formation or droplet evaporation, which in turn affects the amount of charged ion in the gas phase that ultimately reaches the detector [14–16]. The extraction recovery of itopride and domperidone (low, medium and high) from human plasma was evaluated by comparing the peak areas responses from plasma sample spiked with particular standard working solution of analyte before extraction with those from drug-free samples extracted and spiked with the same concentration of analyte after extraction. The recovery of IS at a single concentration of 100 ng mL^{-1} was determined in the same way. The number of replicates for each concentration was six.

The effect of plasma constituents over the ionization of analyte and IS was measured by matrix effect. It was determined by comparing the responses of the post-extracted spiked QC sample with the response of analyte from neat samples at equivalent concentration. Matrix effect was calculated as per the following equation

Matrix effect = (analyte peak area of post extracted spiked plasma QC samples/ analyte peak area of neat QC sample at same concentration) × 100%

Stability

Long and short term stability Three aliquots of each low and high QC sample were kept in a deep freezer at −20 ± 5 °C for 1 month. The samples were processed and analyzed and the concentrations obtained were compared to the actual value of QC samples to determine the long-term stability of analyte in human plasma.

Three aliquots each of high and low unprocessed QC samples were kept at ambient temperature 20–30 °C for 8 h in order to establish the short term stability of the analytes. The samples were analyzed and the concentrations obtained were compared to the actual values of QC samples. Samples were concluded stable if the % RSD of the stability samples was within ±15% of the actual value.

Post preparative stability Three aliquots each of high and low QC samples were stored at 10 °C in an autosampler for 24 h, analyzed and the concentrations were compared to the actual values. Stability was concluded when the % RSD was within ±15% of the actual value.

Stock solution stability Separate standard stock solutions containing 500 ng mL^{-1} of itopride, domperidone, and IS were prepared and stored at 2–8 °C for 30 days. The response obtained from the three drugs was calculated and compared to that of the freshly prepared solutions of the same concentration with an acceptable limit of ±2% [17].

Freeze–thaw stability The stability of the analytes after three freeze and thaw cycles was determined at low, high QC samples. The samples were stored at −20 °C for 24 h and thawed unassisted at room temperature. After completely thawing, the samples were refrozen for 12–24 h. After three freeze–thaw cycles, the concentration of the samples were analyzed.

Dry state stability Three aliquots each of low and high QC samples were stored at −20 °C without reconstitution after extraction (i.e. in dry state). The samples were analyzed after 24 h and a deviation of ± 15% was found.

Results and Discussion

Internal Standard

A stable isotope labeled analyte had to be used as an IS to deal with sample matrix effects. Since such an IS is not available commercially, an alternative approach has been used. IS chosen should match the chromatographic properties, recovery and ionization properties of the analyte [18]. Pantoprazole was found to match these criteria and therefore was chosen as an IS. Other internal standards like rabeprazole and metoclopramide were also tried but rejected because of their low recovery and inefficient extraction. Pantoprazole was selected because of its high recovery and also the intensity of itopride and molecular ion peaks in mass spectrometric analysis remained unaffected as compared to rabeprazole. Good chromatographic separation was another reason for its selection.

LC–MS–MS Analysis

LC–MS–MS was employed for the simultaneous quantification of itopride hydrochloride and domperidone in human plasma. To the best of our knowledge there is no LC–MS–MS method reported for the simultaneous determination of these drugs in human

Table 1. Detection in Multiple Reaction Monitoring (MRM)

Analyte	Quantifier MRM	Qualifier MRM	Signal Qual/signal Quant (Mean)	MRM ratio range (20%)
Itopride HCl	359.1/72.3	359.1/165.1	0.16	0.13–0.19
Domperidone	426.2/147.2	426.2/176.2	0.75	0.60–0.90
Pantoprazole	384.1/138.1	384.1/200	0.17	0.14–0.20

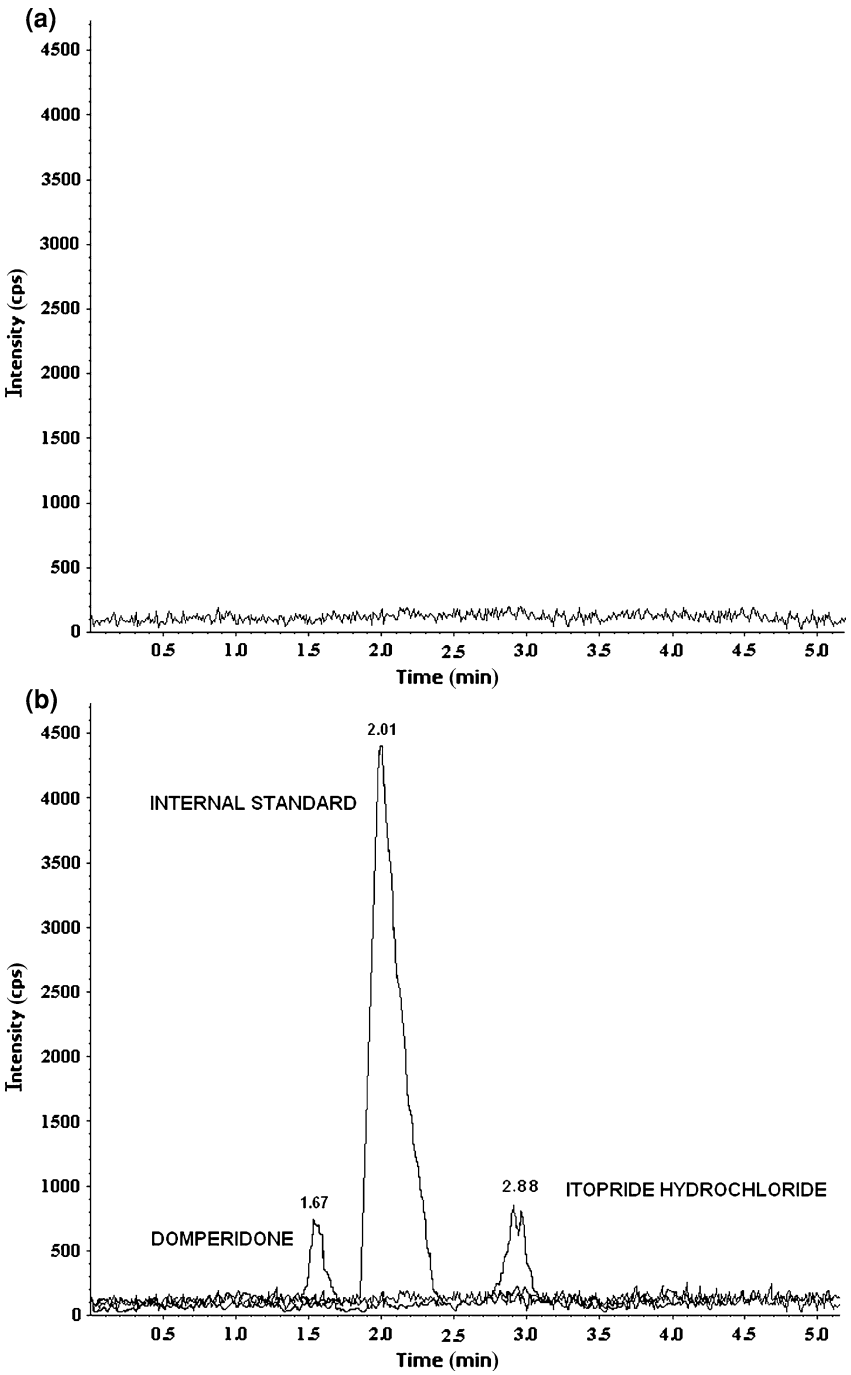

Fig. 3. **a** Representative chromatogram of blank plasma. **b** Representative chromatograms of extracted ion chromatogram (XIC) of blank plasma spiked with itopride and domperidone (LOQ, 3.33 ng mL^{-1}) and pantoprazole (IS) (100 ng mL^{-1})

Table 2. LOD, LOQ and calibration results

Analyte	Regression equation	R^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Range (ng mL^{-1})
Itopride HCl	$y = 0.0190x - 0.014$	0.9954	1	3.33	3.33–500
Domperidone	$y = 0.0064x + 0.024$	0.9952	1	3.33	3.33–100

Table 3. Intra and inter-day precision and accuracy for itopride Hcl and domperidone ($n = 5$)

Analyte	QC sample (ng mL^{-1})	Intra-day variation			Inter-day variation		
		Mean ± SD	RSD (%)	Accuracy (%)	Mean ± SD	RSD (%)	Accuracy (%)
Itopride HCl	5	4.86 ± 0.31	6.37	97.20	4.89 ± 0.25	5.11	97.8
	250	250.05 ± 4.25	1.7	100.02	249.09 ± 17.18	6.89	99.63
	400	395.85 ± 4.25	1.07	98.96	398.11 ± 45.16	11.34	99.5
Domperidone	5	5.12 ± 0.32	6.4	102.4	4.98 ± 0.25	5.02	99.6
	50	49.26 ± 1.25	2.53	98.52	49.92 ± 1.25	2.50	99.84
	80	78.94 ± 1.74	2.20	98.67	79.33 ± 3.12	3.93	99.16

% Relative standard deviation (RSD) = (SD/mean) × 100
SD standard deviation

plasma. The LC–ESI-MS–MS in MRM mode provided a highly selective method for the simultaneous determination of itopride and domperidone in human plasma. ESI source provided a better ionization of the compounds as compared to the atmospheric chemical ionization (APCI). The positive mode of ionization was selected because the intensity of the molecular ion peaks was higher in the positive mode.

The exact mass of itopride, domperidone and IS were found to be 358.1, 425.2 and 383.1, respectively. Figure 2 shows the product ion mass spectra of itopride, domperidone and IS respectively.

The method used two multiple reaction monitoring (MRM) transitions for each target compound for both quantization and confirmation. The ratio of the intensity of quantifier ion and qualifier ion is stable and an excellent tool for structural confirmation of target compounds. The most intense product ions of itopride, domperidone and pantoprazole were found to be m/z 72.3, 247.2 and 138.1, respectively. The transition m/z 359.0 → 72 (Quant), 426.2 → 147.2 (Quant), and 384.1 → 138.1 (Quant), was selected for the quantification of itopride HCl, domperidone and the IS respectively. A similar choice was done for the qualifier, leading to the transition m/z 359.0 → 136 (Qual), 426.2 → 136(Qual), 384.1 → 200 (Qual), in the case of itopride HCl, domperidone and the IS respectively. For routine analysis, the presence of analytes is confirmed only when the ratio signal Qual/signal Quant matches with the relative intensity observed in the data of the CID-spectra; in the example depicted in Fig. 2a–c. Due to some variation in the ionization yield and the internal energy of the protonated molecule, the ratio is not rigorously constant and some tolerance limits should be defined to surround the target value. In the commission decision of 12 August 2002, the EU has set up some tolerances based on the relative transition intensities [19]. Taking into account the performance criteria of the EU [19] the ratio should range with a limit of ±20%.

For elimination of any subjectivity, the normal variation interval of ±20% should be validated simultaneously. We have taken the MRM ratio data from each sample for linearity, precision, accuracy and stability analysis and taken the average of the MRM ratio. The average ratio signal Qual/signal Quant (MRM ratio) was found to be 0.16, 0.75 and 0.17 for itopride, domperidon and IS respectively (Table 1). The MRM ratio for each analyte sample from linearity, precision, accuracy was found with a range of ±20% and thus the normal variation was validated. The ratio signal Qual/signal Quant was applied for each unknown plasma sample and found within the limit (±20%) of the mean MRM ratio. This approach is also valid for the IS, as the quantitation of analytes in human plasma was based on the detector response ratio of analytes to IS. In case of IS the average MRM ratio and the range were quantified and validated with six IS solutions of concentrations at 100 ng mL^{-1}.

Monitoring the ratio for each target compound added an additional order of confidence when looking at unknown plasma samples, therefore the possibility of false positive detection was reduced. Total run time set for the samples tested was 5 min as shown in Fig. 3b. The results indicated that a run time of 3 min was sufficient for sample analysis. On repeated injection of the samples, the retention time never shifted beyond 10–15 s. Itopride, domperidone and IS were eluted at retention times of 2.86, 1.65 and 2.03 min respectively (Fig. 3b). The main analytes itopride and domperidone were separated with good resolution. Complete chromatographic separation of itopride and IS was not achieved under the set analytical conditions. However due to the high selectivity of tandem MS, complete chromatographic separation is not necessary any more [20]. The main advantage of this method is that a relatively large number of samples can be

analyzed in short time thus increasing the output.

Linearity

The calibration curves were found to be linear over a range of 3.33–500 ng mL^{-1} itopride and 3.33–100 ng mL^{-1} for domperidone. Table 2 summarizes the results of calibration. The average correlation coefficients obtained were 0.9954 and 0.9952 for itopride and domperidone respectively. The LOQ was found to be 3.33 ng mL^{-1} and LOD was found to be 1 ng mL^{-1} for both analytes.

Specificity

No significant peak was observed at the retention times of itopride, domperidone and IS in human plasma spiked with the analytes. Representative chromatograms of blank plasma (Fig. 3a), blank plasma spiked with itopride (LOQ), domperidone (LOQ), IS (100 ng mL^{-1}) are shown (Fig. 3b).

Accuracy and Precision

Table 3 summarizes the mean values of accuracy and precision for both intra- and inter-day assays. Both precision and accuracy were within the acceptable ranges for bioanalytical purpose. Intra-day precision ranged from 1.7 to 6.37% for Itopride and 2.2 to 6.40% for domperidone. Inter-day precision ranged from 5.11 to11.37% for itopride and 2.50 to 5.02% for domperidone. The percentage of accuracy was in the range of 97.20–100.02% for itopride and between 98.52 and 102.40% for domperidone. The assay method demonstrated a high degree of accuracy and precision.

Extraction Recovery and Matrix Effect

Recovery results presented in Table 4 show that the maximum recovery was achieved with internal standard (83.29%) followed by domperidone and itopride hydrochloride. The extraction recovery was found to be satisfactory as it was consistent, precise and reproducible. Thus single step liquid–liquid extraction procedure used in this method proved to be efficient and simple enough to extract three drugs (including IS) simultaneously from human plasma.

Table 4. Extraction recovery of itopride HCl and domperidone ($n = 3$)

Analyte	QC sample (ng mL^{-1})	Extraction recovery (%)	RSD (%)
Itopride HCl	5	75.25	10.25
	250	78.25	7.65
	400	75.36	5.88
Domperidone	5	76.25	5.25
	50	77.35	8.35
	80	77.32	5.12
Internal standard	100	83.29	4.14

% Relative standard deviation (RSD) = (SD/mean) × 100

The endogenous components were mainly the cause of ion suppression effects during electrospray ionization. The extent of this effect was mainly dependent on the sample extraction procedure and was also compound dependent [21]. Ion suppression of itopride and domperidone by matrix constituents was investigated by comparing peak areas of itopride and domperidone working solutions with processed blank samples reconstituted with these working solutions. Ion suppression for internal standards was performed in the same manner. Mean ion suppressions of itopride and domperidone and internal standard were -10.25 ± 3.75, 7.25 ± 3.52 and 5.50 ± 2.65% in human plasma. The negative ion suppression of itopride indicated that the presence of matrix components facilitated ionization leading to enhanced ion formation. The results indicated that the matrix components did not alter or deteriorate the performance of the proposed method as the % RSD of three QC samples was less than 7.53, 4.82 and 2.05% for itopride, domperidone and IS respectively. The matrix effect on the estimation of the analytes was found to be negligible.

Stability

Table 5 summarizes the results of the stability study performed under various conditions. Both analytes were found to be stable at ambient temperature (20–30 °C) for at least 8 h in human plasma. The percentage of accuracy obtained was higher than 95.70 and 96.40% for itopride hydrochloride and domperidone respectively. The LQC and HQC samples of both analytes remained unaffected at −20 °C for one month. In an autosampler maintained at 10 °C, plasma samples of itopride hydrochloride and domperidone were stable for more than 24 h. The freeze thaw stability results showed that Itopride hydrochloride and domperidone were stable for at least three freeze thaw cycles. Stability results indicated that human plasma samples could be thawed and refrozen without compromising the integrity of the samples.

Extracted and dried residues were stable for 24 h without any change in the concentration. Working solutions of itopride, domperidone and IS were stable and the deviation was less than ±2%. There was scarcely any degradation in the solutions even after 30 days.

Application in Clinical Pharmacokinetics

Study Design

Twelve non-smokers, normal, healthy, Indian male volunteers aged between 18 and 45 years (24.6 ± 3.54 years) with a body mass index between 18 and 24 (21.72 ± 3.22) took part in the study. They had neither previously participated in any other clinical trial nor donated blood during the preceding 3–4 months, and had no history of alcohol or drug abuse. None had received prescription or

Table 5. Stability summary of Itopride HCl and domperidone ($n = 3$)

Stability	QC sample (ng mL^{-1})	Itopride HCl			QC sample (ng mL^{-1})	Domperidone		
		Mean ± SD	RSD (%)	Accuracy (%)		Mean ± SD	RSD(%)	Accuracy (%)
Long term (30 days)	5	5.02 ± 0.25	5.00	100.4	5	5.15 ± 0.24	4.66	103.0
	400	395.15 ± 9.14	2.32	98.78	80	79.96 ± .24	1.55	97.49
Short term (8 h)	5	4.79 ± 0.19	3.96	95.8	5	4.95 ± 0.25	5.05	99.75
	400	389.95 ± 16.68	4.27	97.48	80	76..36 ± 1.54	2.01	95.45
Post preparative (24 h)	5	4.89 ± 0.12	2.45	97.8	5	4.85 ± 0.14	2.88	97.02
	400	392 ± 18.09	4.61	98.00	80	79.96 ± 1.89	2.36	99.95
Freeze thaw	5	4.95 ± 1.23	6.16	99.75	5	5.06 ± 0.25	4.94	101.20
	400	389.96 ± 11.25	2.88	97.49	80	78.56 ± 1.98	11.31	97.14
Dry Extract (24 h)	5	4.85 ± 0.35	7.20	97.00	5	5.01 ± 0.29	5.18	100.20
	400	388.36 ± 24.73	6.36	97.09	80	78.89 ± 1.11	1.40	98.61

% Relative standard deviation (RSD) = (standard deviation/mean) × 100
SD standard deviation

Table 6. Pharmacokinetic parameters obtained after oral administration of FDC of Itopride HCl 50 mg and Domperidone 20 mg

Pharmacokinetic parameters	Itopride HCl (Mean ± SD)	Domperidone (Mean ± SD)
C_{max} (ng mL^{-1})	234.25 ± 45.23	70.2 ± 14.23
AUC_{0-t}	779.36 ± 102.32	226.41 ± 54.32
$AUC_{0-\infty}$	819.11 ± 114.56	251.46 ± 61.45
T_{max} (h)	0.800 ± 0.21	0.800 ± 0.35
$T_{1/2}$ (h)	4.60 ± 1.32	5.88 ± 1.02

over the counter drugs for at least 4 weeks prior to the study day. All of them underwent complete physical, biochemical, and haematological tests before enrolling for the study. Informed consent was obtained from all the subjects prior to the start of the study. The study was only initiated after the protocol and subject information forms had been approved by the Drugs Control General of India (DCGI), New Delhi and Institutional Ethical Committee (IEC) of Jadavpur University, Kolkata, India. It was designed as a randomized, single-dose, two-period, and two-sequence crossover study under fasting conditions with a 1-week wash out period.

Drug Administration and Sample Collection

All the subjects assembled in the Clinical Pharmacology Unit (CPU) ward at 6 AM on the study day of each session, after overnight fasting of 10 h. They did not consume caffeinated or alcoholic beverages for at least 72 h prior to drug administration or during the study days. The total pulse rate and blood pressure was recorded. They received the study preparations and served as their own control. According to US Food and Drug Administration (FDA) (US FDA, 2000) and European Agency for the Evaluation of Medicinal Products (EMEA 2002) regulations, the sampling schedule should be planned to provide a reliable estimate of the extent of absorption (CPMP 1991). This is generally achieved if AUC_{0-t} is at least 80% of $AUC_{0-\infty}$. Usually the sampling time should extend to at least three terminal elimination half-lives of the active ingredient. The time periods between sampling should not exceed one terminal half-life. A total of eleven blood samples were collected at 0 h (before drug administration) and at 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 4.0, 6.0, 12.0 and 24.0 h (after drug administration) in the test tubes containing EDTA at each time point. Breakfast, lunch, and dinner were provided after 3, 6, and 13 h, respectively, after drug administration. Collected blood samples were centrifuged immediately; plasma was separated and stored frozen at −20 °C with appropriate labeling of the subject code number, study date, and collection time, till the date of analysis.

Evaluation of Pharmacokinetics Parameters

The method described above was successfully applied to phase 1 pharmacokinetics studies of FDC of itopride HCl and domperidone in human plasma. The values of the main pharmacokinetic parameters are shown in Table 6. All the pharmacokinetics parameters of itopride and domperidone were found to be similar to those of published pharmacokinetic parameters.

Conclusion

The method described here was highly specific due to the inherent selectivity of tandem-mass spectrometry. The method demonstrated high throughput capability because of the short time required for analysis. Both analytes were found to be stable in human plasma for 30 days when stored at −20 °C. A simple and convenient extraction procedure made this method more feasible for the bioanalysis of itopride hydrochloride and domperidone. It is expected that this method can also be applied to pharmacokinetic studies.

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