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Development and validation of a liquid chromatographic method for determination of related-substances of mosapride citrate in bulk drugs and pharmaceuticals

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Abstract

An isocratic reversed-phase high-performance liquid chromatographic (RP-HPLC) method for determination and evaluation of purity of mosapride citrate in bulk drugs and pharmaceuticals has been developed using Waters Symmetry C_{18} column with acetonitrile:0.024 M orthophosphoric acid (28:72, v/v) adjusted to pH 3.0 with triethylamine and photodiode array detector set at 276 nm. The method is simple, rapid, selective and capable of detecting all process related impurities at trace levels in the finished products with detection limits ranging in between 0.2×10^{-8}g and 6.4×10^{-8}g. The method has been validated with respect to accuracy, precision, linearity, ruggedness, and limit of detection and quantification. The linearity range was 125–1000 μg/ml. The percentage recoveries from pharmaceutical dosages were ranged from 95.53 to 100.7. The method was found to be suitable not only for monitoring the reactions during the process development but also quality assurance of mosapride citrate.

Keywords: Mosapride citrate; Gastroprokinetic; Process-related impurities; Bulk drugs; RP-HPLC

1. Introduction

Mosapride citrate, known as 4-amino-5-chloro-2-ethoxy-*N*-[[4-(4-fluorobenzyl)-2-morpholinyl]methyl]benzamide citrate dihydrate is a novel gastroprokinetic agent and plays an important role in conjunction with life-style modifications in short and long term management of gastroesophageal reflux disease and dyspepsia in many of the Asian countries [1]. Unlike the conventional gastroprokinetic agents, it is free of dopamine D2 receptor antagonist and neither stimulates colon motor activity nor causes adverse effects such as central nervous system depression and extra pyramidal syndrome in man [2–4]. It behaves as a selective 5-HT4-receptor agonist and enhances only upper gastroprokinetic motor activity [5,6].

A thorough literature search has revealed that only a few analytical methods are available for determination of mosapride citrate in bulk drugs and pharmaceuticals [7,8]. Its pharmacokinetic profiles in rats [9], dogs, monkeys [10] and in healthy subjects [11] have been well characterized. Yokoyama et al. [12], have developed a method for simultaneous enantiomeric determination of mosapride and its metabolites in plasma using α_1-acid glycoprotein HPLC column. The effect of mobile phase pH and column temperature on the retention behavior of enantiomers of mosapride citrate using chiral-AGP was studied [13]. Recently, Kumar et al. [14] have studied the application of LC-MS/MS for detection of a polar impurity in the bulk drugs of mosapride citrate. However, none of these methods address to the problem of separation and determination of all process related impurities,

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which are most likely to be present in the finished products of mosapride citrate. Further, to the best of our knowledge, mosapride citrate is not yet official in any of the pharmacopoeia and no method for determination of its impurities has been reported either in bulk drugs or pharmaceuticals. Thus, there is a great need for analytical methods, which will be helpful to monitor the levels of impurities in the finished products of mosapride citrate during process development. In present study, the separation and determination of mosapride citrate and its process related impurities were examined by reversed-phase high-performance liquid chromatography (RP-HPLC) using a C_{18} column and PDA detector at ambient temperature. Two new impurities viz.; 2,4-dichloro-5-ethoxyaniline (VIII) and 4-amino-3-chloro-2-ethoxy-*N*-[[4-(4-fluorobenzyl)-2-morpholinyl]methyl]benzamide citrate (VII) which is chloro isomer of mosapride citrate have been detected by LC-PDA and identified by LC-MS. Their mass spectral characterization has been reported for the first time in the present paper.

2. Experimental

2.1. Materials and reagents

All reagents were of analytical-reagent grade unless stated otherwise. Glass-distilled and de-ionized water (Nanopure, Barnsted, USA), HPLC-grade acetonitrile, triethylamine (Ranbaxy, SAS Nagar, India) and orthophosphoric acid (S.D. Fine chem, Mumbai, India) were used. Samples of mosapride citrate (VI), its reference standard and process intermediates viz., 2-[(4-flurobenzyl)amino]ethanol (II), citric acid (V), 2-amino-4-(4-flurobenzyl)morpholine (III), *N*-[2,3-epoxypropyl]phthalimide (I) and 4-amino-5-chloro-2-ethoxybenzoicacid (IV) (sponsored by Glenmark pharmaceuticals, R&D center, Mumbai, India) were used.

2.2. Apparatus

The HPLC system composed of two LC-10AT VP pumps, an SPD-M10Avp diode array detector an SIL-10AD VP auto injector, a DGU-12A degasser and SCL-10A VP system controller (all from Shimadzu, Kyota, Japan). A reversed-phase Symmetry C_{18} (Waters, USA) column (25 cm × 4.6 mm i.d.; particle size 5 μm) was used for separation. The chromatographic and the integrated data were recorded using HP-Vectra (Hewlett Packard, Waldron, Germany) computer system.

2.3. Chromatographic conditions

The mobile phase was 0.024 M *ortho* phosphoric acid–acetonitrile (72:28, v/v), the buffer pH was adjusted to 3.0 with triethylamine (Et_3N). Before delivering into the system it was filtered through 0.45 μm, PTFE filter and degassed using vacuum. The analysis was carried out under isocratic conditions using a flow rate of 1.0 ml/min at room temperature (28 °C). Chromatograms were recorded at 276 nm using a SPD-M10Avp diode array detector.

2.4. Analytical procedures

Solutions (500 μg/ml) of mosapride citrate and its intermediates were prepared in the mobile phase by dissolving known amounts of the components in the mobile phase. These solutions were adequately diluted to study the accuracy, precision, linearity and limit of detection and limit of quantitation.

2.4.1. System suitability

The system suitability was conducted by using 0.1% of all impurities spiked to the mosapride citrate and evaluated by making five replicate injections. The system was deemed to be suitable for use if the tailing factor for mosapride and its impurities are less than or equal to 1.2, the resolution was greater than 1.5 or higher and column plate numbers for main peak are more than 20,000. Synthetic mixtures and process samples were analyzed under identical conditions. The quantities of impurities and assay of mosapride were calculated from their respective peak areas.

3. Results and discussion

3.1. Optimization of chromatographic conditions

The chemical structures of mosapride citrate (VI) and all its process related intermediates (II, III, IV, and V) including starting material (I) are shown in Fig. 1. It could be seen from Fig. 1 that there are six compounds, including the starting material and intermediates that could be present as potential impurities in the finished products of mosapride citrate. The present study is aimed at developing a chromatographic system capable of eluting and resolving mosapride citrate and its impurities originated from the synthesis. All the impurities of mosapride citrate were subjected to separation by reversed-phase HPLC on a Symmetry C_{18} column with water–acetonitrile as eluent. Three compounds viz., II, III and V were merged when the concentration of acetonitrile was kept below 50%. However, on increasing the concentration of acetonitrile, four compounds were well separated except IV and V. In another attempt, water was replaced with 0.024 M orthophosphoric acid buffer and the effect of concentration of organic modifier viz., acetonitrile and buffer pH on separation was studied (Figs. 2 and 3). It could be seen from Figs. 2 and 3, when the concentration of acetonitrile was 28% and buffer pH 3.0 all the compounds were eluted and separated with good peak shapes. A typical chromatogram of mosapride citrate spiked with 0.1% each of all related substances is shown in Fig. 4. The peaks were identified by injecting and comparing with the retention times of the individual compounds and the absorption spectra recorded using PDA detector. Impuri-

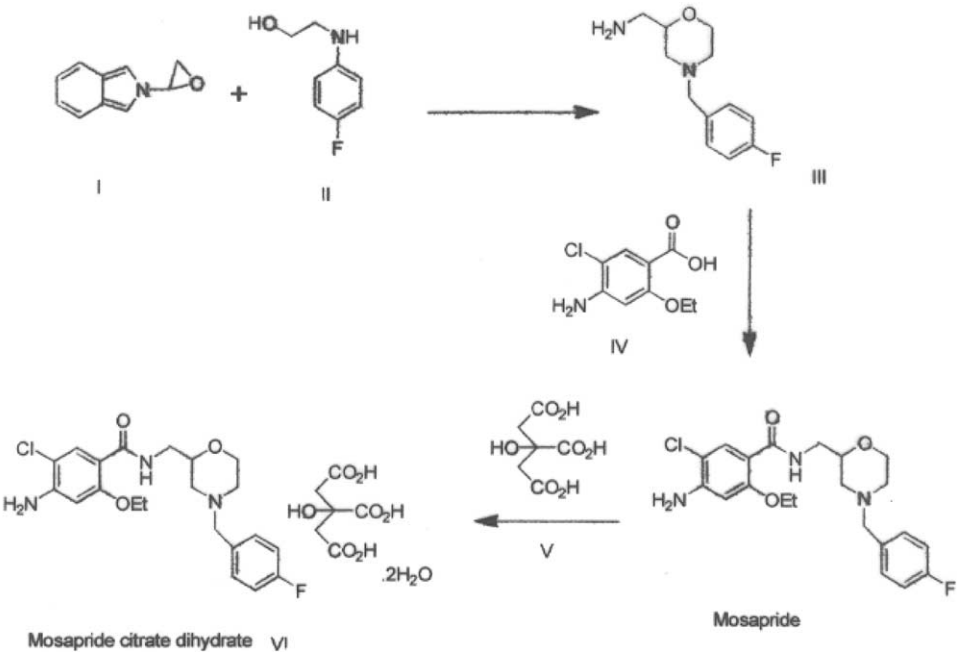


Fig. 1. Chemical structures of mosapride citrate and its process intermediates.

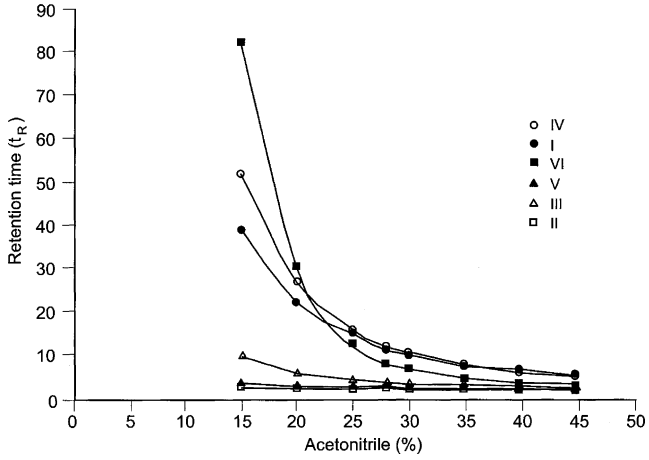


Fig. 2. Effect percentage of acetonitrile on the retention of the compounds under study (pH was kept constant at 3.0).

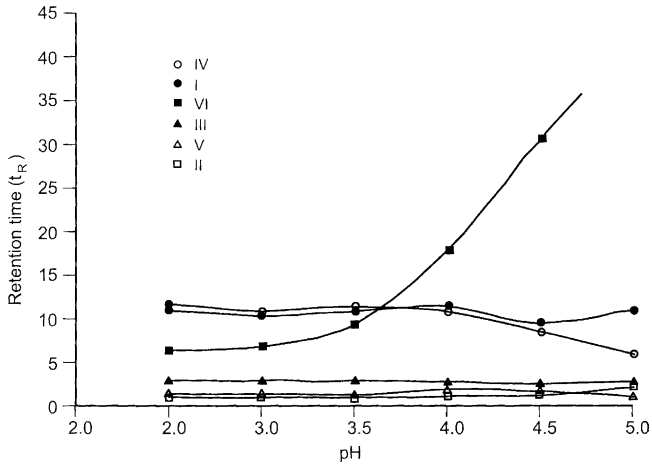


Fig. 3. Effect of pH on retention of mosapride citrate and its process intermediates (acetonitrile content was kept constant at 28%).

ties eluted at retention time (t_R) 2.45 min (0.32 RRT), 3.5 min (0.45 RRT) and 8.68 min (1.12 RRT) are >0.1%, out of which 0.32 RRT impurity was matched with citric acid process intermediate, 0.45 and 1.12 RRT did not match with any of the process intermediates. These two are later identified by LC-MS. Mosapride citrate and its starting materials/process intermediates have been subjected to UV analysis and the spectra recorded in the range of 190–400 nm are shown in Fig. 5. From Fig. 5 it could be seen that mosapride citrate (VI) has shown three well defined absorption bands at 215, 276, and 305 nm. Similarly the intermediate IV has also absorption bands at 215, 220, 270 and 300 nm. Other starting materials (II) and intermediates (III, V) have lower absorption at 276 nm. This means that their molar percentage would be

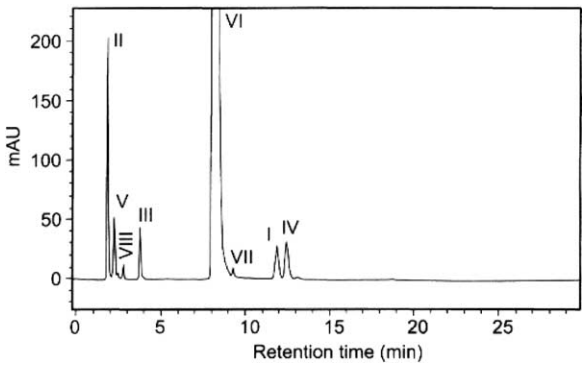


Fig. 4. Typical chromatogram of mosapride citrate and its related compounds. For identification of peaks see text.

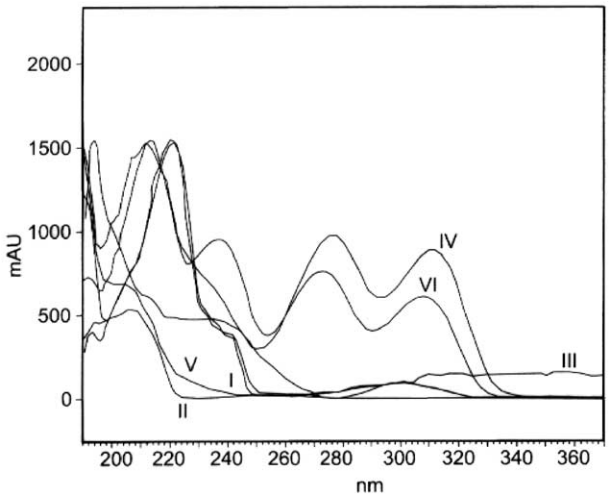


Fig. 5. UV absorptions spectra of mosapride citrate and its process intermediates.

higher when compared to the percentage of their respective peaks. However, this particular wavelength has been chosen for detection and quantitation through out the study. This is not only because of the strong absorption of isomeric impurities at 276 nm but also possible interference of solvent impurities at 215 nm. It was observed that 0.1% of all impurities could be detected and determined with good accuracy at 276 nm. System suitability studies were carried out and the results are recorded in Table 1. The selectivity was found to be more than 0.50 with resolution more than 1.30 for all the compounds.

3.2. Assay

The assay of mosapride citrate was estimated using a working standard and the method is validated by the following parameters.

3.2.1. Specificity

Specificity is the ability of the method to measure the analyte response in presence of all potential impurities. The results are recorded in Table 2 PDA was used to evaluate the homogeneity of the peaks in the chromatogram. Chromatographic peak purity was determined using wavelength comparison at 215 and 276 nm. The plot with flat top showed that

Table 1
System suitability data

Compound	k'	Tailing factor	RRT	Rs	Thereotical plates (N)
I	6.61	1.05	0.75	11.34	92254
II	0.40	1.12	0.27	1.50	6022
III	1.60	1.10	0.50	2.15	23823
IV	6.98	1.08	1.54	1.35	62347
V	0.65	1.03	0.32	2.03	10147
VI	4.16	1.08	1.00	4.77	24902
VIII	1.30	1.10	0.45	2.11	24182

RRT: relative retention time; Rs: resolution; k': capacity factor.

Table 2
Specificity data

S. no.	Assay (%)	
	Unspiked sample	Sample spiked with impurities
1	99.85	99.42
2	99.80	98.80
3	99.65	99.45
Mean	99.77	99.22
S.D.	0.10	0.37
R.S.D. (%)	0.10	0.37

mosapride exhibited a homogeneous peak with no detectable impurities embedded in it. The specificity was checked by stressing the pure sample under UV light at 254 nm, 65 °C temperature for 24 h and extreme conditions such as 0.1N HCl, 0.1N NaOH, and 3% H_2O_2. In UV light and thermal conditions no change in the sample purity was observed, but in alkaline conditions, the degraded products were formed and well separated from mosapride. In formulations, it was observed that the excipient peaks did not interfere with the peaks of interest (Fig. 6). Thus, the method was found to be applicable for quantitative determination of mosapride in pharmaceuticals dosages.

3.2.2. Accuracy

The recoveries of I, II, III, IV and V were determined by spiking each impurity at six different levels ranging from 25 to 150% with respect to the concentration of mosapride citrate (VI) at a specified level. The recovery range and R.S.D. for all impurities were found to be 93.42–101.22% and 0.15–2.25%, respectively (Table 3). Similarly the accuracy in determination of the assay of mosapride citrate was checked at six concentration levels i.e. 125, 250,375, 500, 625, and 750 μg/ml each in triplicate for 3 days and the percentage recoveries are recorded in Table 4. The R.S.D. values are found to be below 1.7%.

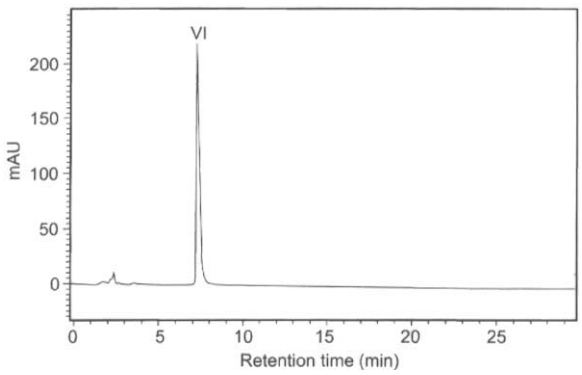


Fig. 6. Typical chromatogram of a formulation containing 5 mg of mosapride citrate.

Table 3
Recovery data

	Nominal 0.1% of impurity spiked to mosapride citrate					
	25	50	75	100	125	150
Amount added (μg/ml)	0.125	0.25	0.375	0.50	0.75	0.875
%Recovery (±R.S.D., %)[a]						
(a) I	99.79 ± 0.68	98.92 ± 1.66	98.65 ± 1.66	97.59 ± 1.79	96.49 ± 0.63	95.72 ± 0.50
(b) II	101.22 ± 1.42	98.12 ± 2.15	93.46 ± 0.54	95.45 ± 1.17	93.42 ± 1.17	94.66 ± 0.15
(c) III	98.06 ± 2.25	101.17 ± 1.50	97.94 ± 1.68	99.08 ± 0.63	100.40 ± 3.55	99.07 ± 1.03
(d) IV	97.96 ± 0.73	101.11 ± 0.73	94.68 ± 2.5	99.44 ± 0.79	94.48 ± 0.68	93.84 ± 1.17
(e) V	100.5 ± 0.95	99.40 ± 0.61	98.08 ± 1.23	100.10 ± 0.45	95.10 ± 0.47	95.70 ± 0.50

[a] $n = 3$.

Table 4
Accuracy data

S. no.	Concentration of mosapride citrate (mg/ml)			
	Taken	Recovered ($n = 3$)	%Recovery	R.S.D. (%)
1	0.125	0.1246	99.40	0.34
2	0.250	0.2524	99.90	1.38
3	0.375	0.3780	99.30	1.18
4	0.500	0.5001	100.0	0.07
5	0.625	0.6188	99.50	0.63
6	0.750	0.7560	99.90	1.61

$n = 3$: average of three determinations; R.S.D.: relative standard deviation.

3.2.3. Precision

The precision of the method was tested by six ($n = 6$) injections of mosapride citrate spiked with 0.1% (w/w) of each impurity and the R.S.D. of retention time (t_R), peak area were determined. The R.S.D. ranges from 0.23 to 2.52% (Table 5). The precision in determination of assay was studied by repeatability, intermediate precision and reproducibility (ruggedness). Repeatability is the intra-day variation in assay obtained at different concentration levels of mosapride citrate and expressed in terms of R.S.D. calculated for each day. The R.S.D. values were found to be below 2.0%, indicating a good repeatability (Table 6). The intermediate precision is the inter-day variation at the same concentration level determined on successive days. The inter-day variations calculated for each concentration level from the data of 3 days are expressed in terms of R.S.D. values. At each concentration level, the R.S.D. values were below 1% indicating a good intermediate precision. The ruggedness of the method is defined as the degree of reproducibility obtained by the analysis of the same sample under a variety of conditions at different labs, different analysts, different instruments, and different lots of reagents. The same samples of three concentrations were analyzed in triplicate on 2 days by another instrument (LC-10A Module HPLC system containing pump and UV-visible detector) by a different analyst with different lots of reagents and columns. The data obtained were within 2% R.S.D.

Table 5
Precision data

Compound	Retention time (t_R)		Peak area	
	Average[a]	R.S.D. (%)	Average[a]	R.S.D. (%)
I	11.23	0.23	57038	1.45
II	2.00	0.52	15018	1.61
III	2.23	0.81	9704	0.75
IV	12.23	0.63	60687	2.52
V	2.83	0.44	183368	0.73
VI	7.89	0.72	26079642	0.90

[a] Average of six determinations.

Table 6
Inter and intra-day assay variation of mosapride citrate

Intra-day			
Day 0			
Mean of concentration (mg/ml: $n = 3$)	0.1246	0.2522	0.3748
S.D.	0.0004	0.0033	0.0044
R.S.D. (%)	0.34	1.38	1.18
Day 1			
Mean of concentration (mg/ml: $n = 3$)	0.12	0.2500	0.3740
S.D.	0.0004	0.0019	0.0022
R.S.D. (%)	0.33	0.77	0.58
Day 2			
Mean of concentration (mg/ml: $n = 3$)	0.1253	0.2471	0.3747
S.D.	0.0008	0.0026	0.0023
R.S.D. (%)	0.64	1.03	0.20
Inter-day			
Mean of concentration (mg/ml: $n = 3$)	0.1246	0.2471	0.3745
S.D.	0.0007	0.0026	0.0004
R.S.D. (%)	0.52	1.04	0.12

Table 7
Linearity data

Compound	Range (μg/ml)	Regression equation	r^2
I	0.125–1.00	$Y = 13681x + 869$	0.999
II	0.125–1.00	$Y = 25387x + 617$	0.998
III	0.125–1.00	$Y = 29384x + 400$	0.999
IV	0.125–1.00	$Y = 118577x + 1839$	0.998
V	0.125–1.00	$Y = 5189x + 648$	0.999
VI	125–1000	$Y = 2566x + 9477$	0.999

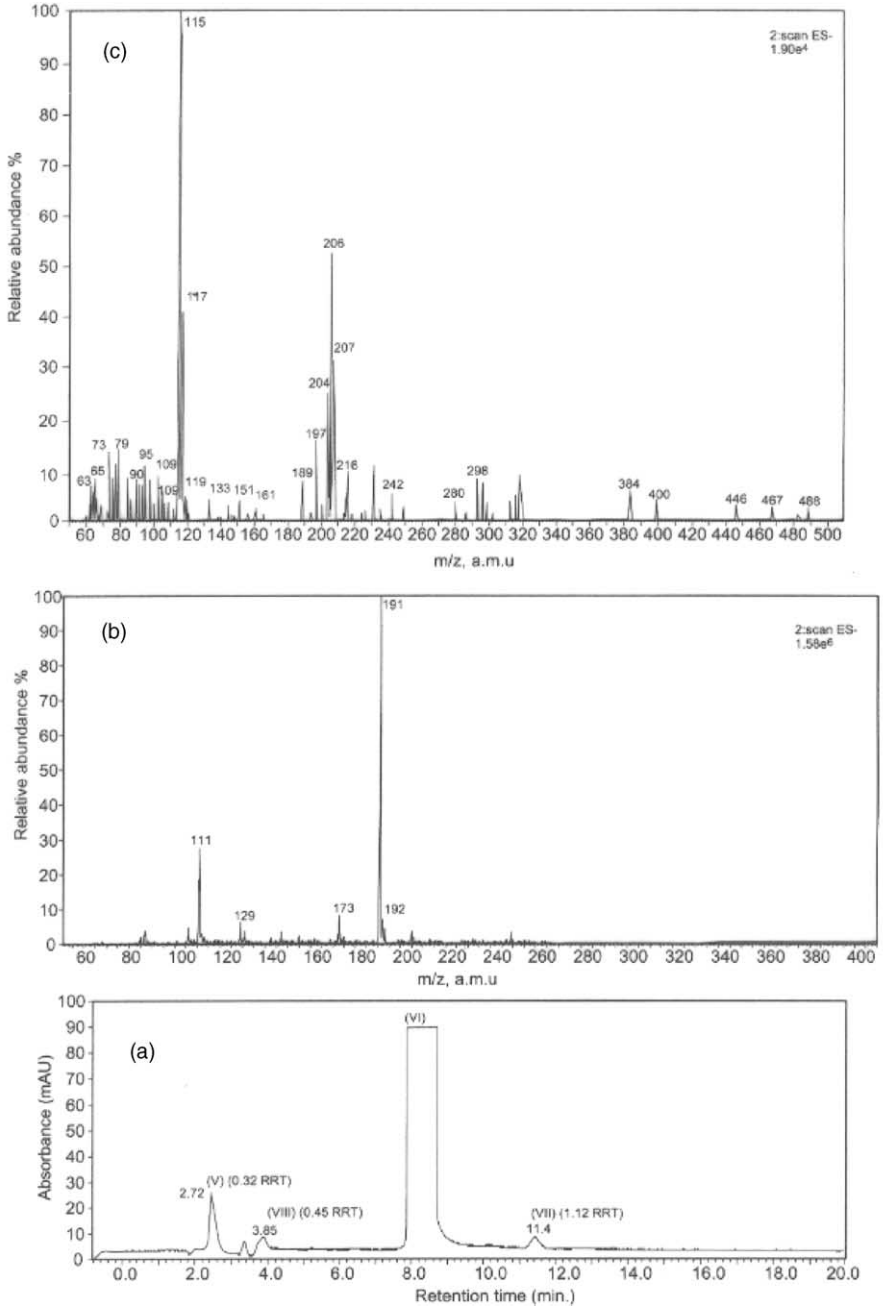


Fig. 7. LC-ESI-MS (a), chromatogram of mosapride citrate dihydrate (VI), (b), and (c) corresponding mass spectra impurities V (0.32 RRT) and VIII (0.45 RRT).

Table 8
Limits of detection and quantitation

S. no.	Compound	LOD ($a \times 10^{-8}$ g)	LOQ ($b \times 10^{-8}$ g)
1	I	0.42	1.26
2	II	0.80	2.20
3	III	0.80	2.40
4	IV	0.20	0.80
5	V	6.40	19.6
6	VI	0.64	2.48

a, *b* are variables.

3.2.4. Linearity

The linearity of detector response to different concentrations of impurities were studied by analyzing mosapride citrate spiked with each impurity at eight levels ranging from 25 to 200% (0.125–1.00 μg/ml). Similarly, linearity of mosapride citrate was also studied by preparing standard solutions at eight different levels ranging from 125 to 1000 μg/ml. The data were subjected to statistical analysis using a linear-regression model, the standard deviation of slope and intercept are calculated and shown in Table 7. The results have indicated good linearity.

Table 9
Assay of mosapride citrate from Moza-5 M tablets

S. no.	Number of injection	Concentration of mosapride citrate (mg/ml)			%R.S.D.
		Taken	Recovered	%Recovery	
I	1	0.0989	0.0988	–	–
	2	0.0989	0.0984	99.73	0.21
	3	0.0989	0.0987	–	–
II	1	0.0495	0.0495	–	
	2	0.0495	0.0499	100.70	0.58
	3	0.0495	0.0501	–	–
III	1	0.0247	0.0244	–	–
	2	0.0247	0.0243	98.92	0.62
	3	0.0247	0.0246	–	–

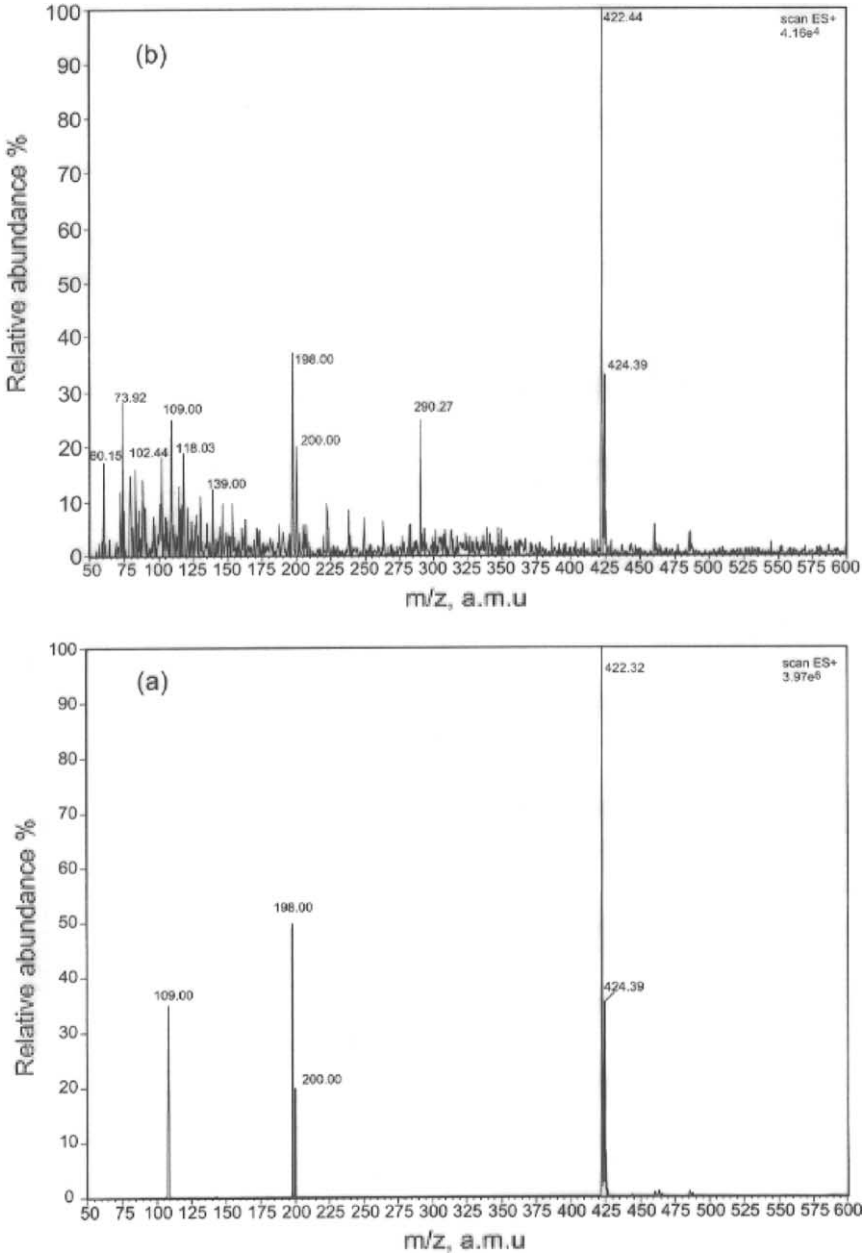
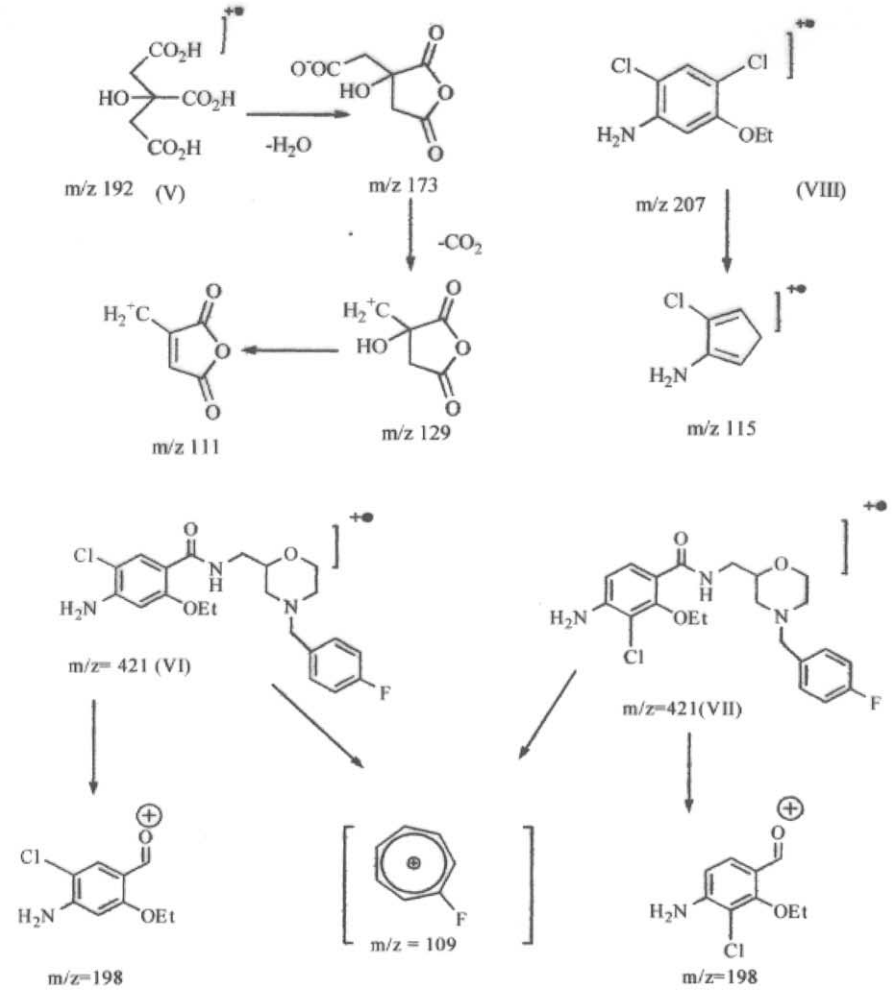


Fig. 8. Mass spectra of (a) mosapride citrate dihydrate (VI), and (b) its chloro isomer VII (1.12 RRT).



V	VI	VII	VIII
M^+=192	M^+=421	M^+ =421	M^+ =207
m/z= 173, 129, 111	m/z= 198, 109	m/z=198, 109	m/z= 115

Fig. 9. Mass spectral fragmentation patterns of V, VII and VIII.

3.2.5. Limits of detection and quantitation

Limit of detection (LOD) and limit of quantitation (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratio of 3 for LOD and 10 for LOQ, respectively. LOD and LOQ were determined by measuring the magnitude of analytical background by injecting blank samples and calculating the signal-to-noise ratio for each compound by injecting series of solutions until the S/N ratio 3 for LOD and 10 for LOQ. The results are given in Table 8.

3.2.6. Assay of mosapride citrate in tablet formulations

Ten weighed tablets of Moza-5 (equivalent to 5 mg each of mosapride citrate) were ground to powder and an equivalent of 50 mg of active ingredient dissolved in acetonitrile was taken in 100 ml volumetric flask, ultra sonicated for about 5 min and diluted with the mobile phase. The supernant liquid was diluted with mobile phase to required concentrations and analyzed. The results of the extracted sample at three different independent concentrations were found to be comparable with the claimed values (Table 9).

3.2.7. Bulk drug analysis

Different batches of mosapride citrate were analyzed and the results are recorded in Table 10. Three impurities have more than 0.1% area at retention times 2.45 min (0.32 RRT), 3.5 min (0.45 RRT), and 8.68 min (1.12 RRT) were detected.

Table 10
Results of analysis of bulk drugs by HPLC

S. no.	Sample	Impurities (%)							Assay (w/w)
		I	II	III	IV	V	VI	VIII	
1	GPL-3000/1001	nd	nd	nd	nd	0.12	0.15	0.09	99.35
2	GPL-3000/1002	nd	nd	nd	nd	0.19	0.15	0.13	99.15
3	GPL-3000/1003	nd	nd	nd	nd	0.13	0.14	0.10	99.56
4	GPL-3000/1005	nd	nd	nd	nd	0.13	0.12	0.09	99.65

nd: not detected.

Out of which one impurity at 0.32 RRT has perfectly matched with the retention time of citric acid (V) and the later at 0.45 RRT, and 1.12 RRT did not match with any of the process intermediates studied in the present investigation. In order to characterize these impurities LC-MS was used. LC-MS analysis was carried out using 0.05 M ammonium acetate buffer–acetonitrile (50:50, v/v), buffer pH adjusted to 4.0 with acetic acid, as a mobile phase in positive and negative modes using electro spray ionization technique. The TIC chromatogram showed a peak eluted at 2.72 min with *m*/*z* 191 (100%) and daughter ions *m*/*z* 173, 129, and 111 formed due to the loss of H_2O, CO_2, and H_2O, respectively. This has supported the impurity as citric acid, which has eluted at 0.32 RRT in HPLC. Another impurity at 3.85 min showed *m*/*z* 206 with a stable daughter ion *m*/*z* 115 formed due to the loss of –Cl, $–C_2H_5$ and –CO has been identified as 2, 4-dichloro-5-ethoxyaniline (VIII), chromatogram and mass spectra are shown in Fig. 7. In positive mode mosapride citrate has eluted at 8.92 min and shown as a protonated molecular ion at *m*/*z* 422 while the impurity eluted at 11.48 min (1.12 RRT) also gave *m*/*z* at 422 with similar fragments shown in Fig. 8. It could be a positional isomer of mosapride citrate (VII) (chlorine could be in the 3rd position instead of 5th position in benzamide moiety). Formation of these three impurities were rationalized by citric acid (V) directly coming from process and the later viz., 2,4-dichloro-5-ethoxyaniline (VIII), and 4-amino-3-chloro-2-ethoxy benzoic acid as probable impurities of 4-amino-5-chloro-2-ethoxy benzoic acid (IV) used in the synthesis. The condensation IV with 2-amino-4-(4-flurobenzyl)morpholine (III) may form chloro isomer, viz., 4-amino-3-chloro-2-ethoxy-*N*-[[4-(4-fluorobenzyl)-2-morpholinyl]methyl]benzamide citrate (VII). The chemical structures and mass spectral fragmentation pattern of the three impurities are shown in Fig. 9.

4. Conclusions

An isocratic RP-LC method has been developed and validated for evaluation of purity and determination of mosapride citrate in bulk drugs and pharmaceuticals. The developed method has been found to be selective, sensitive, precise and stability indicating. The method is also capable of detecting intermediates and other process-related impurities, which may be present at trace level in the finished products. The unknown impurities have been identified by LC-ESI-MS in the bulk drugs.

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