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Reverse Phase High Performance Liquid Chromatographic method for the simultaneous estimation of Esomeprazole and Itopride in Capsule

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Abstract

Most of the drugs in multi component dosage forms can be analyzed by HPLC method because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. An accurate, simple, reproducible and sensitive method for the simultaneous estimation of Esomeprazole and Itopride in the capsule was developed. This method employed Buffer (Ammonium Acetate, pH-5.5): Water: Methanol (25:15:60) as mobile phase. Phenomenex C_{18} column was used and the Spectrophotometric determination was done at 275 nm. The method was validated for linearity, precision, solution stability, repeatability, reproducibility, accuracy, robustness, system suitability, limit of detection and limit of quantification.

Keywords: Esomeprazole, Itopride, HPLC, Validation

Introduction

Proton-pump inhibitors (PPIs) have emerged as the drug class of choice for treating patients with acid-related diseases, including gastro esophageal reflux disease (GERD), duodenal ulcer, and gastric ulcer. PPIs are also effective in treating patients with Barrett's esophagus and Zollinger-Ellison syndrome. These agents inhibit gastric acid secretion by targeting the gastric acid pump, H^+ K^+ adenosine triphosphatase (ATPase), in the canalicular membrane of the parietal cell. [1-5] The regulation of acid secretion is a complex process involving many cell types, hormones, and mediators, but these processes converge in a final common step involving H^+ K^+ ATPase. As a

result, PPIs effectively inhibit acid secretion in a manner independent of the processes that stimulate the parietal cell. Each member of the PPI class omeprazole, lansoprazole, pantoprazole and rabeprazole has been shown to be effective and well tolerated in randomized, controlled clinical trials.

Esomeprazole and Itopride are newer proton pump inhibitor molecules and there are very few methods for validation are available for them especially in their combined capsule form. [1-5, 12]

Results and Discussion

Selection of mobile phase

To optimize the HPLC assay parameters, the mixtures of Ammonium Acetate, methanol and water in different combinations at various flow rates were tried. The optimum wavelength for detection was set at 275 nm at which much better detector responses for both drugs were obtained. The mixture of Ammonium Acetate, methanol and water (25:60:15) at 1.5 ml/min flow rate, proved to be better than the other mixtures in terms of resolution and peak shape. As shown in Figure 1, the retention times were 5.824 min for ESO and 2.325 min for ITO. The calibration graphs for ESO and ITO were constructed by plotting the peak area versus their corresponding concentrations, respectively; good linearity for both was found over the range 1-20 µg/ml.

Results obtained by applying the RP-HPLC method showed that the concentrations of ESO and ITO could be simultaneously determined in prepared mixtures. The proposed method has been applied to the assay of ESO and ITO in capsule. The validity of the method was further assessed by applying the standard addition technique. The results obtained indicate the additives present do not interfere with analysis of the studied mixtures.

Chromatogram

Several mobile phases were tried to accomplish good quantization and separation of ESO and ITO. After optimizing all parameters chromatographic condition were obtained which gave satisfactory chromatogram with sharp peak and best resolved of ESO and ITO.
A representative chromatogram has been shown in Figure 1. Parameters of chromatogram are shown in Table 1. Better Shape of the peak with clear base line separation was found.

Linearity

The seven-point calibration curves that were constructed were linear over the selected concentration range for both ESO and ITO ranging between 1-20 µg/ml. Each concentration was repeated three times. The assay was performed according to the experimental conditions previously described. The linearity of the calibration graphs and adherence of the system to Beer's law were validated by the high value of the correlation coefficient and the intercept value. [11, 15]

Accuracy

Accuracy of the methods was assured by use of the standard addition technique, involving analysis of formulation samples to which certain amounts of authentic drugs were added. The resulting mixtures were assayed, and the results obtained for both drugs were compared to those

expected. The good recoveries with the standard addition method (Table 2) prove the good accuracy of the proposed methods.

The percent recoveries obtained were 99.13 to 102.03 % for ESO and 96.28 to 102.34 % for ITO. The results of recovery study are given in Table 2. The low value of S.D indicates that the proposed method is accurate. [11, 15]

Precision

For evaluation of precision, repeatability of the results for a concentration of 4µg/ml was evaluated by six replicate determinations. For evaluation of intermediate precision, the results over the concentration range 1-20 µg/ml. was evaluated by three replicate determinations to estimate intra-day variation and another three replicate determinations on three days to estimate inter-day variation. The percentage RSD values at these concentration levels were calculated. [11, 15]

Relative standard deviation of all the parameters is less than 2% (Table 2), which indicates that the proposed method is repeatable.

The low percentage CV values of intra-day (0.018-0.566 for ESO and 0.141-0.93 for ITO) and inter-day (0.049-0.270 for ESO and 0.053-0.527 for ITO) precision reveal that the proposed method is precise (Table 4 and 5). [15]

Limit of detection and Limit of quantitation

The limit of detection for ESO and ITO was found to be 0.0362 µg/ml and 0.0565 µg/ml respectively.

The limit of quantification for ESO and ITO was found to be 0.1098µg/ml and 0.171µg/ml respectively. [15]

Selectivity

Assay results obtained by proposed method are in good agreement with labeled value, which indicates no interference of excipients present in the formulation of ESO and ITO capsule. (Table 8) [15]

Robustness

Variation in the composition of mobile phase by ± 0.1 unit and its organic strength by ± 0.1% did not have a significant effect on the chromatographic resolution. [15]

System suitability

Percentage RSD of all parameters is less than 2 % (Table 6) which indicates that the proposed method is repeatable. [15]

Application to the capsule

The proposed validated method was successfully applied to determine ESO and ITO in capsule dosage forms. Results are given in Table 8. No interference of the excipients with the peaks of interest appeared.

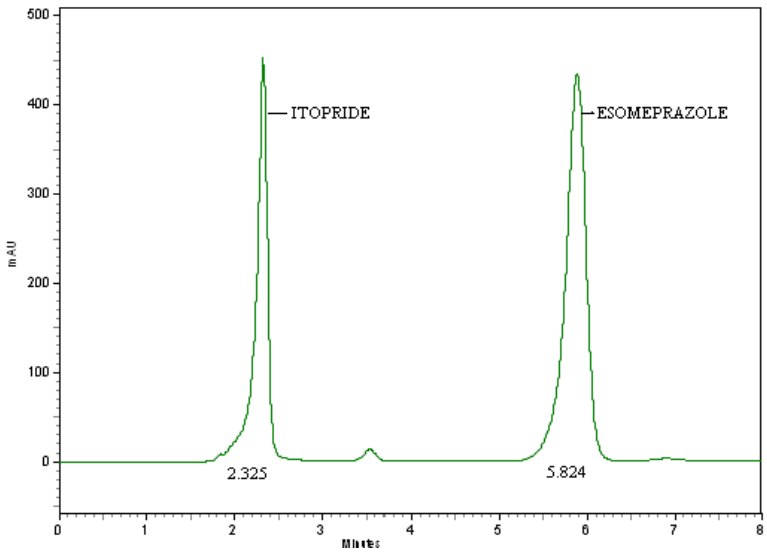

Figure 1: HPLC Chromatogram of ESO (10 µg/ml) and ITO (10 µg/ml)

Table 1: Parameters of chromatogram

Parameters	**ESO**	**ITO**
Retention Time	5.824	2.325
Tailing factor	0.93	0.97
Asymmetry factor	0.89	0.85
Theoretical plate	2265.84	2184.97

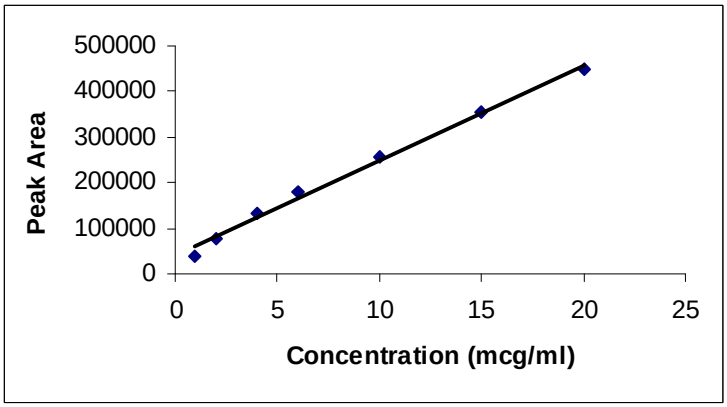

Figure 2: Calibration curve of ESO

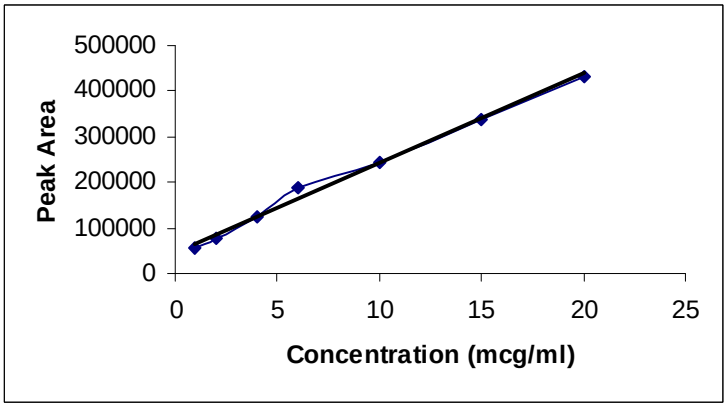

Figure 3: Calibration curve of ITO

Table 2: Data of Recovery study for ESO and ITO by HPLC method

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery ± S.D (n=3)
ESO	4	1	5.00	100.05 ± 1.200
	4	2	6.117	102.03 ± 1.407
	4	4	7.93	99.13 ± 1.145
ITO	4	1	4.81	96.28 ± 1.184
	4	2	6.13	102.34 ± 1.532
	4	4	8.1	101.28 ± 1.250

Table 3: Method Precision data for analysis of ESO and ITO by HPLC method

Parameters	Area of ESO	% Assay	Area of ITO	% Assay
1st	124565	102.1	124028	100.1
2nd	123219	100.5	124320	100
3rd	125770	103.54	125415	101.85
4th	122636	99.8	123285	99.15
5th	123570	100.92	123554	99.50
6th	124220	101.69	125870	102.43
Mean	-	101.425	-	100.505
S.D.	-	1.322	-	1.325
%CV	-	1.30	-	1.31

Table 4: Intra-day Precision data for analysis of ESO and ITO by HPLC method

Concentration of ESO and ITO (µg/ml)	Intra-day Precision			
	ESO		ITO	
	Mean ± S.D (n=3)	% RSD	Mean ± S.D (n=3)	% RSD
1	38491 ± 218.073	0.566	53500 ± 76.544	0.143
2	76501 ± 311.896	0.407	75128.33 ± 703.342	0.93
4	134282.7 ± 265.902	0.198	124564 ± 845.766	0.678
6	180314 ± 229.105	0.127	198338.3 ± 908.893	0.48
10	256721.7 ± 229.674	0.089	244544.3 ± 560.835	0.228
15	353795 ± 341.756	0.096	339735.7 ± 688.88	0.203
20	448296.3 ± 82.718	0.018	4333258 ± 747.446	0.172

Table 5: Inter-day Precision data for analysis of ESO and ITO by HPLC method

Concentration of ESO and ITO (µg/ml)	Inter-day Precision			
	ESO		ITO	
	Mean ± S.D (n=3)	% RSD	Mean ± S.D (n=3)	% RSD
1	38342.67 ± 39.627	0.103	53787.33 ± 182.544	0.342
2	76423.33 ± 192.055	0.251	75690.33 ± 247.243	0.346
4	134584 ± 362.873	0.270	124799.3 ± 685.516	0.527
6	180294.3 ± 252.702	0.14	189363.7 ± 502.26	0.265
10	256973 ± 256.568	0.099	244513.3 ± 256.582	0.105
15	353728 ± 284.428	0.0804	339459.3 ± 256.528	0.076
20	448009.7 ± 223.061	0.049	433379.3 ± 231.916	0.053

Table 6: Statistical analysis of parameters required for system suitability testing of the HPLC method

Parameters	Mean		S.D.		% RSD	
	ESO	ITO	ESO	ITO	ESO	ITO
Retention Time	5.823	2.324	0	0	0	0
Area	38491	53500	218.073	76.544	0.566	0.143
Asymmetric Factor	0.8833	0.8533	0.0051	0.0052	0.58	0.61
Tailing Factor	0.9366	0.9717	0.0082	0.0075	0.875	0.75

Table 7: Assay parameters and method validation obtained by applying the proposed methods for determination of ESO and ITO in binary mixtures

Parameters	ESO	ITO
Calibration range	1-20µg/ml	1-20µg/ml
Detection limit	0.0362 µg/ml	0.0565 µg/ml
Quantitation limit	0.1098 µg/ml	0.171 µg/ml
Slope	20954	19720
Intercept	38982	45072
Mean	101.425	100.505
Standard deviation	1.322	1.325
% RSD	1.30	1.31
Correlation coefficient	0.9922	0.9923
Intraday RSD, %	0.018-0.566	0.141-0.93
Interday RSD, %	0.049-0.27	0.053-0.527

Table 8: Application of the proposed method to the capsules

ESO			ITO		
Amount taken (mcg/ml)	**Amount found (mcg/ml)**	**% Amount Found ± S.D. (n=3)**	**Amount taken (mcg/ml)**	**Amount found (mcg/ml)**	**% Amount Found ± S.D. (n=3)**
2	1.99	99.76 ± 1.31	7.5	7.623	101.67 ± 1.09
4	4.11	102.90 ± 0.36	15	14.98	99.89 ± 0.079

Materials and Methods

Chemicals and materials:

Esomeprazole (ESO) and Itopride (ITO) were supplied by Torrent Research Center (Gandhinagar, India). Acetonitrile, Ammonium acetate and Methanol (HPLC grade, S.D. Fine

Chemicals Ltd., Mumbai), Triple distilled water, nylon 0.45 µm - 47 mm membrane filter (Gelman laboratory, Mumbai, India).

Chromatographic conditions:
Phenomenex C_{18} column (250 mm x 4.6 mm i.d., 5 µm particle size), The mobile phase: Buffer (Ammonium Acetate, pH 5.5) -Water-Methanol (25:15:60 v/v/v), Flow rate: 1.5 ml/min, Filter: Nylon 0.45 µm - 47 mm membrane filter, Mobile Phase was degassed before use, Detection wavelength: 275 nm, the injection volume: 20 µl, and Temperature: 20 ± 3^0 C. [6, 7,13]

Preparation of the mobile phase:
Accurately weighed Ammonium Acetate (2.5 gm) was transferred to a beaker (500 ml) and dissolved in tripled distilled water (500 ml).

The mobile phase was Buffer (Ammonium Acetate, pH-5.5): Water: Methanol (25:15:60). The mobile phase was filtered through nylon 0.45 µm- 47 mm membrane filter and was degassed before use. [6, 7, 13, 14]

Preparation of standard stock solution (500µg/ml) and working solution (100 µg/ml):
Accurately weighed ESO (50 mg) and ITO (50 mg) were transferred to two separate 100 ml volumetric flask. Methanol (50 ml) was added to the flask. The drug was dissolved with sonication and the final volume was adjusted with methanol up to the mark to prepare a 500 µg/ml stock solution of both drugs. The solution (20 ml) transferred into a 100 ml volumetric flask and makes the final volume with methanol to prepare 100 µg/ml working solutions. [9, 13, 14]

Preparation of Sample solution:
Twenty capsules were weighed accurately and emptied. Accurately a quantity of the powder equivalent to about ESO (10 mg) and ITO (10 mg) into 10 ml measuring flask was weighed and sonicated for 20 minutes. The solution was filtered through Whatmann filter paper No. 41 and the residue was washed thoroughly with methanol. The filtrate and washings were combined in a 10 ml volumetric flask and diluted to the mark with methanol. 1ml of extract was then transferred into 10 ml volumetric flask and diluted to the mark with methanol to get a final concentration of 100 µg/ml. [8, 9, 10, 13, 14]

Determination of wavelength of maximum absorbance:
The standard solution of ESO and ITO were scanned in the range of 200-400 nm against mobile phase as a blank. ESO and ITO showed maximum absorbance at 275 nm. So the wavelength selected for the determination of ESO and ITO was 275 nm. [9,10, 13, 14]

Pre-treatment of column
Phenomenex C_{18} column was properly washed with of Acetonitrile (HPLC grade previously filtered with Nylon 0.45 µm - 47 mm membrane filter and degassed properly) for 30 min at 1.5 ml/min of flow rate. [9, 10, 13, 14]

Chromatographic separation
With the help of micro liter syringe and loop, 20 µl of each working standard solutions and sample solution were injected into the column through loop at 1.5 ml/min flow rate. The Peaks of

ESO and ITO were detected at 275 nm and retention times were found to be 5.824 and 2.325 minutes respectively. [9, 10, 13, 14]

Calibration curve of standard ESO and ITO

A calibration curves were plotted over a concentration range of 1 - 20 µg/ml for ESO and ITO. Accurately measured standard stock solutions of ESO and ITO (0.1, 0.2, 0.4, 0.6, 1.0, 1.5 and 2.0 ml) were transferred to a series of 10 ml corning volumetric flasks and the volume in each flask was adjusted to 10 ml with mobile phase. The resulting solution was injected into the column and the peak area obtained at retention time 5.824 and 2.325 minutes and flow rate 1.5 ml/min were measured at 275 nm for ESO and ITO respectively. Calibration curves were constructed for ESO and ITO by plotting peak area versus concentration at 275 nm. Each reading was average of five determinations. [9, 10, 13, 14]

Quantitation of ESO and ITO in its Capsules

The powder was weighed and collected from 20 capsules. Accurately, a quantity of the powder equivalent to about 40 mg of ESO and 150 mg of ITO was weighed and transferred into 100 ml measuring flask and mixed with mobile phase and sonicated for 20 minutes. The solution was filtered through Whatman filter paper No. 41 and the residue was washed thoroughly with mobile phase. The filtrate and washings were combined in a 100 ml volumetric flask and diluted to the mark with mobile phase. 25 ml of extract was transferred into 100 ml volumetric flasks and diluted to the mark with mobile phase to get a final concentration of 100 µg/ml of ESO and 375 µg/ml of ITO. For final test solution, 0.2 and 0.4 ml of the above solution was transferred to 10 ml volumetric flask and diluted up to the mark with mobile phase (ESO was 2 and 4 µg/ml and ITO was 7.5 and 15 µg/ml). The solution was analyzed by preparation of calibration curve as described above. [9, 10, 13, 14]

Conclusion

From the results obtained by applying the suggested procedures, it is obvious that the proposed method can be applied successfully in routine analysis for the determination of ESO and ITO in combined dosage form without interference from commonly encountered excipients and additives and with good sensitivity.

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