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SIMULTANEOUS ESTIMATION OF ESOMEPRAZOLE MAGNESIUM TRIHYDRATE, ITOPRIDE HYDROCHLORIDE AND MOSAPRIDE CITRATE BY RP-HPLC METHOD: OPTIMIZATION, DEVELOPMENT, VALIDATION AND APPLICATION TO LABORATORY SAMPLE.

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Keywords:

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ABSTRACT: A simple, rapid, accurate, precise and economical RP-HPLC method with UV detection was optimized, developed and validated as per ICH-Q2 guideline for the simultaneous estimation of Esomeprazole magnesium trihydrate (ESO), Itopride hydrochloride (ITO) and Mosapride citrate (MOSA) in laboratory sample. An optimum condition of separation and detection was developed on a reverse-phase Supelco 516 C_{18} DB column (250mm×4.6mm i.d., 5µ particle size), using a mobile phase composition of phosphate buffer (20mM, pH-7.4 adjusted with sodium hydroxide):acetonitrile:methanol in the ratio of a 20:20:60 (%v/v) at a flow rate of 1.0 ml/min with UV detection at 275 nm within 6 min with retention time of 3.09, 3.89 and 5.19 for ESO, ITO and MOSA respectively. The standard curves were linear over the concentration range of 2-12 µg/mL, 7.5-45 µg/mL and 1.5-9 µg/mL for ESO, ITO and MOSA respectively with R^2 more than 0.999. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantification. From the validation results it was concluded that proposed method can be used for the estimation of three drugs in laboratory sample.

INTRODUCTION: Esomeprazole Magnesium Trihydrate (ESO) is chemically known as bis{6-methoxy-2[(S)-{(4-methoxy-3,5-dimethylpyridine-2-yl)methane}sulfinyl]-1H-1,3-benzimidiazole, magnesium trihydrate [**Figure 1(a)**] and used as a proton pump inhibitor for the symptomatic treatment of hyperacidic condition.

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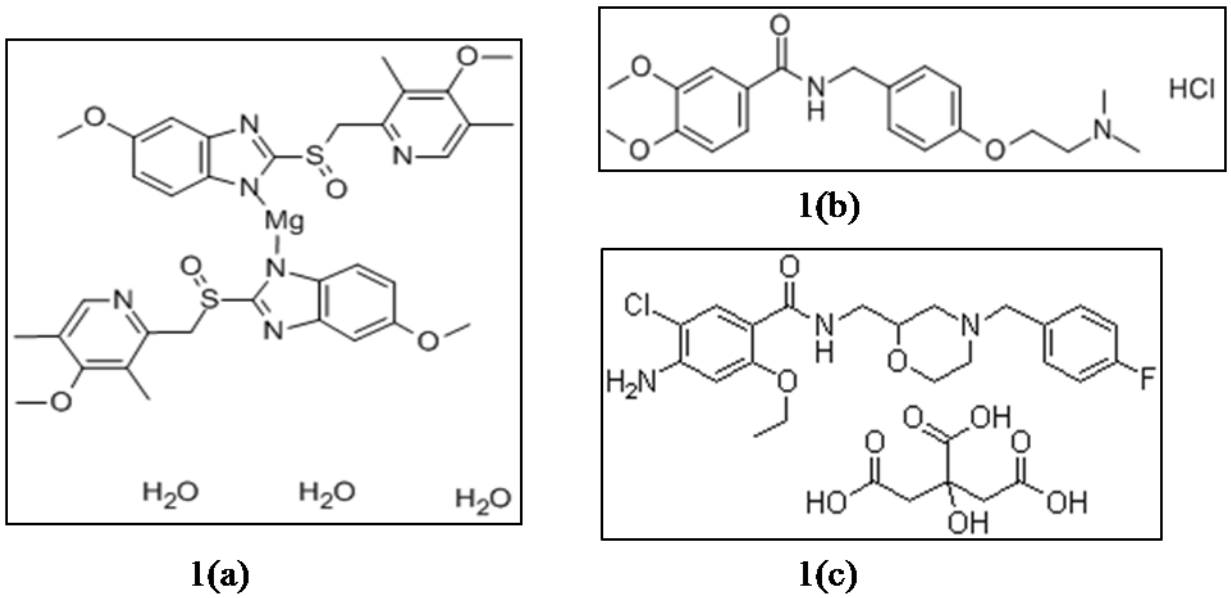
Itopride hydrochloride (ITO) is chemically known as N-[[4-(2-dimethyaminoethoxy)phenyl]methyl]-3,4-dimethoxybenzamide hydrochloride) [**Figure 1(b)**], it inhibits dopamine and has a gastrokinetic effect. Itopride is indicated for the treatment of functional dyspepsia and other gastrointestinal conditions. Mosapride citrate (MOSA) is chemically known as 4-amino-5chloro-2-ethoxy-N-[[4-[4-flurophenyl)methyl]morpholin-2-yl]methyl] benzamide 2-hydroxypropane [**Figure 1(c)**].

It is a gastroprokinetic agent that acts as a selective 5HT-4 agonist, which accelerates gastric emptying and is used for the treatment of acid reflux, irritable bowel syndrome and functional dyspepsia.



1(a) 1(b) 1(c)

FIGURE 1: CHEMICAL STRUCTURE OF (1A) ESO, (1B) ITO AND (1C) MOSA

The literature survey revealed that there are several analytical methods reported for ESO, ITO and MOSA either individually or in combination with other drugs like spectrophotometric method [1-11], RP-HPLC [11-15], stability indicating UPLC method [16], stability indicating HPTLC method [17], determination of itopride hydrochloride in human plasma by RP-HPLC with fluorescence detection [18]. However, there is no analytical method reported for the simultaneous determination of these drugs in a pharmaceutical formulation. The main aim of the present work is to develop a simple, rapid, accurate and precise RP-HPLC method for simultaneous determination of ESO, ITO and MOSA in laboratory sample. The proposed method was validated as per ICH guidelines [19].

During the development process of the present method, effect of mobile phase pH (which influences the ionization state of compounds) and mobile phase composition (which influences peak separation) were studied on various chromatographic parameters such as resolution, plates, asymmetry factor, and retention time.

MATERIALS AND METHODS:

Instrumentation: Chromatographic separation was performed on Shimadzu (Shimadzu Corporation, Kyoto, Japan) LC system equipped with Shimadzu LC-20AT pump and Shimadzu SPD-20AV detector and Rheodyne 7725 injector with fixed loop of 20 µl. Data acquisition and integration was performed using Spinchrome software.

A reverse-phase Supelco 516 C_{18} DB column with dimension of 250mm×4.6mm i.d., 5µm particle size was used.

Chemicals and reagents: Reagents such as HPLC grade acetonitrile and HPLC grade methanol were purchased from Spectrochem Pvt. Ltd. (Mumbai, India). Potassium dihydrogen phosphate and sodium hydroxide were purchased from Loba Chemicals Pvt. Ltd. (Mumbai, India). All the solutions were prepared in double distill water. Unless otherwise specified, all solutions were filtered through a 0.2 µm Ultipor® N66® Nylon 6, 6 membrane filter (Pall Life Sciences, USA) prior to use.

Year of Experiment: 2013.

Site: Quality Assurance Laboratory, Centre of Relevance and Excellence in Novel Drug Delivery System, G. H. Patel Building, Donor's Plaza, The Maharaja Sayajirao University of Baroda, Fatehgunj, Vadodara – 390 002, Gujarat, India.

Chromatographic conditions: The HPLC analysis was performed using isocratic conditions with the mobile phase phosphate buffer (20mM, pH-7.4 adjusted with sodium hydroxide): acetonitrile: methanol in the ratio of a 20:20:60 (%v/v) and at the flow rate of 1.0 ml/min. The mobile phase was premixed, filtered with 0.2 µm Nylon 6, 6 membrane filter and degassed by using sonicator before use.

Sensitivity of three drugs was found optimum at 275 nm as compared to the other wavelengths hence 275 nm was considered as the detection wavelength. Zero order overlain of UV spectra is shown in **Figure 2**.

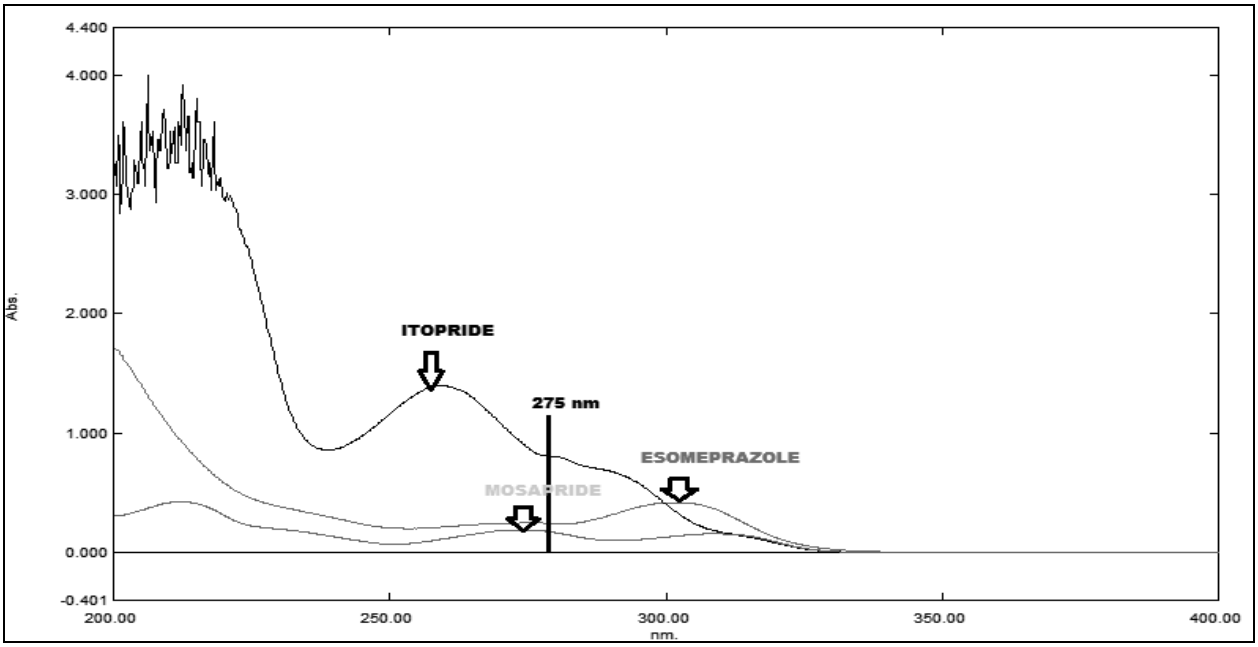


FIGURE 2: ZERO ORDER OVERLAIN SPECTRUM OF ESO, ITO AND MOSA

Preparation of standard solutions: Stock solutions of 100 µg/ml of pure ESO, ITO and MOSA were freshly prepared in the mixture of water: methanol (50:50). Aliquots of these solutions were diluted stepwise with the mobile phase to obtain 10 µg/ml of ESO, ITO and MOSA. These solutions were used for the optimization and trials of the proposed method.

Preparation of Laboratory Sample: The combined dosage formulation of ESO, ITO and MOSA is the product of Orbit Life Science Pvt. Ltd [20] which is not yet available in the market, so a laboratory sample was prepared using the excipients and by following the standard procedure[21]. The formula for laboratory sample used for analysis is mentioned in **Table 1**. 250 mg of laboratory sample was weighed and dissolved in methanol: water (50:50) to make up the volume upto 100 ml. The solution was filtered through whatman filter paper No. 41 and 0.45 µm membrane filter to remove undissolved substances. From this filtrate, 0.5 ml of aliquote was taken and diluted upto 50 ml to give resultant sample solution which was injected in HPLC. The chromatogram of the laboratory sample solution has been shown in **Figure 3**.

TABLE 1: Formula for Synthetic Mixture

Sr. No	Chemical	Quantity (mg)
1	Esomeprazole Magnesium Trihydrate	30
2	Itopride Hydrochloride	150
3	Mosapride citrate	40
4	Eudragit L-100	14
5	Hydroxy Propyl Methyl Cellulose	14
6	Talc	2
	Total	250

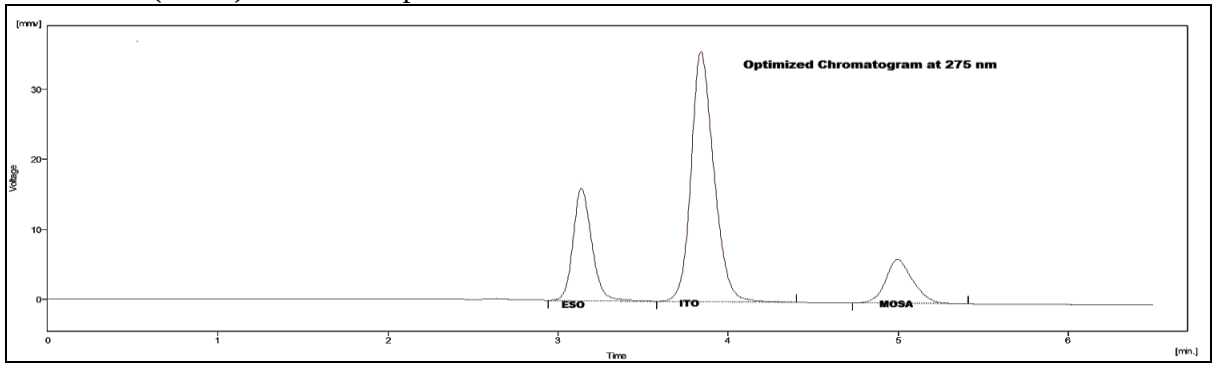


FIGURE 3: OPTIMIZED CHROMATOGRAM OF ESO, ITO AND MOSA

Validation parameters: The proposed methods were validated as per ICH guidelines for linearity, precision, accuracy, sensitivity, robustness.

1. **Linearity:** Linearity was determined by taking six different concentrations of ESO, ITO and MOSA in triplicate and calibration curves were plotted in specified range of 2-12 µg/ml, 7.5-45 µg/ml and 1.5-9 µg/ml for ESO, ITO and MOSA respectively. Calibration curve was evaluated using the least square method within Microsoft Excel® program.

2. **Precision and accuracy:** Intra-day precision and inter-day precision for developed method was measured in terms of percentage relative standard deviation (% R.S.D). The experiments were repeated three times a day for intra-day precision and on three different days for inter-day precision. For the developed method the accuracy was studied using standard addition method at the levels of 50%, 100% and 150%.

3. **Sensitivity:** Sensitivity of the method was determined by calculating LOD and LOQ using following equation:

$$LOD = 3.3*\sigma/S \quad \text{..... (1)}$$

$$LOQ = 10*\sigma/S \quad \text{..... (2)}$$

Where: σ = Standard deviation of the response and S = Slope of the calibration curve

4. **Robustness**: Robustness of methods was determined in form of % R.S.D by small deliberate changes in flow rate, mobile phase ratio and pH of mobile phase.

5. **System suitability parameters:** System suitability parameters were studied to verify that resolution and reproducibility of the system are adequate for the analysis. Parameters such as selectivity, theoretical plates, asymmetry, and resolution and capacity factor have been determined.

RESULTS AND DISCUSSION:

Optimization of method:

(1) Effect of mobile phase pH: As with the aim of the optimization of mobile phase pH (7.0, 7.4, and 7.8), the remaining factors were kept constant, i.e. mobile phase composition phosphate buffer (20mM, pH-7.4 adjusted with sodium hydroxide): acetonitrile: methanol in the ratio of a 20:20:60 (%v/v) and flow rate of 1 ml/min. The number of theoretical plates as well as resolution between ESO and ITO as well as ITO and MOSA were found to be higher at pH 7.4. Thus, the best chromatographic separation was achieved at pH 7.4, and hence was considered to be optimum.

(2) Effect of mobile phase composition: The mobile phase composition, i.e. phosphate buffer (20mM, pH-7.4 adjusted with sodium hydroxide): acetonitrile: methanol was varied at 30:20:50, 20:20:60 and 10:20:70 while keeping the pH 7.4 and flow rate of 1.0 ml/min. Generally, increasing the organic solvent concentration in the mobile phase induces a decrease in distance between the solute molecule and terminal carbon atoms (C_{18}) in the ODS ligand and results in lower retention time. This may be explained by elution power of mobile phase decreased at 30:20:50 v/v; hence as the relative amount of organic phase decreased, the three drug components were eluted at higher retention time.

The elution power of mobile phase was increased at 10:20:70 v/v composition, resulting in the elution of ESO and ITO at 2.23 and 2.75 min retention time respectively (this low retention time could not give proper resolution between the peaks from sample matrix and so this ratio was not selected). However, at 20:20:60, v/v composition, proper resolution was achieved along with appropriate elution time and hence this composition was considered as optimum.

Validation:

1. **Linearity:** Method was found to be linear over concentration range of 2-12 µg/ml, 7.5-45 µg/ml and 1.5-9 µg/ml for ESO, ITO and MOSA respectively. The linear regression equations were calculated by the least squares method using Microsoft Excel® program.

Results of linearity are shown in **Table 2** and the R^2 values were found to be 0.9993, 0.9993 and 0.9991 for ESO, ITO and MOSA respectively.

2. **Precision and Accuracy**: The results of precision were found to be with less than 2% relative standard deviation for the drugs ESO, ITO and MOSA respectively for intra-day and inter-day precision. These results suggest that the developed analytical method is precise and reproducible. In order to examine the accuracy of method, recovery studies were carried out by standard addition method at different levels of 50%, 100% and 150% and the recovery was found to be between 98-102%. Precision and recovery results are explained in **table 2 and table 3** which conclude that the method has enough reproducibility and accuracy so that it can be applicable to laboratory sample.

TABLE 2: VALIDATION PARAMETERS OF RP-HPLC METHOD FOR ESO, ITO AND MOSA

Parameters	ESO	ITO	MOSA
Detection Wavelength		275 (nm)	
Linearity range (µg/ml)	2-12	7.5-45	1.5-9
Regression Coefficient (R^2)	0.9993	0.9993	0.9991
Regression equation (y=mx+c)	y=15.522x +7.231	y= 10.412x +14.714	y= 9.9728x +6.3917
LOD(µg/ml)	0.0733	0.0598	0.3047
LOQ (µg/ml)	0.2227	0.2904	0.9236
Intra-day Precision (%RSD)	0.2746	0.5206	0.7712
Inter-day Precision (%RSD)	0.9079	1.0351	1.7339

TABLE 3: RESULTS OF RECOVERY STUDY OF ESO, ITO AND MOSA BY RP-HPLC METHOD

% Amount	Name of drug	C_{ACTUAL} (µg/ml)	C_{ADDED} (µg/ml)	C_{FOUND}† (µg/ml)	%RECOVERY ±S.D.
50	ESO	4	2	6.01	100.16 ± 0.3353
	ITO	15	7.5	22.43	99.68 ± 0.5376
	MOSA	3	1.5	4.56	101.33± 0.3463
100	ESO	4	4	7.98	99.75 ± 0.2921
	ITO	15	15	29.89	99.63 ± 0.5325
	MOSA	3	3	5.96	99.33 ± 0.3421
150	ESO	4	6	10.09	100.9 ± 0.2935
	ITO	15	22.5	37.56	100.16 ± 0.3181
	MOSA	3	4.5	7.54	100.53 ± 0.6538

† Mean value of three determinations

3. **Sensitivity:** To study the sensitivity of optimized method LOD and LOQ were determined using blank replicate injections by equation 1 and 2. LOD values for ESO, ITO and MOSA were found to be 0.0733 µg/ml, 0.0598 µg/ml, 0.3047 µg/ml and LOQ values were 0.2227 µg/ml, 0.2904 µg/ml, and 0.9236 µg/ml respectively. These results show that method is enough sensitive for the analysis of laboratory sample. Results of LOD and LOQ are incorporated in **Table 2**.

4. **Robustness:** Robustness of the method was determined by small deliberate changes in flow rate, mobile phase ratio, pH of mobile phase and detection wavelength. The method was found to be enough robust and will provide accurate results in normal quality control labs even if there is some sort of experimental error by human or system.

5. **System suitability parameters:** System suitability parameters were studied to verify the optimum conditions. Different parameters have been evaluated such as resolution, capacity factor, separation factor, theoretical plats and asymmetry. The results obtained are summarized in **Table 4**.

TABLE 4: SYSTEM SUITABILITY PARAMETERS VALIDATED RP-HPLC METHOD

SST Parameters	ESO	ITO	MOSA
Retention Time	3.097	3.890	5.187
Capacity factor (k)	1.62	2.21	3.17
Separation factor (α)	2.5808	1.256	1.333
Theoretical plates (USP)	3863	4779	5107
Resolution (R_s)	-	3.187	4.406
Asymmetry (A_s)	1.303	1.376	1.305

Analysis of the laboratory sample: As the preliminary validation parameters show satisfied results hence the method was applied to laboratory sample. In the assay of laboratory sample, percentage purity was found to be 101.5462 ± 0.3883 % w/w, 98.7254 ± 0.4553 % w/w and 99.4632±0.3243% w/w for ESO, ITO and MOSA respectively. The results for percentage purity are also shown in **Table 5**.

TABLE 5: PERCENT PURITY (ASSAY) OF ESO, ITO AND MOSA IN SYNTHETIC MIXTURE

ESO±S.D	ITO±S.D	MOSA±S.D
99.75 ± 0.2921	99.63 ± 0.5325	99.33 ± 0.3421

*Mean value of three determinations ± Standard Deviation

CONCLUSION: The proposed RP-HPLC method can be applied for the simultaneous determination of ESO, ITO and MOSA. Moreover this method is simple, rapid, accurate, precise, reliable and economic. This method can be used for routine quantitative estimation of three components in laboratory sample.

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