Single validated HPLC method for the estimation of Esomeprazole, Pantoprazole, Rabeprazole, Itopride and Levosulpiride in Capsule dosage forms

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Abstract:

Proton pump inhibitors like Esomeprazole (ESP), Pantoprazole (PNP), Rabeprazole (RBP) are used in combination with prokinetic agents Itopride (ITP) and Levosulpiride (LSP) to improve patient compliance in gastric disorders. Many pharmaceutical companies are manufacturing one or more of these combination drug products. The current research article focussed on development and validation of single analytical method with short run time for the estimation of all the mentioned five drugs in capsule dosage forms. Separation was achieved on Zodiac C18 column (150mm X 4.6mm) and 3.5 µM particle size, using mobile phase (KH2PO4) adjusted to pH 3 using Ortho phosphoric acid and acetonitrile in the ratio of 63:37 %v/v run at flow rate of 0.7 mL/min. All the five peaks are eluted within 8 minutes. The retention times are 2.50 min, 3.46 min, 4.85 min, 5.91 min, 6.91 min for ITP, LSP, RBP, ESP, PNP respectively. The compounds are monitored at 290nm. The method is validated as per ICH Q2(R2) guideline. The % recovery values are between 98.0% to 102.0% for all the mentioned five drugs. The %RSD less than 2.0% indicates the method precision. The correlation coefficient values greater than 0.998 for all the drugs indicates the good linear relationship between concentration and peak response. Based on the validation results the method is successfully applied for the estimation of mentioned drugs in commercially available capsule dosage forms.

Key words:

Capsule dosage forms, HPLC, Proton pump inhibitors, Prokinetic agents, Validation.

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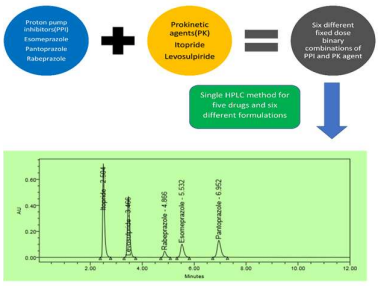
Graphical Abstract



1.Introduction:

GERD (gastric oesophageal reflux disease) is the chronic disorder of upper gastro intestinal tract. The main principles of the treatment include control of acid secretion and improvement of gastric motility. Proton pump inhibitors are the substituted benzimidazoles (PPI's) viz Pantoprazole (PNP), Esomeprazole (ESP), Rabeprazole (RBP) are drugs which irreversibly inhibit proton pump (H+/K+ ATPase) function and are the most potent gastric acid-suppressing agents in clinical use [1]. Prokinetic agents viz Levosulpiride (LSP) and Itopride (ITP) have potential usefulness as adjunctive treatment of GERD by increasing lower oesophageal sphincter pressure, enhancing gastric emptying, and

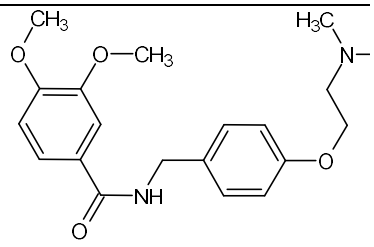
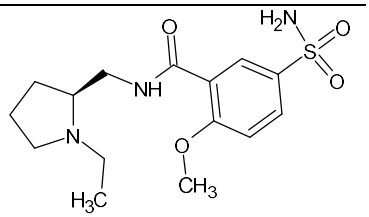
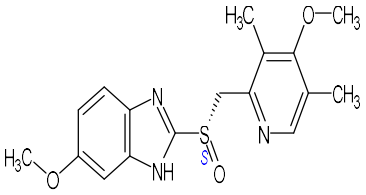
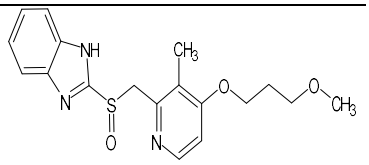
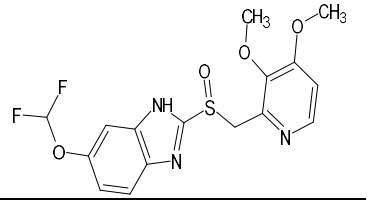
improving peristalsis [2]. An improved patient compliance is observed with combination of prokinetic agents with the PPI than the monotherapy [3]. Currently following combinations drug products in capsule dosage forms are available in market with the above mentioned five drugs.

1. Esomeprazole and Levosulpiride
2. Pantoprazole and Levosulpiride
3. Rabeprazole and Levosulpiride
4. Esomeprazole and Itopride
5. Pantoprazole and Itopride
6. Rabeprazole and Itopride

Many pharmaceutical industries manufacture their drug products either in combination drug products of PPI's and prokinetic agents or as single dosage forms. Quantitation of drugs is required in routine quality control, stability and in process tests like assay, dissolution and content uniformity. Most of the industries use different analytical methods for different formulations which is time consuming and costly.

As per the literature [4-8] there are several HPLC and LCMS/MS methods were reported for determination of proton pump inhibitors in pharmaceutical drug products. There are also reported analytical methods available for the mentioned prokinetic agents for single and simultaneous estimation with proton pump inhibitors [9-14], however to the best of our knowledge there is no HPLC method available for simultaneous estimation of all mentioned five drugs. The proposed analytical method with the shorter run time of 12 minutes serves as single run method for the separation and quantitation of all the above mentioned five drugs PNP, RBP, EMP, ITP, LSP in 6 combined formulations of PPI's and prokinetic agents.

Table 1: Chemical structures

Name	Chemical structure
Itopride	
Levosulpiride	
Esomeprazole	
Rabeprazole	
Pantoprazole	

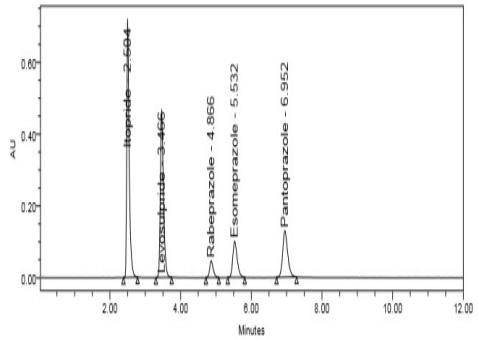
2. Materials and methods

2.1 Chemicals and reagents:

Analytical reagent grade (AR) Potassium dihydrogen phosphate, phosphoric acid, HPLC grade water and acetonitrile were purchased from local Sigma-Aldrich suppliers. Rabeprazole, Esomeprazole, Pantoprazole, Levosulpiride, Itopride working standards were obtained as gift samples from local pharmaceutical companies with greater than 99.5% purity.

2.2 Equipment:

HPLC waters e 2695 model equipped with e2695 PDA detector



2.3 Standard solutions details:

Standard Stock solutions were prepared by dissolving about 37.5mg, 18.75mg, 10mg, 10mg, 5mg of ITP, LSP, PNP, ESP, RBP respectively in 50mL amber volumetric flask by dissolving them in

20 mL methanol sonicated to dissolve and diluted to volume with diluent containing water: acetonitrile in the ratio of 50:50% v/v. From this stock solution working standard solution containing 150 µg/mL of ITP, 75 µg/mL of LSP, 40 µg/mL of PNP, 40 µg/mL of EMP, 20 µg/mL of RBP was prepared by diluting 10.0mL of this stock solution to 50mL with diluent in 50mL amber volumetric flask. This solution is considered as 100% solution and stored at refrigerator conditions at 2-8°C.

2.4 Method development:

Method development trials were done on C8 and C18 columns with 150 mm diameter and 4.6 mm ID and 3.5µm particle size at pH 3.0, pH 6.0 and pH 9.0 using linear gradient experiments starting from 10% to 90% acetonitrile with a flow rate 1.0 mL /min and run time of 15 minutes by keeping column temperature at 30°C. With pH 6.0 and 9.0 peak shapes for the prokinetic agents were not good and resolution also compromised. Resolution between Levosulpiride and Rabeprazole is not good with C8 column. Best resolution and peak shapes were obtained at pH 3.0 on C18 column. Flow rate, column temperature, injection volume was further optimized to get better peak shapes and less run time. Wavelength of 290nm was selected where all the five drugs shown good absorbance. All the five peaks were separated at less than 40% of acetonitrile. Final optimized chromatographic conditions mentioned in section 2.5.

2.5 Optimized Chromatographic conditions:

Zodiac C18 column (150mm X 4.6mm), 3.5 µM particle size was used for separation of the drugs and column temperature was maintained at 30°C. Auto sampler temperature maintained at 10°C. The output signal was monitored and processed by using Empower software. Mobile phase consisting of 10mM Potassium dihydrogen phosphate buffer of pH 3.0 adjusted with phosphoric acid solution: Acetonitrile in the ratio 63:37% v/v run with a flow rate of 0.7 mL/min in isocratic mode. The injection volume was set at 10 µL. The elute was monitored at 290nm.

2.6 Degradation sample preparation details:

Acid, Base, Peroxide, Heat, Hydrolytic and Photo degradation samples were prepared in 250 mL volumetric flasks by spiking the enteric coated and sustained release placebo powders with about 10mg ESP, 5mg RBP, 10mg PNP, 37.5mg ITP, 18.75mg LSP and subjected to degradation. Acid, Base, Hydrolytic and Oxidation degradation samples were subjected to degradation by adding 10 mL of 0.01N hydrochloric acid, 10mL 0.01N sodium hydroxide, 10mL water and 10mL 3% hydrogen peroxide respectively kept at room temperature for 2 hours, light degradation sample is placed in photolytic chamber at 1.2 million Lux hrs, heat degradation sample kept in oven at 60°C for 2 hrs. After degradation acid and base samples are neutralized and all the degradation samples prepared same as per the procedure mentioned for formulation sample solution.

2.7 Formulation sample solutions preparation procedure:

Weighed 20 capsule contents of each formulation separately and triturated to make powder, weighed an amount of capsule powders equivalent to 5 mg RBP and 37.5 mg of ITP, 10 mg ESP, 10 mg PNP, 18.75 mg of each formulation into 6 different 250 mL volumetric flasks, added 50 mL of methanol, sonicated for about 30 minutes to dissolve, diluted to volume with solvent (50:50 % v/v water: acetonitrile% v/v), mixed well, filtered through 0.45µm PTFE filter and filled into HPLC vials and injected.

2.9 Method validation:

Method validation was performed as per ICHQ2(R2) guideline[15]. Specificity, precision, linearity, accuracy, robustness parameters were studied.

3. Results and discussion:

3.1 Method development results:

The optimized chromatographic method was able to separate all the five drugs with minimum resolution greater 3.0 and maximum USP Asymmetry less than 1.6. Theoretical plate count was more than 5000 for all the peaks.

Figure 1 Optimized chromatogram standard solution

3.2 Method validation results

3.2.1 Selectivity:

Selectivity of the method was determined by injecting solvent, placebo solution and forced degradation samples and determined the peak purity values. No interference observed at the retention times of all the mentioned drugs in the solvent and placebo chromatograms. Peak purities of the degradant samples were determined by calculating the purity angle and purity threshold values using empower software. For all the five mentioned drugs purity threshold is more than the purity angle and no degradant peak is interfering with the main peaks.

Figure 2: Acid degradation sample chromatogram

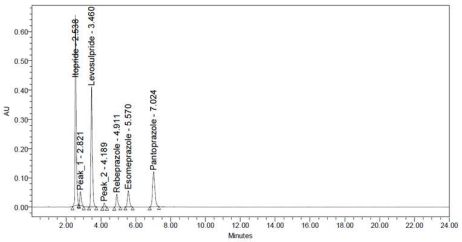


Figure 3: Base degradation sample chromatogram

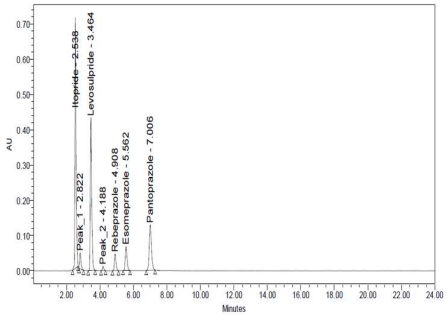


Figure 4: Peroxide degradation sample chromatogram

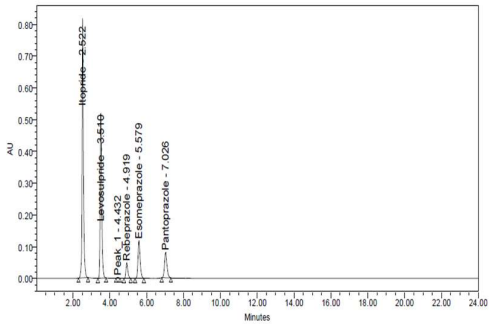
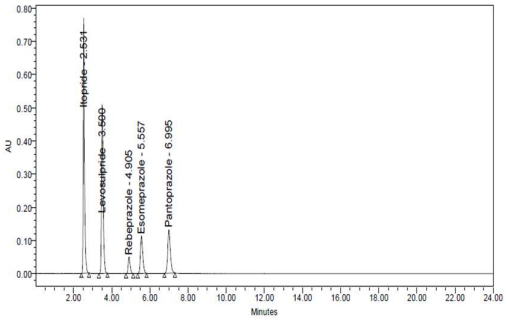


Figure 5: Light degradation sample chromatogram



3.2.2 Method Precision and application to marketed formulation:

Method precision was established by preparing six sample solutions of each formulation by independent weighing and calculated the percent assay values. The %RSD(n=6) of the assay values less than 2.0%, indicates the method precision. The results were reported in table 1

Table 2: Method precision and %Assay results

Marketed formulation	Results: Mean %Assay(%RSD), n=6	
Rabeprazole(20mg) & Itopride(150mg)	Rabeprazole:	100.38(1.19)
	Itopride	100.21(0.37)
Rabeprazole(20mg) & Levosulpiride(75mg)	Rabeprazole:	99.40(1.10)
	Levosulpiride	99.81(0.88)
Pantoprazole(40mg) & Itopride(150mg)	Pantoprazole:	100.75(0.92)
	Itopride	100.59 (0.66)
Pantoprazole(40mg & Levosulpiride(75mg)	Pantoprazole	99.32 (0.79)
	Levosulpiride	99.96(0.61)
Esomeprazole(40 mg) & Itopride(150mg)	Esomeprazole	99.91(0.55)
	Itopride	98.96(0.82)
Esomeprazole(40 mg) & Levosulpiride(75mg)	Esomeprazole	99.50(1.32)
	Levosulpiride	99.72(0.39)

3.3.3 Linearity

The linearity was established for all the mentioned five drugs from 25% to 150% of the analyte concentration. The correlation coefficient values greater than 0.998 indicates good correlation between the concentration and peak areas of the respective analytes.

Linearity results were mentioned in the table 3.

Table 3 linearity results

Drug name	Slope	Y intercept	Correlation coefficient
Itopride	22343	7423	0.9997
Levosulpiride	38083	759	0.9995
Rabeprazole	16097	249.4	0.9998
Esomeprazole	21827	8360	0.9989
Pantoprazole	31452	5312	0.9999

3.3.4 Accuracy:

Accuracy of the method is determined by spiking all five the drug substances to the generic placebo mixture at three different concentration levels in triplicate i.e. 50%, 100% and 150% of the analyte concentration. The mean percentage recovery(n=9) and %RSD(n=9) were calculated at each level and found to be in between 98.0 to 102.0% and %RSD values were less than 2.0% for all the reported drugs.

3.3.5 Robustness:

Robustness of the method was evaluated by performing small and deliberate changes to the Flow rate (0.6 to 0.8mL/min), pH (2.9 to 3.1) mobile phase A composition (66% to 69%), and column temperature (28 to 32°C). The effect of these changes resolution and tailing factor and theoretical plates was evaluated. The method was found robust in the studied ranges with minimum resolution more

than 2.5, USP Asymmetry less than 1.6 and minimum theoretical plates greater than 5000.

4. Conclusion

A new and single green analytical method with short run time was developed which can be used for quantitation of five different drugs PNP, RBP, EMP, ITP, LSP in 6 combined formulations of PPI and prokinetic agents.

The method was validated as per ICH guidelines. As per the validation results method is proven to be selective, precise, accurate, linear and robust. The method can be successfully used for the routine quality control assay, dissolution and content uniformity testing by HPLC.

5. Acknowledgement

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6. Conflict of Interests

The authors declare no conflict of interest.

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