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New method for simultaneous determination of Rabeprazole and Itopride in pharmaceutical dosage form by UV-visible absorption spectrophotometry

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ABSTRACT

A simple, accurate and rapid spectrophotometric method for simultaneous determination of Rabeprazole (RAB) and Itopride (IPD) in binary mixture has been developed. Two calibration curves were carried out; the first was elaborated to determine Rabeprazole in the mixture at 462 nm and the second was used to determine the concentration ratio (CR) in the mixture at 419 nm. This last was plotted by determination of the absorbance ratio (AR) against the concentration ratio $CR = [RAB] / [IPD]$. Finally the concentration of the RAB was deduced $[RAB] = CR_{exp} \times [IPD]$. The developed method has been validated and has given satisfactory results. The results of analysis formulation given as percentage of label claim were found to be 99.85% and 99.67% for RAB and IPD respectively for AR_1 , 99.54 for RAB and 99.74 for IPD for AR_2 . The suggested method could be used in the routine quality control analysis of pharmaceutical formulation

Keywords: UV-visible Spectrophotometry, Rabeprazole, Itopride, Concentration ratio, Absorbance ratio

INTRODUCTION

Itopride Hydrochloride

Itopride hydrochloride is an oral prokinetic agent used in the treatment of gastric motility disorders like dyspepsia of a non-ulcer/dysmotility type. There is evidence

that Itopride may have prokinetic effects throughout the gastrointestinal tract from the stomach to the end of the colon. Very few methods were reported for quantitative analysis of Itopride in biological and non biological samples [6-9].

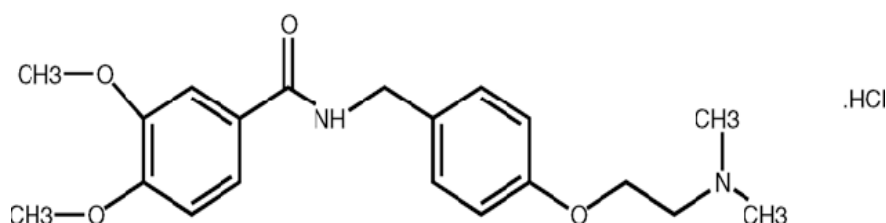


Fig. 1: Chemical structure of Itopride hydrochloride

Rabeprazole Sodium

Rabeprazole is a proton pump inhibitor (PPI) and a potent inhibitor of gastric acidity used in the therapy of gastroesophageal reflux and peptic ulcer disease. Rabeprazole therapy is associated with a low rate of transient and asymptomatic

serum aminotransferase elevations and is a rare cause of clinically apparent liver injury. Very few methods were reported for quantitative analysis of Rabeprazole alone in biological and non biological samples [11-17].

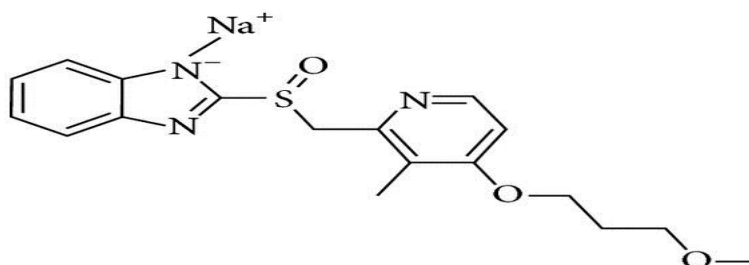


Fig.2: Chemical structure of Rabeprazole Sodium

LITERATURE REVIEW

Rabeprazole Sodium

Sun LN, Shen YW et.al reported LC-MS/MS method for simultaneous quantification of Rabeprazole sodium with the title “Stereo selective pharmacokinetics of (R)-(+)- and (S)-(-)-**Rabeprazole** in human using chiral LC-MS/MS after administration of **Rabeprazole sodium** enteric-coated tablet” [1].

Kumar N et.al reported RP-HPLC method for simultaneous quantification of Rabeprazole sodium with the title “Development and Validation of a Stability-Indicating RP-HPLC **Method** for the Determination of Process-Related Impurities and Degradation Products of **Rabeprazole Sodium** in Pharmaceutical Formulation [2]

Itopride Sodium

Thiruvengada RV et.al reported RP-HPLC method for simultaneous quantification of Itopride sodium with the title “A Simple RP-HPLC Method for quantization of Itopride HCl in Tablet Dosage Form” [3].

Combination of Rabeprazole sodium and Itopride hydrochloride

Mohamed HM et.al reported spectrophotometric methods for the simultaneous determination of Rabeprazole sodium and Itopride hydrochloride with the title “A study of selective spectrophotometric methods for simultaneous determination of Itopride hydrochloride and Rabeprazole sodium binary mixture by resolving sever overlapping spectra” [4]

El-Fataty HM et.al reported HPLC-DAD method for simultaneous determination of Rabeprazole sodium and Itopride hydrochloride with the title “Stability-indicating HPLC-DAD methods for determination of two binary mixtures [5].

MATERIALS AND METHODS

Chemicals and reagents

The combined dosage form of RAB and IPD is available in the form of sustained release capsules available at the local pharmacy. Each

tablet contains 20 mg of RAB and 150 mg of IPD. Water was prepared by using water distillation system.

Instrument

Spectral and absorbance measurements were made on an UV-visible double beam Spectrophotometer Perkin-Elmer Lambda 12 with a spectral band width of 1 nm, a wavelength accuracy of 0.3 nm and automatic correction with two matched 1cm quartz cells.

PREPARATION OF SOLUTIONS

Stock solution

RAB and IPD stock solutions (1mg/ml) were prepared by dissolving 237 mg each of RAB and 330 mg of IPD using distilled water in volumetric flask respectively. This solvent was selected for the current study on the basis of solubility and physicochemical characters of the two drugs. Standard stock fresh solutions were prepared before each test.

Working solutions

For the preparation of working solution containing different ratio of the both drugs, two series of solutions of RAB (1–5 µg/ml) and IPD (0.7–3.5 µg/ml) were prepared from their stock solutions and distilled water.

Binary mixture solutions

Five binary mixture solutions were prepared by mixing 5ml of each of RAB and IPD working solution in the same 10ml volumetric flasks (v/v) to obtain 1–5 µg/ml of RAB and 0.7–3.5 µg/ml of IPD. We calculate concentration ratio $CR = [RAB] / [IPD]$ for the elaboration of the calibration curve.

Selection of Wavelengths

For determination of λ_{1max} of RAB and λ_{2max} of IPD. Standard solution of RAB (50 µg/ml) and IPD (50 µg/ml) were prepared by appropriate dilution of standard stock solutions and scanned separately in the range of 400–800nm against a blank.

Assay of RAB and IPD combined dosage forms (Tablets)

Ten capsules content is taken and powdered. Powder equivalent of 20mg of RAB and 150 mg of IPD was dissolved in distilled water using 100ml volumetric flask, then add 0.2% w/v $FeCl_2$ and 2 drops of 1,10 phenanthroline and subject to heating for 15 min and filtered. An accurate dilution was made using water distilled to prepare sample containing 2.5 µg/ml of RAB and 2.8 µg/ml of IPD.

Validation of the method

The validation of the method was carried out according to International Council Harmonization guidelines [17, 18] (ICH Q2). We studied some parameters of validation such as linearity, accuracy, and precision. Precision was assessed using repeatability.

Determination of concentrations

The concentration of RAB and IPD was determined directly by the sample absorbance measurement and the regression equation of the first curve calibration. On the other hand, the second calibration curve was used to determine the concentration ratio (CR) in the mixture and finally deduce the concentration of the RAB. $[RAB] = CR_{exp} \times [IPD]$

RESULTS AND DISCUSSION

We have developed a spectrophotometric method, for the simultaneous determination of RAB and IPD. Results are summarized in this paragraph.

Overlay absorption spectrum

For the simultaneous determination of two active pharmaceutical ingredients by standard mixture ratio method, wavelengths of the two drugs were determined. The absorption spectrum RAB has a characteristic absorption peak at 462nm, while for IPD the λ_{max} was shown at 419 nm. The overlay absorption spectrum of RAB and IPD separately and in the binary mixture were shown in fig. 3, and 4 respectively.

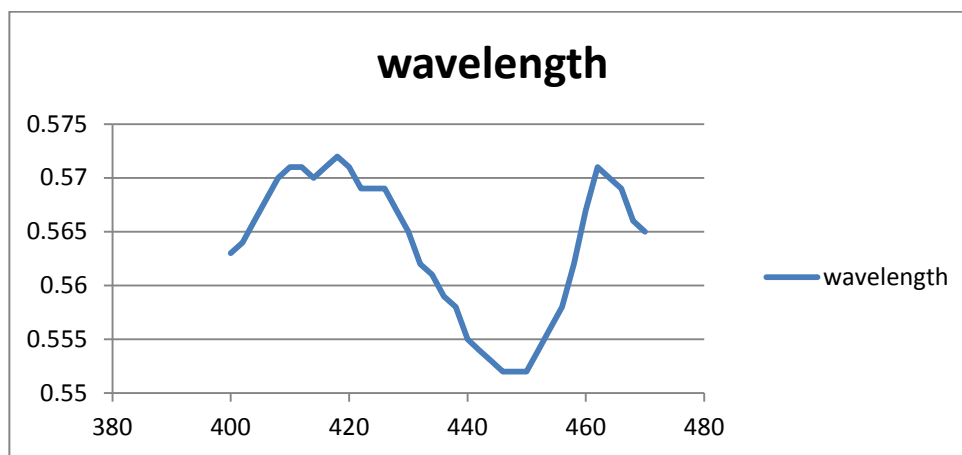


Fig.3: Absorption maximum Spectra of RAB and IPD

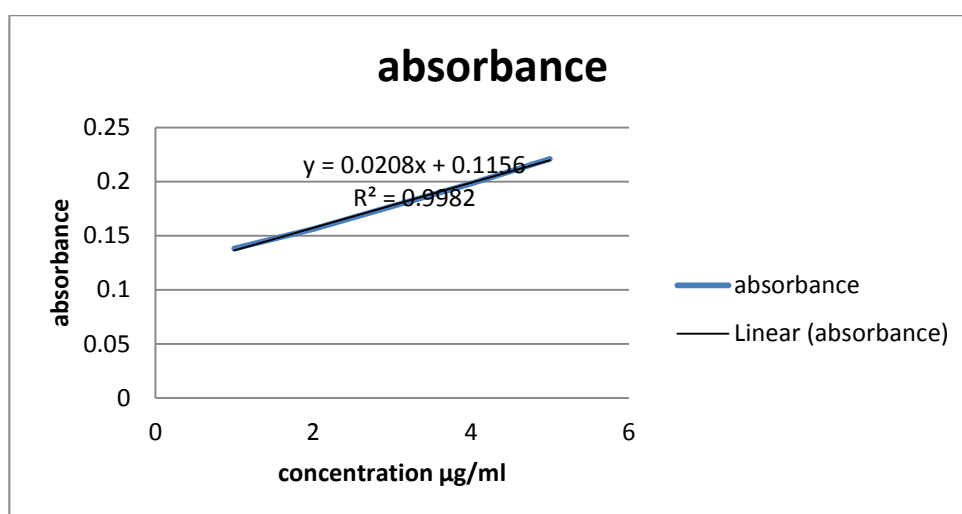


Fig.4: Calibration curve of RAB alone at 462 nm

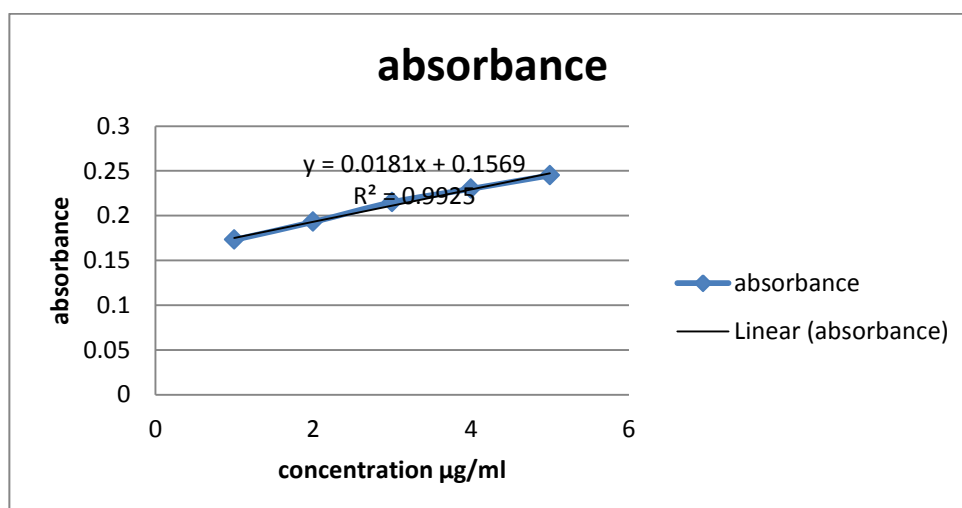


Fig. 5: Calibration curve of IPD in the mixture at 419 nm

The result of the two calibration curves shows that there is no significant difference

between the two slopes, which proves that the peak observed in the spectrum of the mixture at

462 nm corresponds to the RAB and 419 nm corresponding to IPD. Therefore, the concentration of the RAB and IPD can be determined.

Calibration curves

Absorbance of the spectra of standard mixtures solutions were measured at 462 nm corresponding to the concentrations of RAB, and at 419 nm corresponding to the total content of RAB and IPD in the mixture. Two standard calibration curves have been plotted against a blank; the first one was constructed by plotting absorbance of RAB at 462 nm against its concentrations in standard mixture solutions (Fig. 6). The equation of the calibration curve was $A = f([RAB])$ with

A: Absorbance of RAB and [RAB]: concentration of RAB in the mixture

The second calibration curve was plotted by determination of the absorbance ratio (AR) against the concentration ratio CR with $CR = [RAB] / [IPD]$

Different Concentration Ratios AR have been studied (A_1/A_2 , A_2/A_1 , A_1-A_2/A_1 , A_2+A_1/A_2 ...) to develop an appropriate method of analysis, the criteria used being the linearity and the accuracy of the method i.e. the ratio that will give the best results and the best recoveries. Two absorbance ratios AR1 and AR2 were selected as AR for elaboration of calibration curve (Fig. 7 and 8), $AR_1 = A_1/A_2$ and $AR_2 = A_2-A_1/A_2$ with A_1 and A_2 the absorbance of standard mixture solutions in 419nm and 462 nm respectively. The equation of the second standard calibration curve was $AR = f(CR)$ With: AR: Absorbance ratio (AR_1 or AR_2) and CR: Concentration ratio $[RAB] / [IPD]$.

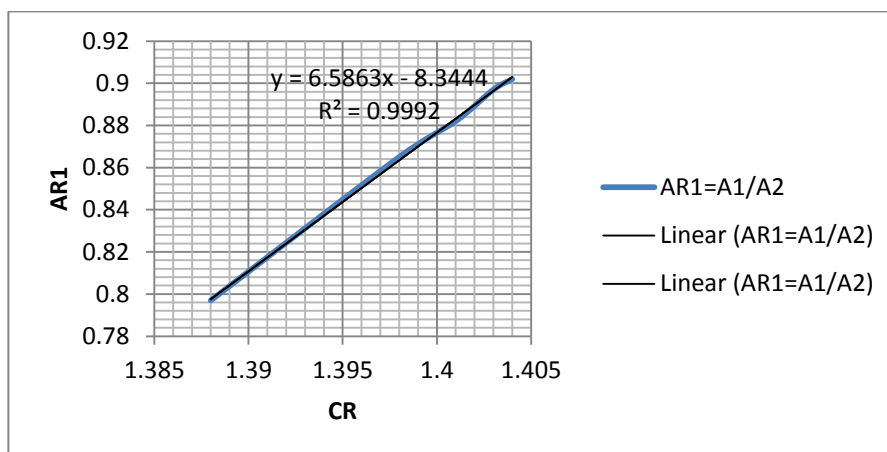


Fig. 6: Calibration curve of standard mixture solutions of RAB and IPD. $A_1/A_2 = f(CR)$

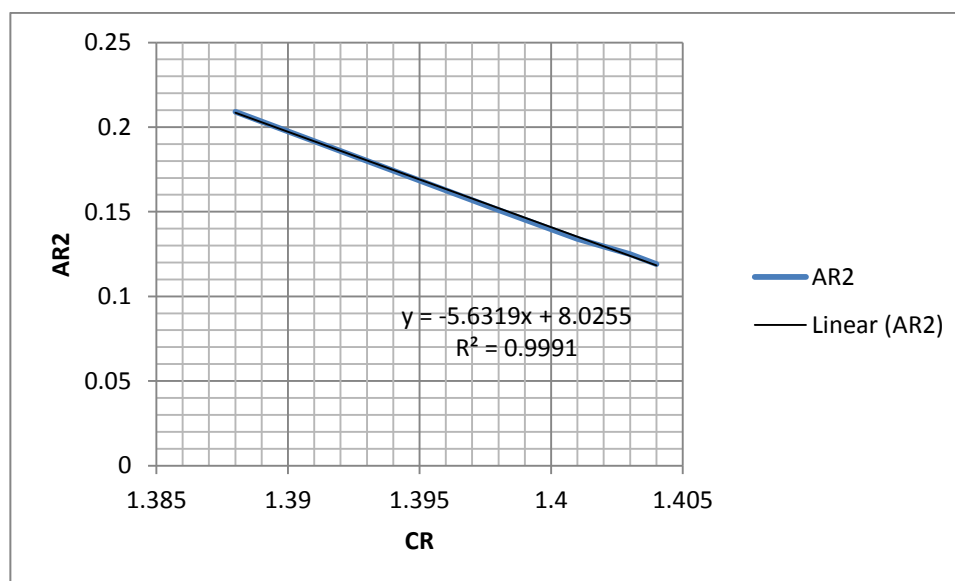


Fig. 7: Calibration curve of standard mixture solutions of RAB and IPD. $(A_2 - A_1) / A_2 = f(CR)$

VALIDATION OF METHOD

Linearity

The linearity of the proposed method was evaluated by analyzing five concentrations of mixture standard solutions of RAB and IPD. The slopes of the regression lines and intercept values for calibration curves $y = 0.020x + 0.115$ ($R^2 = 0.998$) for RAB in the mixture, $y = 0.018x + 0.156$ ($R^2 = 0.992$) for the first ratio of the mixtures AR_1 and $y = 0.7x + 0$ ($R^2 = 1$) for the second ratio of the mixtures AR_2 . The two active pharmaceutical ingredients obeyed Beer Lambert's law in the concentration range of 1-5 $\mu\text{g/ml}$ for RAB and 0.7-3.5 $\mu\text{g/ml}$ for IPD with excellent correlation coefficients indicating good

linearity. The equations obtained to determine the concentrations of RAB and IPD.

ACCURACY

The accuracy is defined as the closeness of agreement between the conventional true value or a reference value and the value found¹⁸. To test the accuracy of this analytical method, recovery studies were performed by the method of assaying a mixture samples at known quantities of the both drug substances at three different levels 80%, 100%, and 120%. The percentage recoveries and the percentages residual standard deviation (RSD) were calculated. Three determinations were made. Results show a good accuracy for the three cases (Table 1).

Table 1: Accuracy values of concentration ratio (RAB/IPD)

Percentage	First Mixture ratio						MET in the mixture		
	Case 1: CR_1			Case 2: CR_2					
	Spiked Ratio CR	% RSD	%Recovery	Spiked Ratio CR	% RSD	%Recovery	Spiked Conc. IPD	% RSD	%Recovery
80%	1	0.142	100.20	1	0.234	100.13	1.5	0.154	99.80
100%	2	0.432	99.84	2	0.432	99.07	2.5	0.165	100.17
120%	3	0.345	99.87	3	0.352	99.34	3.5	0.265	99.65

PRECISION

Repeatability or intra-assay precision of the method is defined as a precision under the same operating conditions in short time. The repeatability of the developed method was assessed by using six determinations of the two active substances at 100% of the test

concentration on the same day¹⁸. Percentages of RSD and Standard deviation SD were calculated. Three determinations were made. The results of intra-assay precision shown in the Table 2 confirmed the precision of the developed method.

Table 2: Precision values of RAB and IPD

Drug	Concentration ($\mu\text{g/ml}$)	Case 1 : CR_1		Case 2 : CR_2	
		SD	RSD%	SD	RSD%
RAB	2.5	0.170	0.65	0.139	0.365
IPD	4.5	0.110	1.6	0.129	0.345

Analysis of marketed formulation (Capsules)

The amount of MET was determined by the sample absorbance measurement and the regression equation $A = 0.07 C_{RAB} + 0$. We used the second regression equation of mixture ratio ($A_1/A_2 = 0.020 (C_{RAB}/C_{IPD}) + 0.115$ or $A_2/A_1 = 0.018(C_{SPY}/C_{MET}) + 0.156$) for determination of experimental concentration

ratio of mixture and finally we deduced the concentration of RAB by the simple calculation

$$[RAB] = CR_{exp} * [IPD]$$

With:

[RAB]: Deduced concentration of RAB

[IPD]: Calculated concentration of IPD

CR_{exp} : Experimental Concentration ratio of mixture

The marketed formulation of studied drugs was evaluated by the suggested method;

satisfactory results were obtained for both drugs in a good agreement with the label claims. The recoveries % were 100.02 (RAB), 99.87 (IPD) for the first case (CR₁) and 100.12 (RAB), 99, 89% (IPD) for the second case (CR₂). The results obtained by the validated method concords with those obtained with other spectrophotometric or chromatographic and electrochemical analysis methods ^{4, 5}. The principal advantage of our method is that it is simpler and more economical than other published analytical methods for the simultaneous determination of RAB and IPD. It can be used for routine quantitative control without sample pretreatment, without too many complications in calculating the unknown concentrations in the mixture and without the use of expensive equipment and materials.

CONCLUSION

To summarize all these steps, once the equations were established, the analysis required measuring the absorbance of the mixture at 462 nm, deducing directly the concentration of RAB and measuring the absorbance of the mixture at 419 nm to calculate the absorbance ratio, and

finally determining the experimental concentration ratio to find the concentration of IPD. The developed method has been validated and has given satisfactory results. The recovery benefits showed that there was no interference of excipients in the tablets. It was successfully applied for the simultaneous determination of RAB and IPD in their binary mixture in pharmaceutical formulations. They were found to be rapid, simple, accurate and reliable method with an excellent accuracy. The suggested method, for the two cases AR₁ and AR₂, could be used as an alternative to others techniques that require expensive or complex instruments used in the routine quality control analysis of pharmaceutical preparations.

CONFLICT OF INTEREST

All authors declare any potential conflict of interest in the research.

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