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Full Paper

Spectrophotometric methods for simultaneous estimation of pantoprazole and itopride hydrochloride in capsules

Krishna R. Gupta, Rajesh B. Chawla and Sudhir G. Wadodkar*

Department of Pharmaceutical Chemistry, S. K. B. College of Pharmacy, New Kamptee 441002, Nagpur (MS) India

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ABSTRACT: *Three simple, accurate and economical methods for simultaneous estimation of pantoprazole and itopride hydrochloride in two component solid dosage forms have been developed. The proposed methods employ the application of simultaneous equation method (Method A), absorbance ratio method (Method B) and multicomponent mode of analysis method (Method C). All these methods utilize distilled water as a solvent. In distilled water pantoprazole shows maximum absorbance at a wavelength of 289.0 nm while itopride hydrochloride shows maximum absorbance at a wavelength of 258.0 nm also the drugs show an isoabsorptive point at a wavelength of 270.0 nm. For multicomponent method, sampling wavelengths 289.0 nm, 270.0 nm and 239.5 nm were selected. All these methods showed linearity in the range from 4-20 µg/mL and 15-75 µg/mL for pantoprazole and itopride hydrochloride respectively. The results of analysis have been validated statistically and by recovery studies.*

Keywords*: pantoprazole; itopride hydrochloride; distilled water; simultaneous equation; absorbance ratio; multi-component mode of analysis*

Introduction

Pantoprazole (PAN) is chemically 5-(Difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole. It is a substituted benzimidazole proton pump inhibitors and used as anti ulcerative. It is official in The Merck Index [1] and Martindale, The Extra Pharmacopoeia [2]. PAN is very soluble in water and methanol. Itopride Hydrochloride (ITH) chemically is, N-{p-[2-(Dimethylamino) ethoxy]benzyl} veratramide hydrochloride. It is a dopamine antagonist and used as prokinetic and

* Corresponding author. E-mail: krishnargupta@rediffmail.com

antiemetic. It is not official in any of the Pharmacopoeias and is listed in Martindale, The Extra Pharmacopoeia [3]. ITH is very soluble in water and freely soluble in alcohol. An extensive literature survey revealed HPLC [4] and UV spectrophotometric [5] methods for the analysis of PAN alone, whereas ITH has been determined by UV spectrophotometric [6], RP-HPLC [7] and HPTLC [8] alone in formulation. So far no spectrophotometric method has been reported for the simultaneous estimation of both these component in a combined dosage form. Spectrophotometric methods are reported for estimation of these drugs alone and would require tedious procedure for analysis if the reported methods are applied for their estimation in combined pharmaceutical formulation, difference spectroscopic method of analysis [5] of PAN in two different media (acidic and basic). The same cannot be applied to ITH. If present in same formulation the methodology has to be modified, or will create interference in the analysis. Similarly involves the formation of a colored chloroform extractable complex of drug (ITH) with bromocresol green in acidic media [6]. However the same complex formation may not be possible with PAN under same specified conditions. Hence both the above methods cannot be applied for the simultaneous estimation of the drugs in combined dose formulations.

Therefore, it was thought to develop a precise, accurate and simple method for estimation of these drugs in combined dosage formulation that would not require any prior separation of the drugs from each other and can be analyzed simultaneously.

Material and Methods

A Shimadzu UV-1700 UV/Vis spectrophotometer with 1 cm matched quartz cell was used for all absorbance measurements. Double distilled water was used as a solvent. PAN and ITH were purchased from Zim Labs. Ltd., Nagpur and Relief Labs., Nagpur.

Preparation of standard stock solution

PAN standard stock solution.

An accurately weighed quantity of pantoprazole sodium sesquihydrate (~25 mg PAN) was transferred in 50.0 mL volumetric flask, dissolved in sufficient quantity of distilled water and volume was made up to the mark with distilled water (concentration: 500 µg/mL).

ITH standard stock solution

An accurately weighed quantity of ITH (~25 mg) was transferred in 50.0 mL volumetric flask, dissolved in sufficient quantity of distilled water and volume was made up to the mark with distilled water (concentration: 500 µg/mL).

Selection of wavelength

These stock solutions were appropriately diluted with distilled water to obtain the

concentration of 10 µg/mL and were scanned in the UV range (200-400 nm) in 1 cm cell against solvent blank. The overlain spectra of both drugs are depicted below in Figure 1.

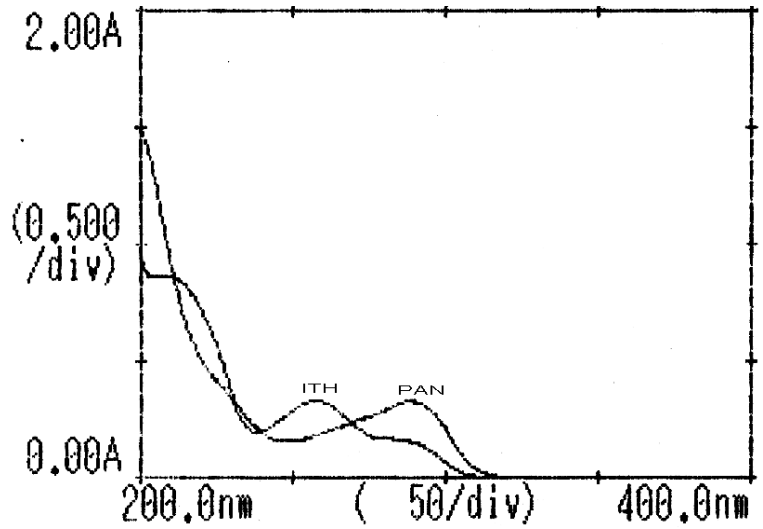

Figure 1. Overlain spectra of pantoprazole (PAN) and itopride hydrochloride (ITH).

PAN shows a well-defined λ_{max} at 289.0 nm and ITH shows a well-defined λ_{max} at 258.0 nm, these two wavelengths were selected for development of simultaneous equation method. Also overlain spectra, shows an isoabsorptive wavelength where both the drugs shows same absorbance at 270.0 nm and 239.5 nm. The wavelengths 289.0 nm and 270.0 nm were considered for estimation of drugs by absorbance ratio method.

The stock solutions of PAN and ITH were further diluted with distilled water in the series from 4-20 µg/mL for PAN and 15-75 µg/mL for ITH respectively. From the overlain spectra depicted in Figure 1, sampling wavelengths 289.0 nm, 270.0 nm, 239.5 nm were selected such that both the drugs have sufficient absorbance. The concentrations of individual drugs were fed to the multicomponent mode of the instrument; all five mixed standards of drug solutions were scanned over a range of 230 nm to 300 nm against solvent blank. The spectrums so recorded of five different mixed standards are depicted below in Figure 2.

Calibration curves were plotted as concentration *versus* absorbance. The optical characteristics such as absorption maxima, Beer's law limit, $A^{1\%}_{1cm,}$ correlation coefficient and limit of detection of both methods were recorded in Table 1.

Application of the proposed methods for determination of PAN and ITH in Capsules:

An accurately weighed quantity of capsule content equivalent to 20.0 mg of PAN (~ 75.0 mg of ITH) was transferred to 100.0 mL volumetric flask, dissolved by shaking for 30 min. with sufficient quantity of distilled water and volume was made up to mark

with distilled water. The contents was filtered through Whatmann filter paper (no.41) and a 5.0 mL portion of the filtrate was further diluted to 100.0 mL with distilled water to get final concentration of about (10.0 µg/mL PAN, 37.5 µg/mL ITH, on label claim basis). The absorbance of the resulting solution was measured at 289.0, 270.0 and 258.0 nm in 1.0 cm cell using solvent blank. The contents of PAN and ITH were calculated by substituting values in the formulae given below for method A, B and C.

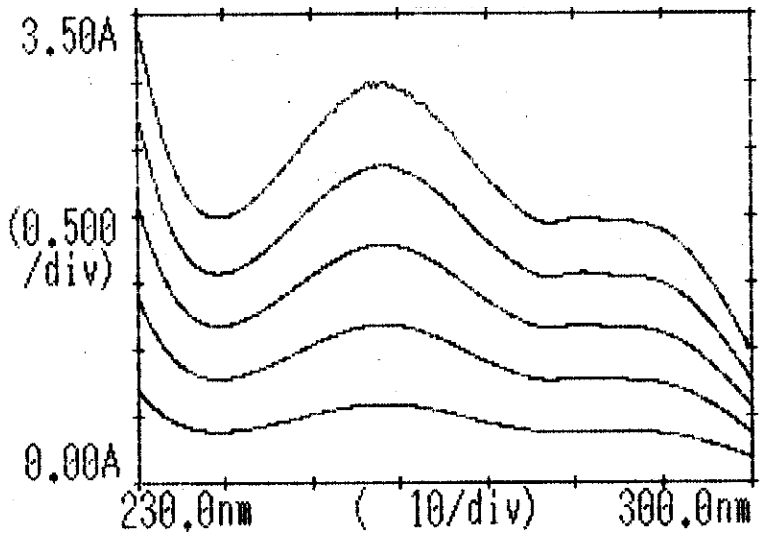

Figure 2. Overlain spectra of five mixed standards.

Table 1. Optical characteristics.

Parameters	*PAN*	*ITH*
λ_{max} and Isobestic	289.0 nm and 270.0 nm	258.0 nm and 270.0 nm
Beer's law limit	4-20 µg/ml	15-75 µg/ml
$A^{1\%}_{1cm}$	379.56 and 278.0	335.90 and 231.0
Correlation coefficient	0.9993 and 0.9997	0.9991 and 0.9997
Limit of detection	67 ng /mL	250 ng /mL

Simultaneous equation method (Method A) [9]

For estimation of PAN For estimation of ITH

$$c_y = \frac{A_1 ax_2 - A_2 ax_1}{ax_2 ay_1 - ax_1 ay_2} \quad c_x = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 - ax_1 ay_2}$$

Where: A_1 and A_2 are absorbance of diluted mixture at 289.0 nm and 258.0 nm respectively; Cx and Cy are the concentrations of PAN and ITH respectively (g/100 mL); ax_1, ax_2 are absorptivities of PAN and ay_1, ay_2 for ITH at 289.0 nm and 258.0 nm

respectively.

Absorbance ratio method (Method B) [9]

Amount of each drug in sample was determined using following formulas:

For estimation of PAN

$$Cx = \frac{Qm - Qy}{Qx - Qy} \times \frac{A}{ax}$$

For estimation of ITH

$$Cy = \frac{Qm - Qx}{Qy - Qx} \times \frac{A}{ay}$$

Where: x= PAN and y= ITH

Cx = Concentration of PAN in g/100 mL; Cy = Concentration of ITH in g/100 mL

Qm = Ratio of absorbance of laboratory mixture at 289.0 nm and 270.0 nm; Qx = Ratio of absorptivity of PAN at 289.0 nm and 270.0 nm; Qy = Ratio of absorptivity of ITH at 289.0 nm and 270.0 nm; ax = Absorptivity of PAN at 289.0 nm; ay = Absorptivity of ITH at 270.0 nm; A = Absorbance of mixture at isoabsorptive point.

From the amount of each drug estimated by above methods, percent estimation for each drug was calculated.

Multi-component mode method (Method C) [9]

Sample solution prepared for above two methods were scanned over the range of 230 nm to 300 nm in multi-component mode against solvent blank and concentration of each drug component was obtained by the analysis of spectral data of sample solution with reference to that of five-mixed standard in terms of concentration in µg/mL

Amt of Drug (mg) = Concentration of drug (mg) X Dilution factor

Results of estimation in marketed formulation are shown in Table 2.

Table 2. Summary of result of estimation in marketed formulation

Sample		***% Label claim estimated****					
		Simultaneous Equation Method		**Absorbance Ratio Method**		**Multicomponent Mode**	
Itop 1	**Mean ±SD CV**	**PAN**	**ITH**	**PAN**	**ITH**	**PAN**	**ITH**
		100.05 0.56 0.56	100.68 1.02 1.02	99.40 0.10 0.10	100.96 0.65 0.65	100.75 0.26 0.26	100.94 0.33 0.33
Itop 2	**Mean** **± SD** **CV** **SE**	99.77 0.61 0.62 0.27	100.12 0.90 0.89 0.40	99.49 0.36 0.36 0.16	100.31 0.89 0.89 0.40	100.62 0.30 0.30 0.13	100.63 0.53 0.53 0.23

*Mean of five observations ± Standard Deviation. Itop 1 and 2: are Marketed formulations used in analysis.

The recovery studies were performed by standard addition method. To a known amount of previously analyzed capsule powder, pure drugs were added at four different levels and the samples were analyzed by proposed methods. The results of recovery studies are recorded in Table 3.

Table 3. Summary of result of estimation in recovery study.

Sample		***% Recovery****					
Itop 1	**Mean ± SD CV**	**Simultaneous Equation Method**		**Absorbance Ratio Method**		**Multicomponent Mode**	
		PAN	**ITH**	**PAN**	**ITH**	**PAN**	**ITH**
		99.29	99.70	99.90	99.73	100.51	99.17
		0.11	0.70	1.20	1.35	0.93	0.67
		0.11	0.70	1.20	1.35	0.92	0.67

* Mean of four observations, Itop 1: is Marketed formulation used in analysis.

Validation of proposed methods [10]

Validation of proposed method was carried out as per ICH guidelines.

Accuracy

Accuracy of the proposed methods was ascertained on the basis of recovery study performed by standard addition method and results are recorded in Table 3 indicate the accuracy of the proposed methods.

Precision

The precision of the method was ascertained by replicate analysis and SD values were found to be within limits. Results are shown in Table 2.

Intermediate Precision

Inter-day precision

It was done by analyzing the solutions by same analyst on alternate days till 7th day. The % RSD (CV) is shown in Table 4.

Intra-day precision

It was done by analyzing the solutions by same analyst within a day. The % RSD (CV) is shown in Table 4.

Specificity studies

An accurately weighed quantities of capsule powder equivalent to 20 mg were transferred to series of 100 mL volumetric flask and kept under following stress conditions viz. 0.5 N NaOH, 0.5 N HCl reflux for 3 h; 6% H_2O_2 24 h at 50 ^{0}C; heat (60 ^{0}C), humidity (75%) and UV exposure for 24 h; direct sun light (photochemical exposure)

for 6 h. After the specified time period volumes were made up to mark with distilled water, filtered and procedure described under marketed formulation was followed. Results of specificity studies are recorded in Table 5.

Table 4. Summary of result of Precision (Intermediate) and Ruggedness.

Time	***% Label Claim***					
	Simultaneous Equation Method		**Absorbance Ratio Method**		**Multicomponent Mode**	
	PAN	**ITH**	**PAN**	**ITH**	**PAN**	**ITH**
0 Hrs	99.25	100.83	99.51	100.65	100.92	100.36
3 Hrs	99.25	100.97	99.70	100.70	101.10	100.82
6 Hrs	99.40	100.72	99.20	100.89	100.54	100.30
Mean	**99.30**	**100.84**	**99.47**	**100.74**	**100.85**	**100.49**
Day	**% Label Claim**					
	Simultaneous Equation Method		**Absorbance Ratio Method**		**Multicomponent Mode**	
	PAN	**ITH**	**PAN**	**ITH**	**PAN**	**ITH**
Day 1	99.25	100.83	99.51	100.65	100.92	100.36
Day 4	93.32	99.97	93.12	100.14	93.96	95.93
Day 7	88.00	99.99	88.25	99.81	90.29	92.51
Mean	**93.52**	**100.26**	**93.62**	**100.20**	**95.05**	**96.27**
Analyst	**% Label Claim**					
	Simultaneous Equation Method		**Absorbance Ratio Method**		**Multicomponent Mode**	
	PAN	**ITH**	**PAN**	**ITH**	**PAN**	**ITH**
Analyst 1	100.22	100.22	99.62	100.02	100.82	100.69
Analyst 2	99.18	99.14	98.79	99.49	99.68	99.66
Analyst 3	100.58	100.24	99.29	99.90	100.27	100.18
Mean	**99.99**	**99.86**	**99.23**	**99.80**	**100.25**	**100.18**

Table 5. Summary of results of Specificity study.

Sample (Treated)	***%Label claim***					
	Simultaneous Equation Method		**Absorbance Ratio Method**		**Multicomponent Mode**	
	PAN	**ITH**	**PAN**	**ITH**	**PAN**	**ITH**
0.5 N NaOH reflux for 3 h	76.18	103.29	75.89	102.69	71.53	99.22
0.5 N HCl reflux for 3 h	115.22	99.37	117.69	99.18	114.52	102.18
6% H_2O_2 for 24 h at 50 ^{0}C	51.49	93.53	50.13	92.89	45.89	76.54
60 °C for 24 h	79.35	99.23	79.13	99.06	82.19	96.79
Humidity (75% RH)	96.78	97.12	96.44	97.03	96.89	97.43
UV-exposure (254.0 nm)	95.64	99.13	95.43	99.47	95.13	100.04
Photochemical	86.78	100.86	86.92	100.09	81.57	95.84

Linearity and Range

An accurately weighed quantity of capsule powder equivalent to 80-120% of label claim was transferred and procedure as described under marketed formulation was followed, graphs were plotted as percentage label claim vs. absorbance for Method A and

B and percentage label claim vs. amount estimated for Method C.

Ruggedness

It was done by analyzing the samples solutions by three different analysts. The % RSD (CV) by proposed methods is shown in Table 4.

Results and Discussion

The well-defined λ_{max} for PAN is at 289.0 nm while ITH shows at 258.0 nm. The drugs show the isobestic point at 270.0 nm, as shown in Figure 1. The absorptivity values of PAN and ITH were found to be 379.56 and 156.57 at 289.0 nm; 212.99 and 335.90 at 258.0 nm; 278.0 and 231.0 at 270.0 nm, respectively. All the proposed methods were found to be rapid, economical and precise for the determination of PAN and ITH in its combined dosage form. The percent label claim was found to be from 99.40-100.75 for PAN and 100.68-100.75 in formulation (Itop 1) while 99.77-100.62 for PAN and 100.12-100.63 for ITH in formulation (Itop 2) using the three proposed methods. The percent recovery was found to be nearly 100%. Specificity study shows degradation of drug under different stressed conditions. Interference studies revealed that the common excipients and other additives usually present in the dosage form did not interfere in all the proposed methods. The values of standard deviation were satisfactory and percentage recovery was close to 100%, indicating the reproducibility and accuracy of the methods. Linearity study showed that all the proposed methods were found to be linear. The Coefficient of correlation at 289.0, 270.0 and 258.0 were found to be 0.9993, 0.9997 and 0.9991, respectively for Method A and B and 0.9996 and 0.9998 for PAN and ITH in Method C.

Our proposed methods do not involve complex formation whose stability is dependent on pH or interference from other drugs/excipients as compared with reported methods for analysis of the either drugs in single formulation. Also no reported methods give little idea about the stability of the drugs under different stress conditions which we have tried to explain to some extent.

Conclusion

All proposed methods can be easily applied to analysis of the said drugs in combined formulation containing PAN and ITH in its dosage forms as they produce comparable results and can be used for precise and accurate analysis of PAN and ITH in its capsule dosage form.

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