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ORIGINAL ARTICLE

Stability indicating high performance thin-layer chromatographic method for simultaneous estimation of pantoprazole sodium and itopride hydrochloride in combined dosage form

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Stability indicating;
Forced degradation

Abstract A specific, precise and stability indicating high-performance thin-layer chromatographic method for simultaneous estimation of pantoprazole sodium and itopride hydrochloride in pharmaceutical formulations was developed and validated. The method employed TLC aluminium plates precoated with silica gel $60F_{254}$ as the stationary phase. The solvent system consisted of methanol:water:ammonium acetate; 4.0:1.0:0.5 (v/v/v). This system was found to give compact and dense spots for both itopride hydrochloride (R_f value of 0.55 ± 0.02) and pantoprazole sodium (R_f value of 0.85 ± 0.04). Densitometric analysis of both drugs was carried out in the reflectance–absorbance mode at 289 nm. The linear regression analysis data for the calibration plots showed a good linear relationship with $R^2 = 0.9988 \pm 0.0012$ in the concentration range of 100–400 ng for pantoprazole sodium. Also, the linear regression analysis data for the calibration plots showed a good linear relationship with $R^2 = 0.9990 \pm 0.0008$ in the concentration range of 200–1200 ng for itopride hydrochloride. The method was validated for specificity, precision, robustness and recovery. Statistical analysis proves that the method is repeatable and selective for the estimation of both the said drugs. As the method could effectively separate the drug from its degradation products, it can be employed as a stability indicating method.

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1. Introduction

Reflux of gastric contents into the oesophagus is a normal phenomenon in most individuals, but it becomes pathological when it causes troublesome symptoms or complications. Since the introduction of proton pump inhibitors (PPIs), the treatment of patients with gastroesophageal reflux disease (GERD) has dramatically improved [1]. GERD is very common and advances in drug development over recent years have markedly improved

GERD management. A wide range of medications are currently used in GERD treatment, including antacids, Gaviscon, sucralfate, histamine-2 receptor antagonists and prokinetics. However, proton pump inhibitors (PPIs) remain the mainstay of treatment for GERD owing to their profound and consistent inhibitory effect on acid secretion. Despite the presence of a wide armamentarium of therapeutic modalities for GERD, many areas of unmet needs remain. Drug development has focused primarily on improving PPI efficacy, reducing the transient lower oesophageal sphincter relaxation rate, attenuating oesophageal sensitivity and developing oesophageal mucosal protectants [2].

Thus, many formulations are available for treatment of GERD. Combination of pantoprazole sodium and itopride hydrochloride is also available for treatment of GERD [2,3]. So, it is necessary to have an analytical method so as to estimate both drugs simultaneously from its combined dosage form.

Literature survey reveals that spectrophotometric [4,5], HPLC [6–9] and high performance thin layer chromatography (HPTLC) [10,11] methods for the estimation of itopride hydrochloride from bulk drugs and pharmaceutical formulation have been developed whereas spectrophotometric [12], HPLC [13,14], RP-HPLC [15], HPTLC [16] methods for the estimation of pantoprazole alone or in combination with other drugs from pharmaceutical formulation have been developed. However, no stability indicating method has been reported so far for simultaneous estimation of both drugs in combined pharmaceutical dosage form by HPTLC. This work presents a stability indicating HPTLC method for the simultaneous estimation of both drugs in their combined pharmaceutical dosage form, which can be used for its routine analysis in laboratory.

The advantage of HPTLC is that large number of samples can be simultaneously analysed in a shorter time period. Unlike HPLC, this method utilises less quantities of solvents, thus lowering the cost of analysis [17].

An ideal stability indicating chromatographic method should estimate the drug and also be able to resolve the drug from its degradation products. Hence an attempt has been made to develop an accurate, rapid and reproducible method for the determination of itopride hydrochloride and pantoprazole sodium in presence of their degradation products for their content analysis in pharmaceutical dosage forms containing this combination as per ICH [18] guidelines.

2. Materials and methods

2.1. Chemicals and reagents

Itopride hydrochloride and pantoprazole sodium were procured as a gift sample. All other solvents and reagents were purchased from S.D. Fine chemicals, Mumbai, India and were of analytical grade.

2.2. Instrumentation

Spotting was done using Camag Linomat 5 sample applicator (CAMAG, Switzerland) and Camag Hamilton Bonaduz microlitre syringe (100 µl) on HPTLC aluminium plates precoated with silica gel $60F_{254}$ (20 cm × 10 cm with 250 µm thickness; Merck, Germany). The plates were prewashed with methanol for 30 min in a Camag twin trough glass chamber closed with lid. The plates were activated at 110 °C for 30 min. The samples were spotted in the form of narrow bands having length of 6 mm. The application position *X* and *Y* were kept at 10 mm and 12 mm, respectively, to avoid edge effect. The distance between the two bands was 10 mm. Spots were applied at a constant rate of 15 nL/s using a nitrogen aspirator. Linear ascending development of chromatogram was carried out in a Camag twin trough glass chamber saturated with the mobile phase for 15 min and chromatogram run was kept up to 90 mm. Spectrodensitometric analysis of the separated components was carried out using Camag TLC Scanner 3 in the reflectance–absorbance mode at 289 nm using a D_2 lamp. The slit dimension used was 6.0 mm × 0.3 mm and sensitivity was kept at auto mode. Scanning speed was 100 nm/s. Integration of the chromatogram was carried out using Planar chromatography manager-winCATS (CAMAG).

2.3. Calibration plots

2.3.1. Calibration plot of pantoprazole sodium in methanol

A total of 10 mg of pantoprazole sodium was dissolved in 100 mL of methanol to obtain stock solution of 100 µg/mL. Appropriate quantities of this stock solution were spotted to obtain the concentration in the range of 100–400 ng.

2.3.2. Calibration plot of itopride hydrochloride in methanol

A total of 10 mg of itopride hydrochloride was dissolved in 100 mL of methanol to obtain stock solution of 100 µg/mL. Appropriate quantities of this stock solution were spotted to obtain the concentration in the range of 200–1200 ng.

2.4. Analysis of marketed formulation

To determine the content of itopride hydrochloride and pantoprazole sodium in marketed capsules (label claim: pantoprazole sodium 40 mg/capsule and itopride hydrochloride 150 mg/capsule), the contents of 20 capsules were weighed and their average weight was determined. The content of capsules containing sustained released pellets was finely powdered.

Solution A: an amount equivalent to average weight of capsule contents was transferred into a 100 mL volumetric flask containing 50 mL methanol. It was sonicated for 10 min and contents were diluted to 100 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and supernatant was collected.

Solution B: 1 mL of solution A was diluted to 10 mL of methanol in a 10 mL volumetric flask. 5 µL of solution B (200 ng of pantoprazole sodium and 750 ng of itopride hydrochloride) was applied on the TLC plate followed by development and scanning. The analysis was repeated for six times.

3. Method validation

3.1. Specificity

The specificity of the HPTLC method was ascertained by analysing standard drug and sample solutions (marketed formulation). The retention factor of pantoprazole sodium

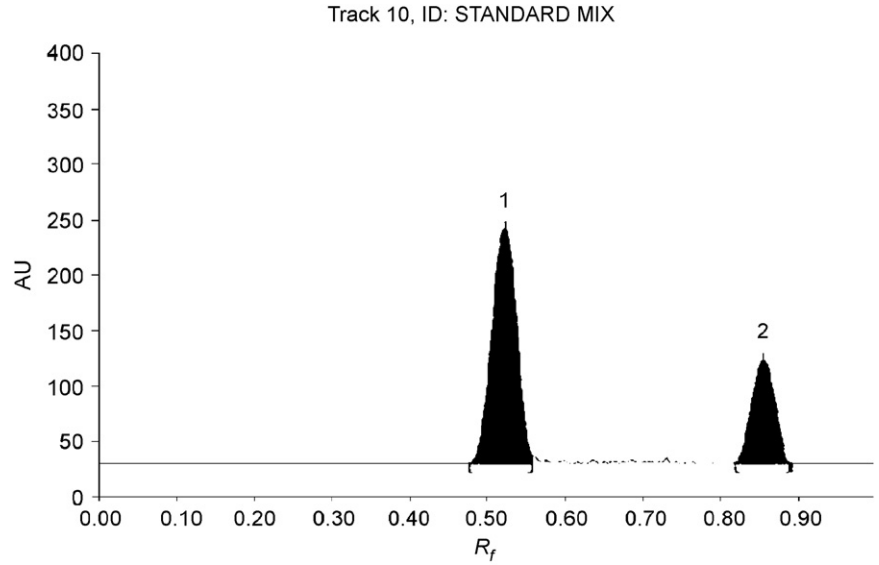

Figure 1 Chromatogram of standard itopride hydrochloride (peak 1, R_f 0.55±0.02) and pantoprazole sodium (peak 2, R_f 0.85±0.03).

Table 1 Linear regression data for calibration plot of pantoprazole sodium and itopride hydrochloride ($n=3$).

Parameters	Pantoprazole sodium	Itopride hydrochloride
Linearity range (ng)	100–400	200–1200
Correlation coefficient (R^2)±S.D.	0.9988±0.0012	0.9990±0.0008
Slope (mean±S.D)	15.2795±0.1542	5.5413±0.0513
Intercept (mean±S.D)	890.0311±21.5457	589.7133±30.2451

Table 2 Intra-day and inter-day precision results for pantoprazole sodium and itopride hydrochloride.

Drug	Pantoprazole sodium			Itopride hydrochloride		
Concentration (ng)	**100**	**200**	**300**	**375**	**750**	**1125**
Intra-day precision						
Mean drug content (%)	99.98	99.42	99.14	99.29	99.89	100.57
% R.S.D.	0.41	0.26	0.38	0.59	0.33	0.44
Inter-day precision						
Mean drug content (%)	99.18	99.13	99.17	99.50	100.05	100.54
% R.S.D.	0.77	0.79	0.34	0.96	0.41	0.31

and itopride hydrochloride in the sample solution was confirmed by comparing with that of the respective standards.

3.2. *Precision*

3.2.1. *Repeatability*

The system repeatability was determined by six replicates of the prepared sample solutions. The repeatability of sample application and measurement of peak area for the drugs were calculated by repeating the assay six times at three different concentration levels of 100, 200 and 300 ng for pantoprazole sodium and 375, 750 and 1125 ng for itopride hydrochloride in the same day for intra-day precision.

Table 3 Recovery data for pantoprazole sodium and itopride hydrochloride.

Drug	Level of % recovery	Mean recovery	R.S.D. (%)
Pantoprazole sodium	80	99.20	0.23
	100	99.32	0.22
	120	99.11	0.17
Itopride hydrochloride	80	99.58	0.25
	100	99.29	0.18
	120	99.29	0.13

3.2.2. Intermediate precision

The intermediate precision was determined by six replicates of the prepared sample solutions. The intermediate precision of sample application and measurement of peak area was obtained by the assay of six sample sets on different days at three different concentration levels of 100, 200 and 300 ng for pantoprazole sodium and 375, 750 and 1125 ng for itopride hydrochloride for inter-day precision.

3.3. Recovery studies

Recovery determination for pantoprazole sodium and itopride hydrochloride was carried out at levels of 80%, 100% and 120%. The analysed samples were spiked with extra 80%, 100% and 120% of the standard drug and the mixture was re-analysed by the proposed method. At each level of the amount, three determinations were performed. This was done

Table 4 Robustness results for pantoprazole sodium and itopride hydrochloride.

Parameters	Pantoprazole sodium		Itopride hydrochloride	
	Mean R_f	% R.S.D. of drug content	Mean R_f	% R.S.D. of drug content
Mobile phase composition				
3.8:0.8:0.5 (v/v/v)	0.83	0.28	0.54	0.87
3.9:0.9:0.5 (v/v/v)	0.83	0.49	0.56	0.19
4.1:1.1:0.5 (v/v/v)	0.86	0.53	0.56	0.10
4.2:1.2:0.5 (v/v/v)	0.86	0.88	0.57	0.17
Volume of mobile phase				
25 mL	0.85	0.41	0.56	0.18
30 mL	0.87	0.31	0.57	0.10

Table 5 Results of analysis of marketed formulation.

Drug	Label claim (mg/capsule)	Amount found (mg)	Label claim estimated[a] (%)	R.S.D. (%)
Pantoprazole sodium	40	39.75	99.37	0.33
Itopride hydrochloride	150	150.14	100.09	0.32

[a]Average of six determinations.

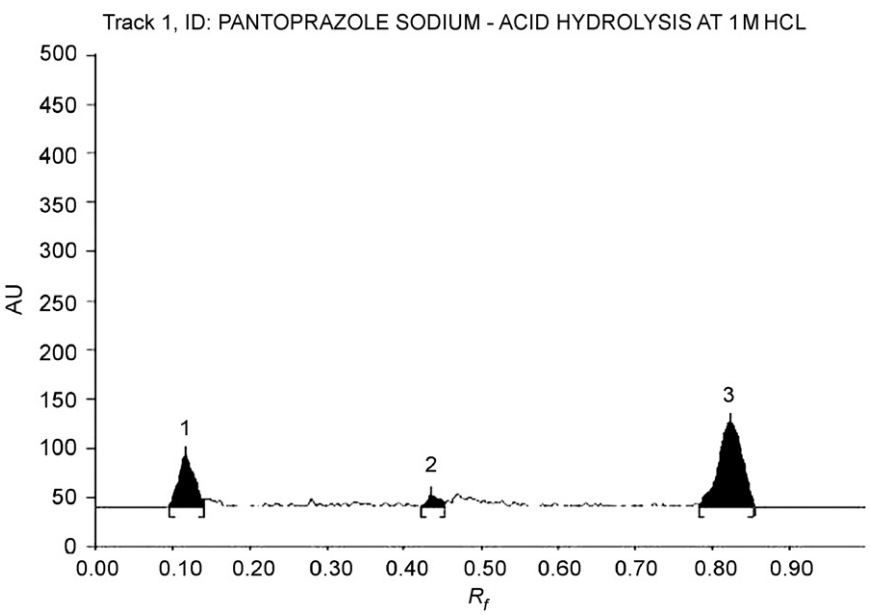

Figure 2 Chromatogram of acid treated pantoprazole sodium. Peak 1: degradant, R_f 0.12, Peak 2: degradant, R_f 0.44 and Peak 3: Pantoprazole sodium, R_f 0.82.

to check the recovery of the drug at different levels in the formulations.

3.4. Robustness

To evaluate the robustness of the developed method, deliberate variations were made in the method parameters such as changing the composition of the mobile phase and volume of mobile phase. 200 ng of pantoprazole sodium and 750 ng of itopride hydrochloride were applied on plates three times under different conditions. The effects of the modified parameters on retention factor and % drug content were calculated.

The following mobile phase compositions were tried (keeping volume of ammonium acetate constant):

- Methanol:water:ammonium acetate; 3.8:0.8:0.5, v/v/v
- Methanol:water:ammonium acetate; 3.9:0.9:0.5, v/v/v
- Methanol:water:ammonium acetate; 4.1:1.1:0.5, v/v/v
- Methanol:water:ammonium acetate; 4.2:1.2:0.5, v/v/v

The following changes were made in the volume of mobile phase used for development of plates in twin trough chamber:

- 25 mL of mobile phase
- 30 mL of mobile phase

4. Forced degradation studies

Pantoprazole sodium and itopride hydrochloride was subjected to various stress conditions to affect their degradation. Thus the acid induced, alkali induced, oxidative and dry heat degradation was attempted. The degradation samples were

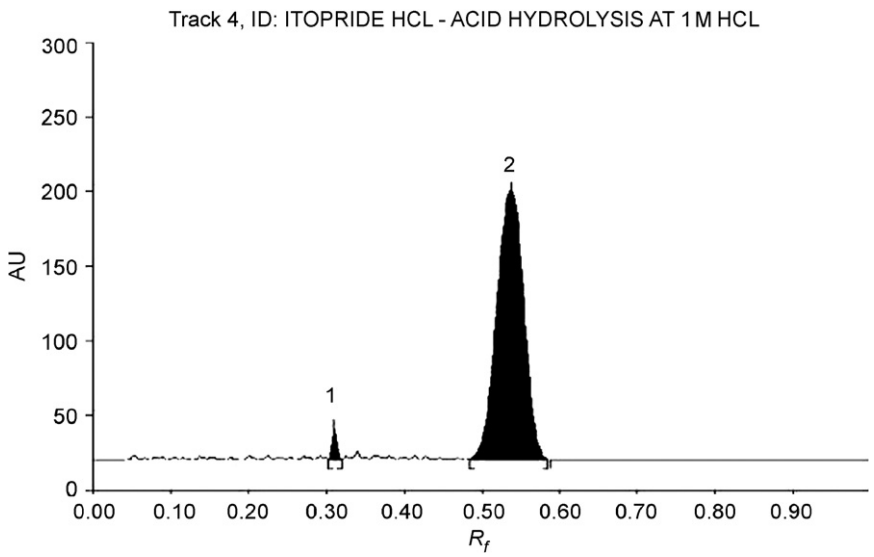

Figure 3 Chromatogram of acid treated itopride hydrochloride. Peak 1: degradant, R_f 0.32, Peak 2: itopride hydrochloride, R_f 0.54.

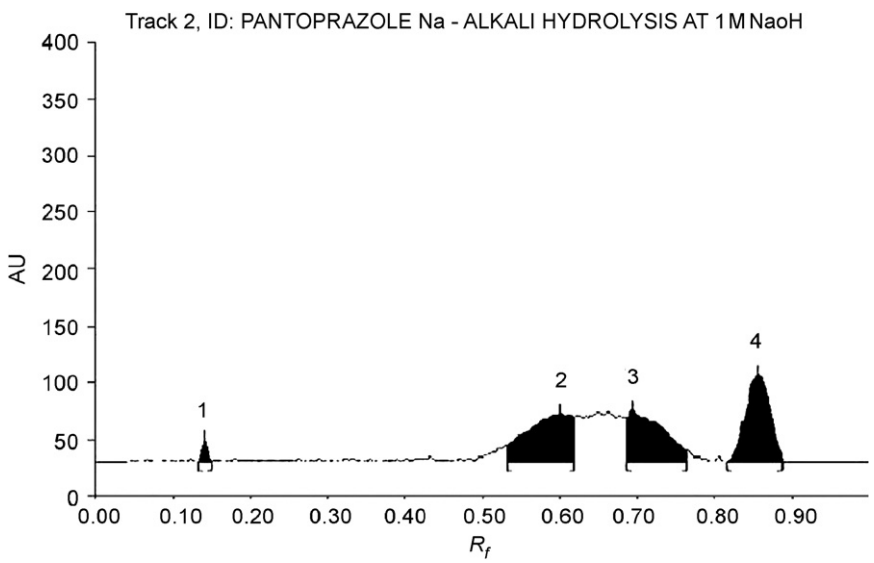

Figure 4 Chromatogram of alkali treated pantoprazole sodium. Peak 1: degradant, R_f 0.14, Peak 2: degradant, R_f 0.60, Peak 3: degradant, R_f 0.69 and Peak 4: pantoprazole sodium, R_f 0.85.

subjected to chromatographic separation to resolve the drug from its degradation products.

4.1. Preparation of acid and alkali induced degradation product

Both the drugs were subjected to forced degradation separately under acidic and alkaline conditions by refluxing with 1 M HCl and 1 M NaOH, respectively, at 80 °C for a period of 6 h. The forced degradation in acidic and alkaline media was performed in the dark in order to exclude the possible degradative effect of light on the drug. A zero time sample and stressed blank sample were also prepared.

4.2. Preparation of hydrogen peroxide induced degradation product

Forced degradation by hydrogen peroxide was performed by refluxing both drugs separately with hydrogen peroxide at 80 °C for a period of 4 h. A zero time sample and stressed blank sample were also prepared.

4.3. Preparation of dry heat degradation product

The standard drug of pantoprazole sodium was placed in a hot air oven at 110 °C for 2 h and itopride hydrochloride was placed for 8 h at 110 °C to study dry heat degradation.

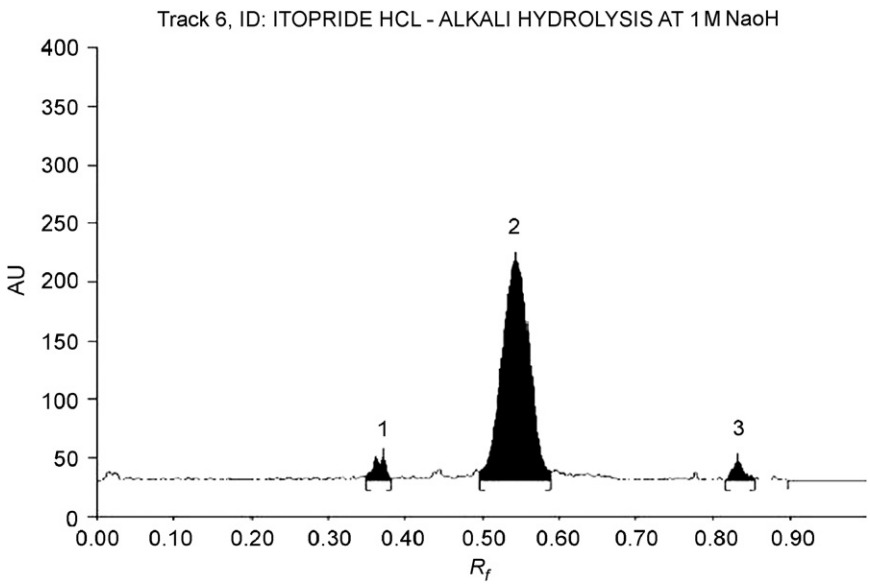

Figure 5 Chromatogram of alkali treated itopride hydrochloride. Peak 1: degradant, R_f 0.37, Peak 2: itopride hydrochloride, R_f 0.54 and Peak 3: degradant, R_f 0.83.

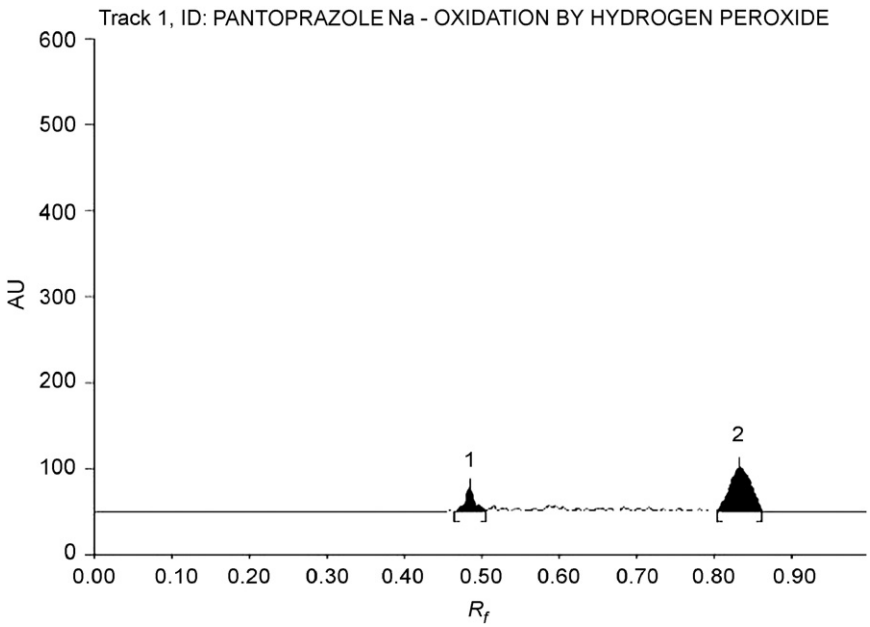

Figure 6 Chromatogram of hydrogen peroxide treated pantoprazole sodium. Peak 1: degradant, R_f 0.48 and Peak 2: pantoprazole sodium, R_f 0.83.

5. Results and discussion

5.1. Optimisation of chromatographic condition

The TLC procedure was optimised with a view to develop a stability indicating assay method. Both the pure drug and the degraded products were spotted on the TLC plates and run in different solvent systems. But mobile phase consisting of methanol:water:ammonium acetate (4.0:1.0:0.5, v/v/v) was able to give characteristic spots for both drugs. By optimizing chromatographic conditions such as having chamber saturation for 15 min, using 20 mL of mobile phase and activating plates before spotting, we obtained a good resolution as well as a sharp and symmetrical peak with R_f value of 0.55 ± 0.02 for itopride hydrochloride and 0.85 ± 0.04 for pantoprazole sodium (Fig. 1).

5.2. Validation of the developed method

5.2.1. Linearity

The calibration plot was found to be linear in the concentration range of 100–400 ng and 200–1200 ng for pantoprazole sodium and itopride hydrochloride, respectively. The linearity was validated by the high values of the correlation coefficient. The results are tabulated in Table 1.

5.2.2. Precision studies

The repeatability (intra-day precision) and intermediate precision (inter-day precision) of sample application and measurement of peak area were expressed in terms of % R.S.D and was found to be less than 2% as depicted in Table 2.

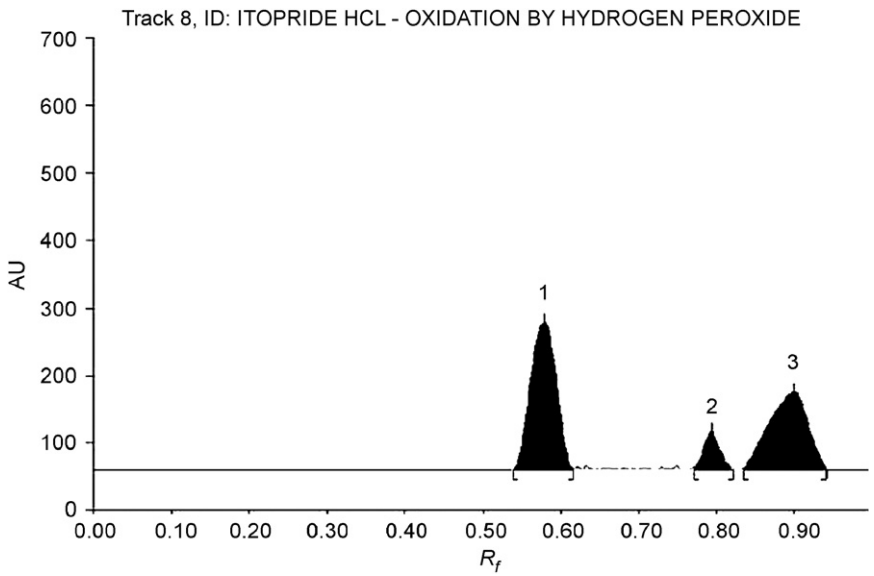

Figure 7 Chromatogram of hydrogen peroxide treated itopride hydrochloride. Peak 1: itopride hydrochloride, R_f 0.57, Peak 2: degradant, R_f 0.79 and Peak 3: degradant, R_f 0.90.

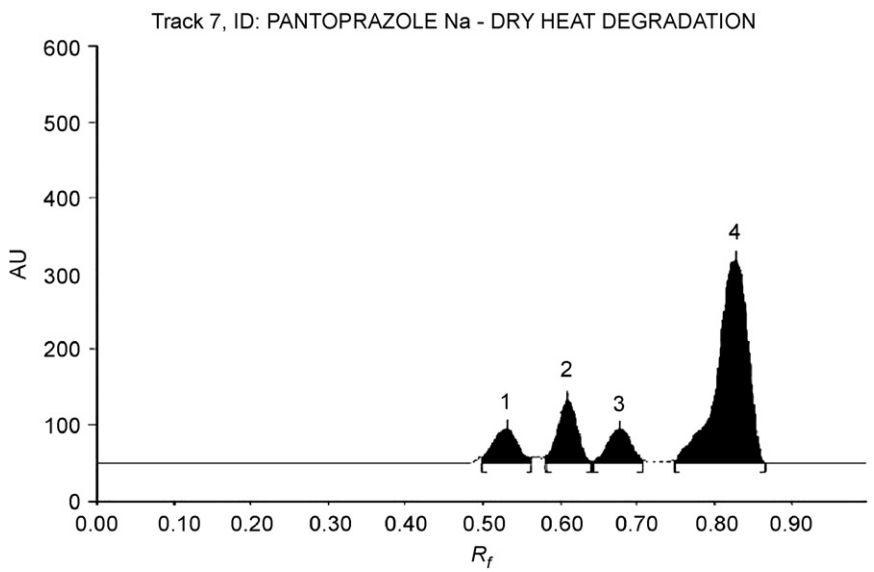

Figure 8 Chromatogram of dry heat treated pantoprazole sodium. Peak 1: degradant, R_f 0.53, Peak 2: degradant, R_f 0.61, Peak 3: degradant, R_f 0.68 and Peak 4: pantoprazole sodium, R_f 0.83.

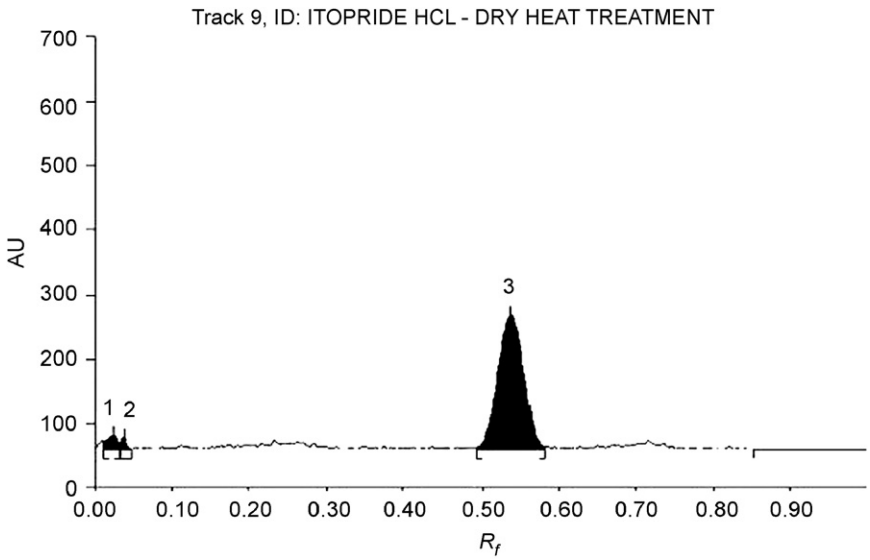

Figure 9 Chromatogram of dry heat treated itopride hydrochloride. Peak 1: degradant, R_f 0.03, Peak 2: degradant, R_f 0.04 and Peak 3: itopride hydrochloride, R_f 0.55.

5.2.3. Recovery studies

The recovery studies were carried out at 80%, 100% and 120% of the test concentration as per ICH guidelines. The results of the recovery studies and its statistical validation are given in Table 3.

5.2.4. Robustness

To evaluate the robustness of the method, selected parameter was varied at different levels. The results presented in Table 4 indicate that R_f and % drug content were unaffected by small variations in the selected method parameters.

Also the low values of % R.S.D. (<2) of % drug content obtained after introducing small changes in mobile phase composition and volume of mobile phase were indicative of the robustness of the method as presented in Table 4.

5.2.5. Specificity

The chromatogram of commercial formulation showed only two peaks at R_f values of 0.85 and 0.55 for pantoprazole sodium and itopride hydrochloride, respectively, indicating that there is no interference of the excipients in the capsule formulations.

5.2.6. Analysis of marketed formulation

The chromatograms of the drug extracted from commercial formulation, exhibited two peaks at R_f value of 0.85 and 0.55 for pantoprazole sodium and itopride hydrochloride, respectively. The mean drug content was found to be 39.75 mg and 150.14 mg for pantoprazole sodium and itopride hydrochloride with a % R.S.D of 0.33 and 0.32, respectively. The results of the analysis of marketed formulation are given in Table 5.

5.3. Forced degradation results

Peaks obtained from samples degraded by treatment with acid, alkali, hydrogen peroxide and dry heat treatment contained well separated spots of the pure drugs and some additional peaks at different R_f values. It is apparent from Figs. 2–9 that the spots of the degradation products were well resolved from those of the drugs. The peaks of itopride hydrochloride and pantoprazole sodium were not significantly shifted in the presence of the degradation peaks, which indicated the stability-indicating nature of the method.

6. Conclusion

HPTLC determination of pantoprazole sodium and itopride hydrochloride from pharmaceutical capsule dosage form revealed no interference between two drugs and excipients of the marketed capsule contents. The method is rapid, allowing a high sample throughput necessary for routine analysis with an added advantage of low solvent consumption. Also the method is simple, rapid, specific and well suited for quantitative estimation of both drugs individually from bulk drug and from pharmaceutical preparations. As the method could effectively separate the drugs from their degradation products it can be employed as a stability indicating method.

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