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Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Amitriptyline Hydrochloride and Pantoprazole Sodium in Capsules

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ABSTRACT:

A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Pantoprazole sodium and Amitriptyline HCl in Capsules has been developed. The separation was achieved by LC- 20 AT, Cosmocil C18 (250mm x 4.6 mm x 2.6 µm) column and Buffer 0.05 M KH2PO4 (pH 4.0): Methanol (60:40v/v) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 242 nm. Retention time of Pantoprazole sodium and Amitriptyline HCl were found to be 3.187 min, and 5.497 min respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Pantoprazole sodium (20-60 µg/ml) and for Amitriptyline HCl (5-15 µg/ml). Developed method was found to be accurate, precise and rapid for simultaneous estimation of Pantoprazole sodium and Amitriptyline HCl in their combined dosage form. The drugs were subjected to stress condition of Acid, Base, oxidation, photolysis and Thermal degradation, Considerable Degradation was found in acidic media for Amitriptyline HCl and photo degradation for Pantoprazole sodium. The degradation of amitriptyline HCl and Pantoprazole sodium in acid, base, Oxidation, thermal and photolytic conditions were found to be 18.221 %, 12.017%, 10.592%, 15.004%, 11.083% and for pantoprazole sodium 11.341%, 10.100%, 13.890%, 12.206%, 17.366% respectively. The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial combined dosage form.

KEY WORDS: Pantoprazole sodium, Amitriptyline HCl, Stability indicating RP-HPLC Method, Anxipan capsules.

INTRODUCTION:

Amitriptyline HCl is an antianxiety drug belonging to the class of antidepressants; more specifically it belongs to tricyclic antidepressants (TCA). Amitriptyline HCl is used in anxiety and depression. The mode of action is thought to increase the synaptic concentration of noradrenaline and serotonin in the central nervous system by inhibiting their re-uptake by the pre –synaptic neuronal membrane.

Pantoprazole Sodium is a Proton Pump Inhibitor (PPI) drug .it is widely used to treat peptic ulcer, acidity, acid reflux and in gerd. It suppresses the final step in gastric acid production by covalently binding to the (H+,K+ - ATPase enzyme system at the secretory surface of the gastric parietal cells .This effect leads to inhibition of both basal and stimulated gastric acid secretion ,irrespective of the stimulus. The binding to the H+ ,K+ - ATPase result in duration of antisecretory effect that persist longer than 24 hours for all doses tested.

Both the drugs in combination in capsule dosage form is used to treat anxiety induced acidity and function heartburn. As per the literature review no any reported method was available for both the drugs in capsules.

MATERIALS AND METHODS:

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APPARATUS:

Model: Shimadzu HPLC System, Pump:-LC-20 AT, Column: Cosmosil C_{18} (250 mm × 4.6 mm, 5 µm) column, Injector: 20 µl fixed loop, Detector: UV Detector, Software: Spinchrom Analytical balance: Electronic Balance (Shimadzu AUX220), UV Spectrophotometer: Systronics 119.

Reagents and Materials:

Amitriptyline HCl and Pantoprazole sodium were procured From Ascent Pharma Pvt. Ltd. Shapar Veraval, Rajkot.

Chromatographic Conditions:

Column: Cosmosil C_{18} (250 mm × 4.6 mm, 5 µm) column, Mobile Phase: 0.05 M KH_2PO_4 Buffer (pH 4.0): Methanol (60:40), Flow Rate: 1.0 ml/min, Detection Wavelength: 242 nm, Run time: 15 min, Injection volume: 20.0 µl

Selection of Detection wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study Standard solution of Pantoprazole Sodium (40 µg/ml) and Standard solution of Amitriptyline HCl (10 µg/ml) in Methanol were scanned between 200-400 nm using UV-visible spectrophotometer. Good response was achieved at 242 nm. So **242 nm** is used as Detection Wavelength.

Preparation of Standard Solutions:

(A) Pantoprazole Sodium standard stock solution: (400 µg/ml)

Forty (40) mg of pantoprazole sodium was weighed and transferred to 100 ml volumetric flask. Volume was made up to the mark with methanol.

(B) Amitriptyline HCl standard stock solution: (100 µg/ml)

Ten (10) mg of amitriptyline HCl was weighed and transferred to 100 ml volumetric flask. Volume was made up to the mark with methanol

(C) Preparation of working standard solution of binary mixtures Of Pantoprazole Sodium (40 µg/ml) and Amitriptyline HCl (10 µg/ml)

One ml from the Pantoprazole Sodium stock solution and 1 ml from Amitriptyline HCl stock solution were transferred to 10 ml volumetric flask and volume was made up to the mark by mobile phase.

(D) Preparation of test solution

Capsules Powder equivalent to 40 mg of Pantoprazole sodium and 10 mg of Amitriptyline HCl were transferred to a 100 ml volumetric flask (Accurately weighed 20 filled capsules, than 20 empty capsules were weighed and weight of empty capsules was substrated from filled capsules weight and fine powder of content filled in the capsules), add 60 ml of Mobile phase and Shake for 15 minutes and made up volume up to the mark with mobile phase. The solution was filtered through Whatman filter paper no. 42 and first few drops of filtrate were discarded. 1 ml of this solution was diluted to 10 ml with mobile phase. The solution was injected 20 µl. The areas of resulting peak were measured at 242 nm.

Preparation of Mobile Phase:

COMPOSITION: 0.05 M KH_2PO_4 Buffer (pH 4.0): Methanol (60:40), (v/v)

PREPARATION OF 0.05 M Buffer (KH_2PO_4)

6.8g of KH_2PO_4 was Dissolved in 800 ml of Water, and add remaining 200 ml of water (initial pH 4.7, then it was adjusted to pH 4.0 with 1 % O.P.A).

PREPARATION: 60ml of 0.05M KH_2PO_4 Buffer (pH 4.0) and 40ml of Methanol were mixed well and Filtered with 0.45µ membrane filter paper. It was further Sonicated for 15 minutes to degas.

STABIITY STUDY:

A. Acid degradation

Acid decomposition studies were performed by transferring 1 ml of stock solution to 10 ml of volumetric flask. Two ml of 0.1 N HCl solution was added and mixed well and kept 3 hours at RT (25^{o}C) for Pantoprazole sodium and Amitriptyline HCl. This solution was neutralized with 0.1N NaOH solution then the volume was adjusted with diluent to get 10 µg/ml for Amitriptyline HCl and 40 µg/ml for Pantoprazole Sodium.

B. Base degradation

Base decomposition studies were performed by transferring 1ml of stock solution to 10 ml of volumetric flask. Two ml of 0.1 N NaOH solution was added and mixed well and kept 4 hours at RT (25^{o}C). This solution was neutralized with 0.1N HCl solution, and then the volume was adjusted with diluent to get 10 µg/ml for Amitriptyline HCl and 40 µg/ml for Pantoprazole Sodium.

C. Oxidative degradation

Oxidation decomposition studies were performed by transferring 1ml of stock solution to 10 ml of volumetric flask. Two ml of 3 % H_2O_2 solution was added and mixed well and kept 3 hours at RT (25^{o}C). Then the volume was adjusted with diluent to get 10 µg/ml for Amitriptyline HCl and 40 µg/ml for Pantoprazole Sodium.

E. Thermal degradation

Amitriptyline HCl Thermal Degradation: TEN (10) mg of amitriptyline HCl was weighed and transferred in a petri dish and put it in the oven at 70 ^{0}C for 4 hours, after time period the amitriptyline HCl was transferred in 100 ml

volumetric flask and volume was made up to with mobile phase, from this solution take 1ml and transferred to 10 ml volumetric flask to make amitriptyline HCl 10 µg/ml.

Pantoprazole sodium Thermal Degradation: Forty (40) mg of Pantoprazole sodium was weighed and transferred it in a Petri dish and kept in the oven at 70 ^{0}C for 4 hours, After time period the Pantoprazole sodium was transferred in 100 ml volumetric flask and volume was made up to mark with mobile phase, From this solution 1ml was transferred to 10 ml volumetric flask to make Pantoprazole sodium 40 µg/ml.

Capsule Thermal Degradation: Capsule powder equivalent to 40 mg of pantoprazole sodium and 10 mg of amitriptyline HCl were weighed and transfer in a petri dish and kept it in the oven at 70 ^{0}C for 4 hours, after time period the capsule powder was transferred in 100 ml volumetric flask and volume was made up with mobile phase, from this solution take 1ml and transferred to 10ml volumetric flask to make pantoprazole sodium 40 µg/ml and amitriptyline HCl 10 µg/ml.

F. Photo degradation

Amitriptyline HCl Photo Degradation: Ten (10) mg of amitriptyline HCl was weighed and transferred in a petri dish and kept in the UV chamber for 12 hours, after time period the amitriptyline HCl was transferred in 100 ml volumetric flask and volume was made up with mobile phase, from this solution take 1 ml and transferred to 10 ml volumetric flask to make amitriptyline HCl 10 µg/ml.

Pantoprazole sodium Photo Degradation: Forty (40) mg of pantoprazole sodium was weighed and transferred in a petri dish and kept in the UV chamber for 12 hours, after time period the pantoprazole sodium was transferred in 100 ml volumetric flask and volume was made up with mobile phase, from this solution 1ml was transferred to 10 ml volumetric flask to make pantoprazole sodium 40 µg/ml.

Capsule Photo Degradation: Capsule powder equivalent to 40 mg of pantoprazole sodium and 10 mg of amitriptyline HCl were weighed in a petri dish and kept in the UV chamber for 12 hours, after time period the capsule powder was transferred in 100 ml volumetric flask and volume was made up with mobile phase, from this solution 1ml was transferred to 10 ml volumetric flask to make pantoprazole sodium 40 µg/ml and amitriptyline HCl 10 µg/ml.

Validation of RP-HPLC Method

Linearity

The linearity for Pantoprazole sodium and Amitriptyline HCl were assessed by analysis of combined standard solution in range of 20-60 µg/ml and 5-15 µg/ml respectively 5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of Pantoprazole sodium (400 µg/ml) and Amitriptyline HCl (100 µg/ml) and transfer to 100 ml volumetric flask and was make up with mobile phase to obtain 20,30,40,50,60 µg/ml and 5,7.5,10,12.5,15 µg/ml for Pantoprazole sodium and Amitriptyline HCl respectively.

In term of slope, intercept and correlation co-efficient value, the graph of peak area obtained verses respective concentration was plotted. Correlation co-efficient for calibration curve of Amitriptyline HCl and Pantoprazole sodium was found to be 0.9997 and 0.9998 respectively

The regression line equation for Amitriptyline HCl and Pantoprazole sodium are as following:

For Amitriptyline HCl: **y = 276.4x - 20.185** and For Pantoprazole sodium: **y = 80.48x - 19.12**

Precision

Results should be expressed as Relative standard deviation (RSD) or coefficient of variance.

A. Repeatability

Standard solution containing Pantoprazole sodium (40 µg/ml) and Amitriptyline HCl (10 µg/ml) was injected six times and areas of peaks were measured and % R.S.D. was calculated.

B. Intra-day precision

Standard solution containing (20, 40, 60 µg/ml) of Pantoprazole sodium and (5, 10, 15 µg/ml) of Amitriptyline HCl were analysed three times on the same day and % R.S.D was calculated.

C. Inter-day precision

Standard solution containing (20, 40, 60 µg/ml) of Pantoprazole sodium and (5, 10, 15 µg/ml) of Amitriptyline HCl were analysed three times on the different day and % R.S.D was calculated.

Accuracy

For Pantoprazole sodium

20 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10 ml. The area of each solution peak was measured at 242 nm. The amount of Pantoprazole sodium was calculated at each level and % recoveries were computed.

For Amitriptyline HCl

5 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10 ml. The area of each solution peak was measured at 242 nm. The amount of Amitriptyline HCl was calculated at each level and % recoveries were computed.

LOD and LOQ

The LOD was estimated from the set of 3 calibration curves used to determine Method linearity. The LOD may be calculated as, LOD = 3.3 × (SD/Slope)

Where, SD= Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

The LOQ was estimated from the set of 3 calibration curves used to determine method linearity. The LOQ may be calculated as, LOQ = 10 × (SD/Slope)

Where, SD = Standard deviation of Y-intercepts of 3 calibration curves.

Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.
2. Ratio of Mobile phase was changed (±2) Buffer: Methanol (62:38) and Buffer: Methanol (58:42)
3. pH of buffer was changed (±0.2) pH 4.2 and pH 3.8

RESULTS AND DISCUSSION

Fig.1: Overlay UV Spectrum of Pantoprazole Sodium (40 µg/ml) and Amitriptyline HCl (10 µg/ml) showing selection of wavelength detection 242 nm.

Fig.2: Chromatogram of Pantoprazole Sodium and Amitriptyline HCl in 0.05 M KH_2PO_4 Buffer (pH 4.0): Methanol (60:40, v/v) (Flow rate-1.0 ml/min)

Table 1: Results for system suitability test.

Parameters	Data observed	
	Pantoprazole Sodium	Amitriptyline HCl
Retention Time (min)	3.187	5.497
Theoretical plates per column	6945	7119
Symmetry factor/Tailing factor	1.381	1.400
Resolution	11.172	

Fig. 3: Chromatogram of Amitriptyline HCl and Pantoprazole Sodium Standard for stability

Fig. 4: Chromatogram of Acid Degradation Blank

Fig. 5: Chromatogram of Pantoprazole Sodium Acid Degradation Standard 3 Hours

Fig. 6: Chromatogram of Amitriptyline HCl Acid Degradation Standard 4 Hours

Fig. 7: Chromatogram of Amitriptyline HCl and Pantoprazole Sodium Acid Degradation Sample 3 Hours

Fig. 8: Chromatogram of Base Degradation Blank

Fig. 9: Chromatogram of Pantoprazole Sodium Base Degradation 4 Hours

Fig.10: Chromatogram of Amitriptyline HCl Base Degradation 4 Hours

Fig. 11: Chromatogram of Amitriptyline HCl and Pantoprazole Sodium Base Degradation Sample 4 Hours

Fig. 12: Chromatogram of Oxidation Degradation Blank

Fig. 13: Chromatogram of Pantoprazole Sodium Oxidation Degradation 3 Hours

Fig. 14: Chromatogram of Amitriptyline HCl Oxidation Degradation 3 Hours

Fig. 15: Chromatogram of Amitriptyline HCl and Pantoprazole Sodium Oxidation Degradation sample 3 Hours

Fig. 16: Chromatogram of Thermal Degradation Blank

Fig. 17: Chromatogram of Pantoprazole Sodium Thermal Degradation 4 Hours at 70 ^{0}C

Fig. 18: Chromatogram of Amitriptyline HCl Thermal Degradation 4 Hours at 70 ^{0}C

Fig. 19: Chromatogram of Pantoprazole Sodium and Amitriptyline HCl Thermal Degradation sample 4 Hours at 70 ^{0}C

Fig. 20: Chromatogram of Photo Degradation Blank

Fig. 21: Chromatogram of Pantoprazole Sodium Photo Degradation 12 Hours

Fig. 22: Chromatogram of Amitriptyline HCl Photo Degradation 12 Hours

Fig. 23: Chromatogram of Pantoprazole Sodium and Amitriptyline HCl Photo Degradation sample 12 Hours

Table 2: Amitriptyline HCl and Pantoprazole Sodium

Drugs	Area
Amitriptyline HCl	2882.867
Pantoprazole Sodium	3182.701

S.T.D for Stability

Table 3: Amitriptyline HCl % Degradation

	Amitriptyline HCl			
Parameter	**Standard**		**Sample**	
	Area	**%Degradation**	**Area**	**%Degradation**
Acid	2355.138	18.306	2357.579	18.221
Base	2507.601	13.017	2536.447	12.017
Thermal	2453.020	14.910	2450.315	15.004
Oxidation	2573.726	10.723	2577.521	10.592
Photo	2567.144	10.952	2563.359	11.083

Table 4: Pantoprazole Sodium % Degradation

	Pantoprazole Sodium			
Parameter	**Standard**		**Sample**	
	Area	**%Degradation**	**Area**	**%Degradation**
Acid	2872.747	9.727	2821.412	11.341
Base	2877.232	9.586	2860.896	10.100
Thermal	2725.854	14.343	2793.866	12.206
Oxidation	2746.342	13.699	2740.274	13.890
Photo	2598.371	18.349	2629.673	17.366

Fig. 24: Overlay chromatogram of different concentrations of binary mixtures of Amitriptyline HCl and Pantoprazole sodium

Fig. 25: Calibration Curve of Amitriptyline HCl (5-15 µg/ml)

Fig. 26: Calibration Curve of Pantoprazole sodium (20-60 µg/ml)

Table 7: Repeatability data for Amitriptyline HCl

	Amitriptyline HCl			
Sr. No.	Conc. (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	10	2759.922 2765.588 2768.121 2806.946 2821.006 2815.324	2789.485±27.812	0.997

Table 8: repeatability data for Pantoprazole sodium

	Pantoprazole sodium			
Sr. No.	Conc. (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	40	3220.823 3240.183 3230.473 3267.063 3292.208 3285.620	3256.062 ±29.833	0.916

Table 9: Intraday precision data for estimation of Amitriptyline HCl and Pantoprazole sodium

	Amitriptyline HCl			**Pantoprazole sodium**		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	5	1378.340 ± 6.168	0.447	20	1611.974± 9.635	0.598
2	10	2736.058± 3.049	0.111	40	3197.445±9.741	0.305
3	15	4084.030± 15.936	0.390	60	4772.158± 9.975	0.209

Table 10: Interday precision data for estimation of Amitriptyline HCl and Pantoprazole sodium

	Amitriptyline HCl			**Pantoprazole sodium**		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	5	1362.019±4.698	0.345	20	1586.522±7.134	0.449
2	10	2732.585±17.839	0.653	40	3194.071±14.896	0.466
3	15	4102.735±14.574	0.355	60	4783.032±17.483	0.366

Table 11: Recovery data for Amitriptyline HCl

SR. NO.	**Conc. Level (%)**	**Sample amount (µg/ml)**	**Amount Added (µg/ml)**	**Amount recovered (µg/ml)**	**% Recovery**	**% Mean Recovery ± S.D**
1	80 %	5	4	4.038	100.955	100.987 ± 1.165
2		5	4	3.994	99.839	
3		5	4	4.087	102.168	
4	100 %	5	5	5.037	100.733	100.795 ± 0.518
5		5	5	5.067	101.342	
6		5	5	5.016	100.310	
7	120 %	5	6	5.996	99.940	99.346 ± 1.527
8		5	6	5.857	97.612	
9		5	6	6.029	100.486	

Table 12: Recovery data for Pantoprazole sodium

SR. NO.	**Conc. Level (%)**	**Sample Amount**	**Amount Added**	**Amount recovered (µg/ml)**	**% Recovery**	**% Mean Recovery ± S.D**
1	80 %	20	16	16.138	100.861	101.578 ± 0.627
2		20	16	16.297	101.854	
3		20	16	16.323	102.020	

4	100 %	20	20	20.134	100.668	100.586 ± 0.706
5		20	20	20.249	101.247	
6		20	20	19.968	99.842	
7		20	24	23.974	99.891	99.708± 0.816
8		20	24	23.716	98.816	
9		20	24	24.100	100.418	

Table 13: Limit of Detection data for Amitriptyline HCl and Pantoprazole sodium

Amitriptyline HCl	Pantoprazole sodium
LOD = 3.3 x (SD / Slope) = 3.3 x (10.645/276.40) = 0.127 µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (9.434/80.480) = 0.387 µg/ml

Table 14: Limit of Quantitation data for Amitriptyline HCl and Pantoprazole sodium

Amitriptyline HCl	Pantoprazole sodium
LOQ = 10 x (SD / Slope) = 10 x (10.645/276.40) = 0.385 µg/ml	LOQ = 10 x (SD / Slope) = 10 x (9.434/80.480) = 1.172 µg/ml

Table 15: Robustness data for Amitriptyline HCl

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at Mobile phase (-2)	Area at Mobile phase(+2)	Area at pH(-0.2)	Area at pH(+0.2)
1	2784.462	2678.018	2757.152	2702.595	2797.713	2648.520
2	2774.305	2672.578	2776.511	2718.890	2794.795	2643.220
3	2803.915	2670.730	2784.962	2710.618	2808.844	2651.050
% R.S.D	0.540	0.142	0.514	0.301	0.265	0.151

Table 16: Robustness data for Pantoprazole sodium

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at Mobile phase (-2)	Area at Mobile phase(+2)	Area at pH (-0.2)	Area at pH (+0.2)
1	3249.347	3125.226	3217.571	3153.824	3264.984	3090.801
2	3262.399	3118.955	3240.139	3157.468	3284.602	3109.380
3	3272.250	3140.691	3259.627	3163.273	3278.011	3093.793
% R.S.D	0.352	0.358	0.650	0.151	0.305	0.322

Table 17: Analysis of marketed formulation

Capsule	Label claim		Assay (% of label claim) Mean ± S. D.	
	Amitriptyline HCl	Pantoprazole sodium	% Amitriptyline HCl	% Pantoprazole sodium
Anxipan	10 mg	40 mg	100.348 ± 0.255	100.536 ± 0.406

CONCLUSION

The degradation behaviour of Pantoprazole sodium and Amitriptyline HCl was investigated under different stress degradation (Hydrolytic, oxidative, photolytic and thermal) conditions recommended by International Conference on Harmonization (ICH) using HPLC. A stability indicating RP-HPLC method was developed that could separate drug from degradation products formed under various stressed conditions. RP-HPLC method was developed for simultaneous estimation Pantoprazole sodium and Amitriptyline HCl. In RP-HPLC method, good resolution and separation of two drugs was achieved. Buffer 0.05 M KH_2PO_4 (pH 4.0): Methanol (60:40) was used as mobile phase. Retention time of Pantoprazole sodium and Amitriptyline HCl were found to be 3.187 min and 5.497 min respectively with a flow rate of 1 ml/min. and Detection wavelength was 242 nm. The proposed method was accurate and precise. Therefore proposed method can be used for routine analysis of Pantoprazole sodium and Amitriptyline HCl in capsule. Validation parameters like Linearity, Accuracy, Precision, Robustness, System suitability, Specificity were tested.Observation of all these parameters leads to the point that developed RP-HPLC method is linear, accurate, precise, specific and robust.It can be successfully adopted for routine quality control analysis of Pantoprazole sodium and Amitriptyline HCl in

Combined dosage form without any interference from common excipients and impurity.

Table 18 Summary of RP-HPLC Method development

SR. NO	Parameters	Amitriptyline HCl	Pantoprazole Sodium
1	Column	Cosmosil C_{18} (250 mm × 4.6 mm, 5 µm) column	
2	Mobile Phase	0.05 M KH_2PO_4 Buffer (pH 4.0): Methanol (60:40)	
3	Flow Rate	1.0 ml/min	
4	Detection Wavelength	242 nm	
5	Injection volume	20.0 µl	
6	Run time	15 min	
7	Resolution	11.172	
8	Retention Time (min)	5.497	3.187
9	Theoretical plates per column	7119	6945
10	Symmetry factor/Tailing factor	1.400	1.381

Table 19 Summary of Validation parameter of developed RP-HPLC method.

SR. NO	Parameters	Amitriptyline HCl	Pantoprazole Sodium
1	Linearity Range	5-15 µg/ml	20-60 µg/ml
2	Slope	276.40	80.480
3	Intercept	20.185	19.12
4	Correlation coefficient	0.9997	0.9998
5	% Recovery	99.346-100.987 %	99.708-101.578%.
6	Repeatability (%RSD)	0.997	0.916
7	Intraday (%RSD)	0.111-0.447	0.209-0.598
8	Interday (%RSD)	0.345-0.653	0.366-0.466
9	LOD (µg/ml)	0.127	0.387
10	LOQ (µg/ml)	0.385	1.172
11	Robustness	0.142-0.540	0.151-0.650
12	Assay (% of label claim)	100.348 ± 0.255	100.536 ± 0.406
13	Specificity	Specific	

Table 20 Summary of forced degradation study: Amitriptyline HCl

Degradation Condition	TIME (hours)	% Degradation STANDARD	% Degradation SAMPLE
Acid degradation (0.1 N HCl)	4	18.306	18.221
Base degradation (0.1 N NaOH)	4	13.017	12.017
Photo degradation	12	10.952	11.083
Oxidative degradation (3 % H_2O_2)	3	10.723	10.592
Thermal degradation (70 ^{0}C)	4	14.910	15.004

Table 21 Summary of forced degradation study: Pantoprazole Sodium

Degradation Condition	TIME (hour)	%Degradation STANDARD	%Degradation SAMPLE
Acid degradation (0.1 N HCl)	3	9.727	11.341
Base degradation (0.1 N NaOH)	4	9.586	10.100
Photo degradation	12	18.349	17.366
Oxidative degradation (3 % H_2O_2)	3	13.699	13.890
Thermal degradation (70 ^{0}C)	4	14.343	12.206

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