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RP-HPLC Method Development and Validation for the Simultaneous Estimation of Amitriptyline Hydrochloride and Pantoprazole Sodium in Bulk and Capsule Dosage Form

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ABSTRACT

A simple, rapid, accurate and precise isocratic reversed phase high performance liquid chromatographic method has been developed and validated for Simultaneous Estimation of Pantoprazole Sodium (PNT) and Amitriptyline Hydrochloride in Bulk and Capsule Dosage Form. In RP-HPLC method, the analyte were resolved by using isocratic program, methanol and phosphate buffer (80:20v/v) was used as mobile phase, at a flow rate of 0.8 ml/min. on HPLC system containing UV-visible detector with Workstation software and cosmosil 18 (250 mm × 4.6 mm,5 µm) column. The detection was carried out at 244nm. The retention times were 3.21 minutes and 4.20 minutes for Amitriptyline Hydrochloride and Pantoprazole Sodium respectively. Calibration plots were linearity was found 0.9995 and 0.9997 for Amitriptyline Hydrochloride and Pantoprazole Sodium. The method was validated for linearity, precision, accuracy, ruggedness and robustness. The proposed method was successfully used for simultaneous estimation of Amitriptyline Hydrochloride and Pantoprazole Sodium in capsule dosage form. Validation studies revealed that the proposed method is specific, rapid, reliable and reproducible. The high % recovery and low % RSD confirms the suitability of the proposed method for routine quality control analysis of Amitriptyline Hydrochloride and Pantoprazole Sodium in bulk and capsule dosage forms.

Keywords: RP-HPLC, Amitriptyline Hydrochloride and Pantoprazole Sodium, methanol, phosphate buffer.

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INTRODUCTION

Amitriptyline hydrochloride is chemically 3-(5,6-dihydrodibenzo[2,1-b:2',1'-f][7]annulen-11-ylidene)-N,N dimethylpropan-1-amine hydrochloride dibenzocyclo heptene-derivative tricyclic antidepressant (TCA) and analgesic. They contain a tricyclic ring system with an alkyl amine substituent on the central ring. In depressed individuals, amitriptyline exerts a positive effect on mood. TCAs are potent inhibitors of serotonin and norepinephrine reuptake.

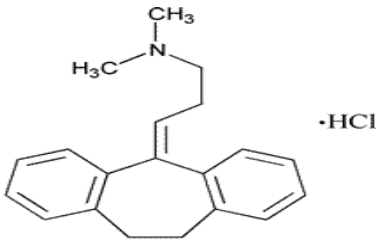

Fig.1: Molecular Structure of Amitriptyline Hydrochloride

Pantoprazole sodium is a proton pump inhibitor (PPI). Chemically it is 5-(difluoromethoxy)-2-[(3,4-dimethoxy pyridin-2-yl)methylsulfinyl]-3*H*-benzoimidazole which block the production of acid in the stomach. Proton pump inhibitors are used for the treatment of conditions such as ulcers, gastro esophageal reflux disease (GERD) and Zollinger-Ellison syndrome. Pantoprazole sodium, blocks the enzyme in the wall of the stomach that produces acid.

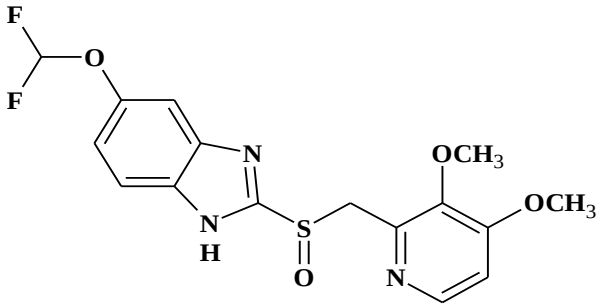

Fig.2: Molecular Structure of pantoprazole sodium

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 reuptake 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 ingerd. 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

Apparatus:

Model: HPLC Binary Gradient System, Make:- Analytical Technologies Ltd. Pump:- P-3000-M Reciprocating (40MPa), Column: Grace C18 (4.6ID×250mm;5µm) , Detector: UV - 3000-M Detector, Software: HPLC Workstation Analytical balance: Wensar High Precision Balance, Model PGB100 pH Meter: Digital pH meter Make:- EU-Tech, ME-302 sonicator: Wensar Ultra Sonicator Model:- WUC-4L Capacity:-4Liter Membrane Filter: Nylon 0.45µm.

Chemicals and solvents:

Amitriptyline HCl and Pantoprazole sodium were procured From Swapnroop Drugs and pharmaceuticals. The marketed formulation of Amitriptyline Hydrochloride Pantoprazole Sodium capsule (Amitriptyline Hydrochloride 10mg Pantoprazole Sodium 40 mg) were procured from local market. HPLC grade water, methanol were purchased from E.Merck Ltd., Mumbai, India.

Chromatographic condition:

Column: Grace C18 (4.6 ID×250 mm;5µm) column, Mobile phase: 10mM KH_2PO_4 Buffer (80:20) Flow Rate: 8.0 ml/min, Detection Wavelength:244 nm, Run time: 8.67min, Injection volume: 20.0 µl.

Detection Wavelength by UV Spectroscopy:

The standard solution was scanned between 400nm to 200nm Analytical wavelength for the examination was selected from the wavelength of maximum absorption from the spectrophotometric analysis and it was 244nm. therefore 244nm considered as an analytical wavelength for further determination.

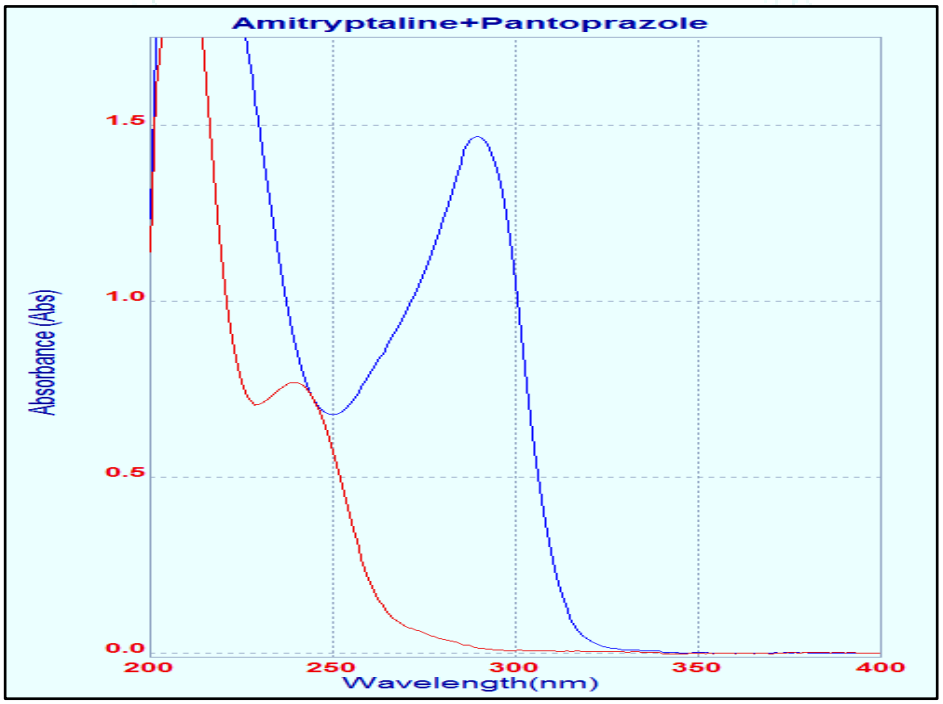

Fig. 3: UV spectrum of Amitriptyline hydrochloride and pantoprazole sodium

Preparation of Standard Solutions:

Standard stock solution of Amitriptyline hydrochloride 1000 ppm.

Accurately weighted about 10 mg of Amitriptyline Hydrochloride and transferred into a 10ml volumetric flask and 7ml of diluent was added and sonicate to dissolve it completely and the volume was adjusted with mobile phase to get stock solution of 1000µg/ml.

Standard stock solution of pantoprazole sodium 1000ppm.

Accurately weighted about 10 mg of pantoprazole sodium and transferred into a 10ml volumetric flask and 7ml of diluent was added and sonicate to dissolve it completely and the volume was adjusted with mobile phase to get stock solution of 1000µg/ml.

Preparation of working standard solution:

capsule powder equivalent to the 10mg of Amitriptyline Hydrochloride and 40 mg of pantoprazole sodium were

transferred to a 100ml of volumetric flask (Accurately weight 10 capsule) add a 65 ml of mobile phase and shake for 20min made up volume up to the mark with mobile phase the solution was filtered through membrane filter. First few drop of filtered were discarded. Form this solution , 0.15 ml of pipette out and transferred into 10ml of volumetric flask and make up the volume to the mark with diluent(15ppm), chromatogram were recorded the responses the content of Amitriptyline hydrochloride and pantoprazole sodium was calculated by comparing a sample peak with that of standard and reported in chromatogram.

Preparation of Mobile Phase:

Composition: Methanol and phosphate buffer (80:20v/v)

Preparation of 10mM Buffer (KH2PO4)

weight accurately 0.136gm of potassium Dihydrogen phosphate (HPLC grade) transferred to 100ml volumetric flask containing some amount of water and volume was made up to the mark after complete dissolution of potassium di-hydrogen phosphate. The resulting mobile phase was filtered through 0.45µ membrane filter and sonicated for three cycle of each of 10 min.

Development and validation of HPLC method

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of Amitriptyline Hydrochloride and pantoprazole sodium in capsule dosage form. The method was validated for the Parameters like system suitability, linearity, accuracy, precision, robustness. Ruggedness, LOD, LOQ.

System Suitability: suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of Amitriptyline Hydrochloride and pantoprazole sodium. Various chromatographic parameters such as retention time, peak area tailing factor, theoretical plates (Tangent) of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters.

Linearity: Several aliquots of standard solutions of Amitriptyline Hydrochloride and Pantoprazole Sodium were taken in different 10 mL volumetric flasks of 0.1, 0.2, 0.3, 0.4, 0.5ml was taken and diluted to 10ml with diluent such that the final concentration of in the range 10 to 50µg/ml. The above solutions were injected into the HPLC system keeping the injection volume constant. The drugs were eluted with UV detector at 244 nm, peak areas was recorded for all the peaks. The linearity curves were constructed by plotting concentration of the drugs against peak areas. The regression equation of this curve was computed.

Precision: Precision for Amitriptyline Hydrochloride and Pantoprazole Sodium were determined in terms of intra-day precision and inter-day precision. Every sample was injected six times. The measurements for peak areas were expressed in terms of % RSD.

Accuracy: The accuracy of the method was assessed by recovery studies of Amitriptyline Hydrochloride and Pantoprazole Sodium at three concentration levels 50%, 100% and 150%. Fixed amount of pre-analyzed sample was spiked with known amount of Amitriptyline Hydrochloride and Pantoprazole Sodium. Each level was repeated three times. The % recovery of Amitriptyline Hydrochloride and Pantoprazole Sodium were calculated.

Robustness of Method: To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the Optimized method parameters were done. The effect of change in flow rate, wavelength, on the retention time and tailing factor were studied. The method was found to be unaffected by small changes in flow rate and changes in wavelength.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the

peak areas using the formulae LOD = 3.3 σ/s and LOQ = 10 σ/s. The results were given in Table

RESULTS AND DISCUSSION

Results of system suitability study are summarized in Table 1. Six consecutive injections of the Standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution For both the drugs which indicate a good system for analysis.

Table No.1: Results of System Suitability Test:

Parameters	Amitriptyline Hydrochloride	Pantoprazole sodium
Retention time (min)	3.21	4.020
Theoretical plates (N)	1990	9631
Tailing factor (T)	1.32	1.27
Resolution (Rs)	5.88	5.88

Table No.2: Results of Linearity:

PARAMETERS	Amitriptyline Hydrochloride	Pantoprazole sodium
Wavelength	244nm	244nm
Linearity range	0.1-0.5 mg/ml	0.1-0.5 mg/ml
Correlation coefficient	0.9995	0.9997
Regression equation	Y=22232x+33899	Y=105682x+241101
Limit of detection(LOD)	0.274969	0.364940
Limit quantification(LOQ)	1.9015	1.105887

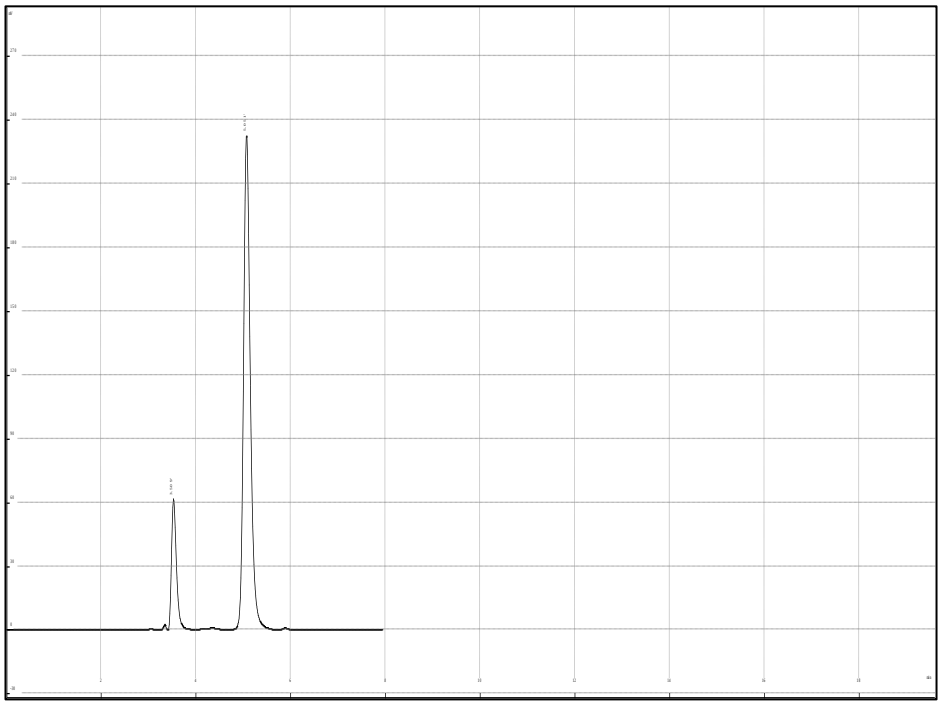

Fig.4: Typical chromatogram of Amitriptyline Hydrochloride and pantoprazole in marketed formulation

Peak name	Retention time	Area	Plate count	Asymmetry
Amitriptyline Hydrochloride	3.509	695653	9202	1.30
Pantoprazole Sodium	5.051	2949371.6	9963	1.26

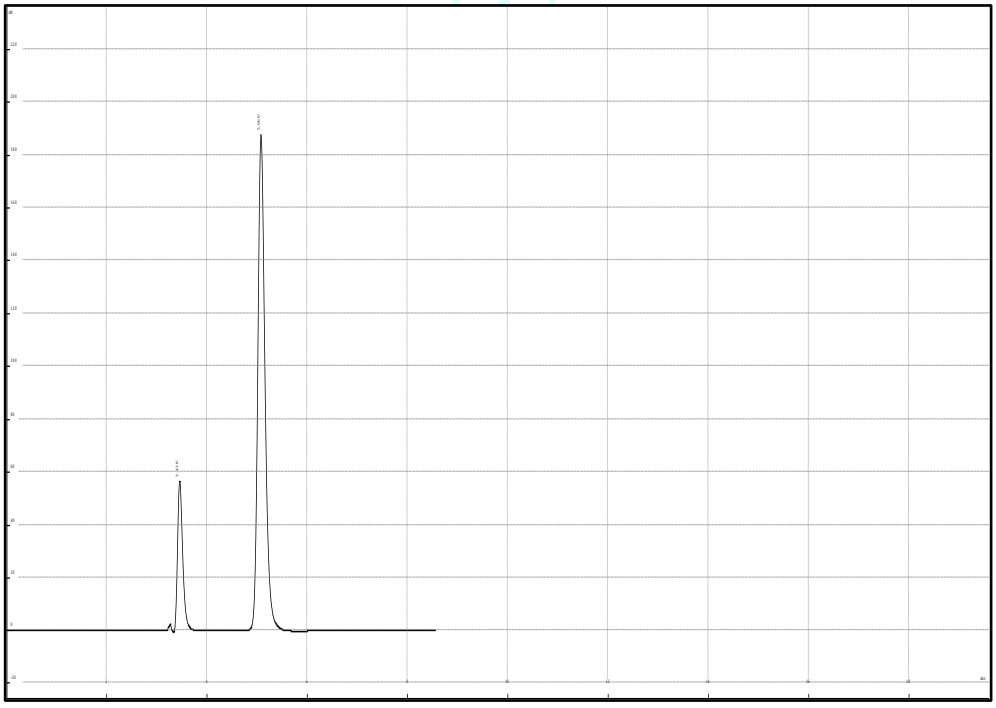

Fig.5: Typical Chromatogram of standard Amitriptyline Hydrochloride and Pantoprazole

Peak name	Retention time	Area	Plate count	Asymmetry
Amitriptyline Hydrochloride	3.438	254911	9552	1.24
Pantoprazole Sodium	5.060	803757	9696	1.36

Chromatograms shown in figure 4 and figure 5 explain that retention time for standard sample and commercial product of Amitriptyline Hydrochloride and pantoprazole sodium are same. This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective. A linear relationship between peak areas versus concentrations was observed for Amitriptyline Hydrochloride and pantoprazole sodium. Correlation coefficient was 0.999 for both the drugs which prove that the method is linear. Calibration curve of Amitriptyline Hydrochloride and pantoprazole sodium are shown in Fig 3 and 4.

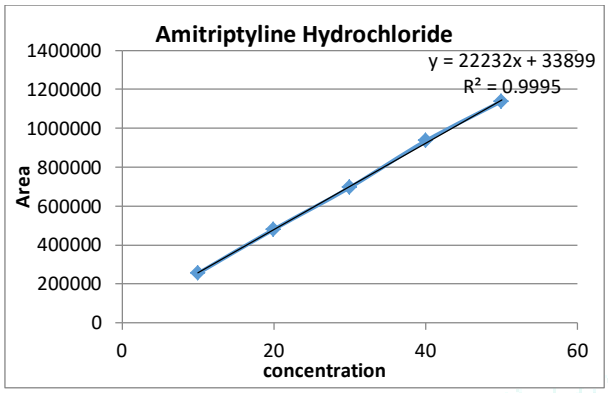

Fig.6: Linearity plot of Amitriptyline Hydrochloride (RP-HPLC)

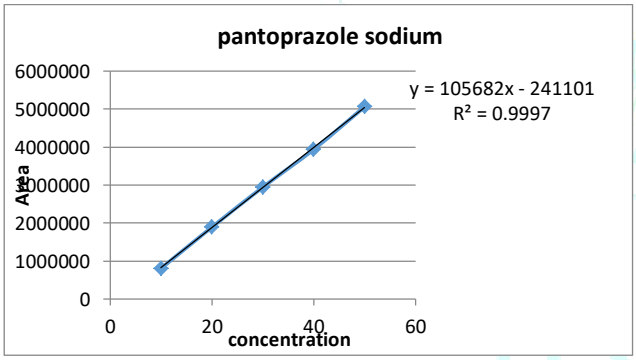

Fig.: Linearity plot pantoprazole sodium

Table.7: Precision data of Amitriptyline Hydrochloride

S. No.	Peak Area	
	Intra-day precision	Inter-day precision
Injection-1	695654	695654
Injection-2	690140	690140
Injection-3	698438	698438
Injection-4	696185	693819
Injection-5	696392	690669
Injection-6	690669	694149
SD	5041.50065	3139.861
%RSD	0.72545	0.452525

Table 3: Precision data of pantoprazole sodium

S. No.	Peak Area	
	Intra-day precision	Inter-day precision
Injection-1	2944683	2944683
Injection-2	2946864	2946864
Injection-3	2942809	2942809
Injection-4	2938262	2945004
Injection-5	2950764	2937185
Injection-6	2955048	2934160
SD	741.5109	778.7222
%RSD	0.107141	0.1122383

Table 4: Accuracy studies of Amitriptyline Hydrochloride

% Concentration level	Area of Standard	Area of Sample	% Recovery
50%	695654	689926	99.17%
100%	935220	939926	100.50%
150%	1138529	1135508	99.73%

Table 5: Accuracy studies of pantoprazole sodium

% Concentration level	Area of Standard	Area of Sample	% Recovery
50%	2944683	2942057	99.910%
100%	3940983	3932057	99.773%
150%	5064419	5052827	99.771%

Table 6. Robustness data relating to change in flow rate

Parameter	Amitriptyline Hydrochloride	Pantoprazole sodium
Flow rat0.7ml/min	482194	1896877
Flow rat 0.8ml/min	477969	1890488
Flow rat 0.9ml/min	480649	1891966
SD	2137.76	3344.69
%RSD	0.445115	0.176677

Table 7. Robustness data relating to change in wavelength

Parameter	Amitriptyline Hydrochloride	Pantoprazole sodium
Wavelength 242	480649	1891966
Wavelength 244	482257	1890329
Wavelength 246	482606	1895453
SD	1043.82	2917.07
%RSD	0.216632	0.138280

CONCLUSION

The present work involved the development of simple, accurate, precise and suitable RP-HPLC method.

Literature surveys revealed that several method have been reported for determination of Amitriptyline Hydrochloride and pantoprazole Sodium individually or in combination with other drug in pharmaceutical dosage forms. Hence, in the present study, a new, sensitive, suitable and cost effective reversed -phase high performance liquid chromatographic method was developed and validated for the determination of Amitriptyline Hydrochloride and Pantoprazole Sodium in bulk and pharmaceutical dosage form.

In RP-HPLC method, the analyte were resolved by using isocratic program and mobile phase was used methanol and phosphate buffer (80:20v/v), at a flow rate of 0.8ml/min, on HPLC system containing UV-visible visible detector with Workstation Software and Greece C18 column (4.6x250mm;5µm). The detection was carried out at

244nm.the method gave the good resolution and suitable retention time.

The result of analysis in the method were validated in terms of linearity, accuracy, precision, robustness, limit of detection and limit of quantitation.

The method has several advantages, including simple mobile phase, low cost solvent, rapid analysis, simple sample preparation and improve selectivity as well as sensitivity. The regression coefficient (r2) for each analyte is not less than 0.999 which shows good linearity. The percentage recovery was acceptable range in capsule dosage form. The % RSD was also less than 2% showing high degree of precision of the proposed method.

Since the method does not require use of expensive reagent and also less time consuming, it can be performed routinely in industry for routine analysis.

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