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Research Article

RP-HPLC METHOD DEVELOPMENT, VALIDATION AND STABILITY INDICATING STUDIES FOR THE ESTIMATION OF AMITRIPTYLINE HYDROCHLORIDE AND PERPHENAZINE IN BULK

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ABSTRACT

A *new, simple, precise, accurate and reproducible RP-HPLC method was validated for the estimation of Amitriptyline Hydrochloride (AMT) and Perphenazine (PRP) & Stability indicating Studies were performed. Separation of Amitriptyline Hydrochloride and Perphenazine was successfully achieved on Hypersil ODS (250x4.6mm) 5µm column in an isocratic mode utilizing Methanol:Water (90:10) at a flow rate of 1.0 ml/min and eluents were monitored at 247 nm with a retention time of 4.235 and 3.490 minutes for and Amitriptyline Hydrochloride and Perphenazine respectively. The method was validated and it was found to be linear and the range is 10-100µg/ml for both drugs. The values of the correlation coefficient were found to 0.9995 for Amitriptyline Hydrochloride and 0.9912 for Perphenazine respectively. The LOD for Amitriptyline Hydrochloride and Perphenazine were found to be 0.23µg/ml and 0.39µg/ml. The LOQ for Amitriptyline Hydrochloride and Perphenazine were found to be 0.72µg/mL and 1.18µg/mL respectively. The mean recoveries obtained were 102.3% and 100% of Amitriptyline Hydrochloride and Perphenazine, which indicates accuracy of the proposed method. Forced degradation of Amitriptyline Hydrochloride and Perphenazine in various conditions like alkaline, acidic, oxidative and thermal degradation was performed in this investigation. The content of degradation of the drugs was quantitatively analyzed by HPLC. The Amitriptyline Hydrochloride is very sensitive drug it was degraded in all conditions while Perphenazine is more stable in alkaline, acidic, and oxidative than thermal conditions. Proposed method was validated for precision, accuracy, linearity & range and robostness according to ICH guidelines. The method was successfully applied to Perphenazine and Amitriptyline Hydrochloride estimation in bulk.*

KEYWORDS: *Amitriptyline HCl, Perphenazine, Hypersil C_{18}, Forced degradation, ICH guidlines.*

INTRODUCTION

Amitriptyline hydrochloride (AMT) belongs to the class of tricyclic antidepressants and its IUPAC name is 3-(10, 11-Dihydro-5H-dibenzo [a, d] cycloheptane-5-ylidine)-N, N-dimethyl-1- propanamine [1]. Its function is to prevent serotonin and noradrenaline from being reabsorbed back into the nerve cells in the CNS. Thus it aids to relieve depression [2].

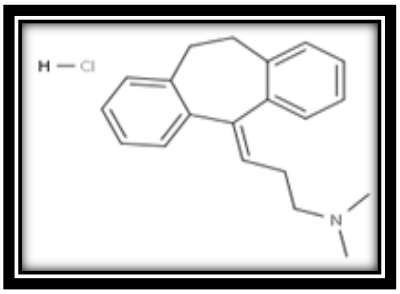

Fig. 1: Structure of Amitriptyline HCl

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Perphenazine (PRP) is 2-(4-(3-(2-Chlorophenothiazine-10yl)propyl)piperazine1-yl) ethanol, an antipsychotic phenothiazine derivative with actions and uses similar to those of chlorpromazine. This compound belongs to the class of organic compounds known as phenothiazines [3]. It acts by binding to the dopamine D1 and dopamine D2 receptors and inhibits their activity. The mechanism of the anti-emetic effect is predominantly due to blockage of the dopamine D2 neurotransmitter receptors in the chemoreceptor trigger zone and vomiting centre. Perphenazine also binds the alpha adrenergic receptor. This receptor's action is mediated by association with G proteins that activate a phosphatidyl inositol-calcium second messenger system [4].

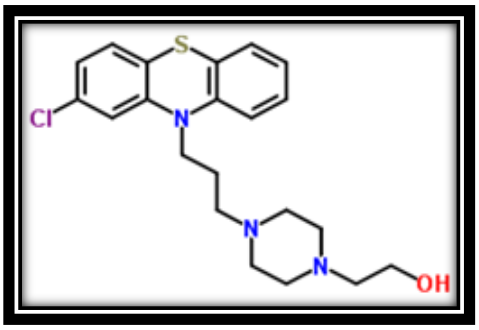

Fig. 2: Structure of Perphenazine

MATERIALS AND METHODS

Standard and samples:

The Amitriptyline Hydrochloride and Perphenazine working standard were provided by Sigma Aldrich Laboratories.

Table No. 1: List of Chemicals and Instruments used

Chemicals	Make	Grade
Methanol	Res Lab Fine Chem	HPLC
Water	Rankem	HPLC
Perphenazine	Sigma Aldrich	AR
Amitriptyline Hydrochloride	Sigma Aldrich	AR
HCl	Res Lab Fine Chem	LR
NaoH	Res Lab Fine Chem	LR
H_2O_2	Merc	LR

Equipments: Instruments used for the present study was weighing balance (Model- AUY220, Shimadzu), Ultra Sonicater water bath (Model-1.5L50, Ultrasonics), UV-VIS Spectrophotometer (Model- UV- 1800, Shimadzu), HPLC (Model- SPD-20A, Shimadzu, UV-Detector, LC Solutions software).

Determination of Isoabsorptivity point:

The standard solutions of PRP (10µg/mL) and AMT (10µg/mL) drugs were dissolved in the HPLC mobile phase and they were scanned separately in the UV region of 200-400nm and the overlay spectra were recorded and the absorbance maxima was determined in Shimadzu UV spectrophotometer using mobile phase as blank.

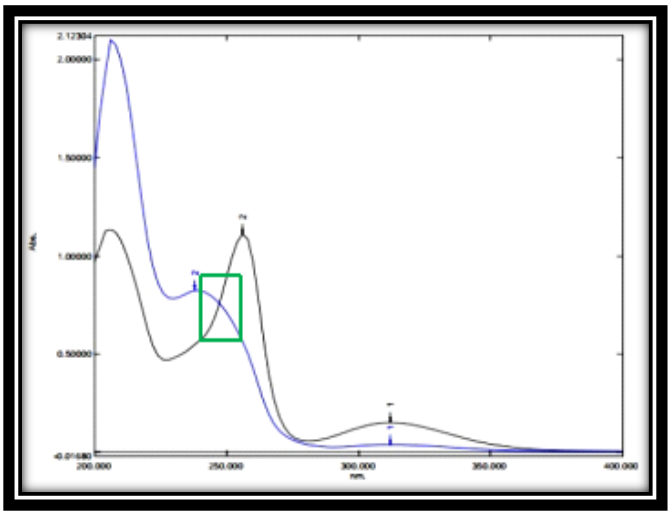

Fig. 3: UV-Spectrum of AMT and PRP

The overlay spectrum (Figure: 3) reveals that PRP has a λmax of 251nm and AMT at 245nm. The isosbestic point was observed at 247nm and chosen for study [5].

Chromatographic conditions:

The chromatograph was operated in the isocratic mode using a mobile phase of Methanol:Water (90:10 %v/v). Eluent was delivered at a flow rate of 1mL/min and Absorbance was monitored at 247nm.

Preparation of mobilephase:

Mix 90 mL of HPLC grade Methanol with the 10mL of HPLC Grade Water and degas in Ultrasonic water bath for 15 minutes

Vehicle: Methanol and Water (90:10 %v/v) used as solvent

Preparation of standard solutions:

Accurately weighed 10mg of AMT and 10mg of PRP were transferred into a 100mL of clean and dry volumetric flask, and about 30mL of mobile phase was added and sonicated to dissolve and degas completely.The volume was made up to the mark with the mobile phase(100µg/mL). Further dilutions like 10, 30, 50, 70, 90,100µg/mL were made using mobile phase [6, 7].

RESULTS AND DISCUSSION

Method development:

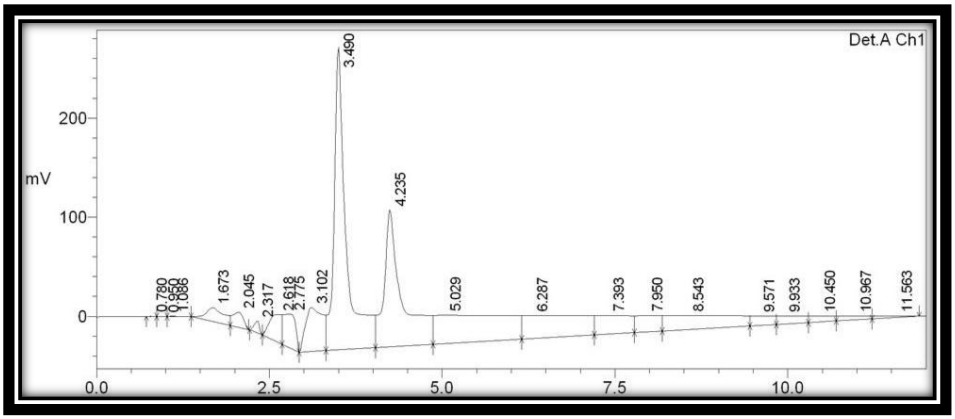

Fig. 4: Chromatogram of optimized method

Table No. 2: Results of the optimized method

Drug	Rt(min)	Peak area	Peak hight	Tailing factor	Theoretical plates/meter
AMT	3.490	3702035	305236	0.00	16961.381
PRP	4.235	2533048	138109	0.00	16111-603

Method validation:

The developed and optimized HPLC method was validated according to ICH guidlines for the following parameters [8-10].

1. Linearity:

Five different concentrations (10-100µg/mL) of AMT and PRP were prepared for linearity studies. The calibration curve obtained by plotting peak area against concentration showed linearity in accordance to beer's law. The correlation coefficient values are 0.999 and 0.991 for AMT and PRP with the range from 10-100 µg/mL respectively. Linearity results were presented in Table No.3.

2. Precision:

The precision of the method was determined by intraday studies. Prepare 30µg/mL solution from a standard solution and injected five times in a day on to analytical column. The percentage relative standard deviation (%RSD) was calculated and lower %RSD indicates that there is less variation and there are high precision in the values. %RSD values of AMT and PRP were 0.24 and 2.71 respectively.

%RSD = (S.D × 100)/mean

Results of Intra-day precision for AMT and PRP are presented in Table No.4.

3. Limit of detection:

The limit of detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula:

LOD = 3.3×(Standard deviation/Slope of calibration curve)

4. Limit of quantification:

The limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the formula:

LOQ = 10 × (Standard deviation/Slope of calibration curve)

LOD and LOQ results were presented in Table No.5.

5. Accuracy:

The accuracy of the method was determined by recovery experiments. The recovery studies were performed by the regular addition method. At 50%, 100%, 150% level, the percentage recovery was calculated. For both the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. Results were presented in Table No.6.

6. Robostness:

Robustness of the method was studied by making slight changes in chromatographic conditions, such as mobile phase ratio and mobile phase flow rate. Results were presented in Table No.7.

Forced degradtion study:

Forced degradation of AMT and PRP in various conditions like acidic, basic,oxidation and thermal degradation was observed. Chromato grams for these studies were represented in Fig. 5-11. Results for Forced degradation study of AMT and PRP were presented in Table No. 3.

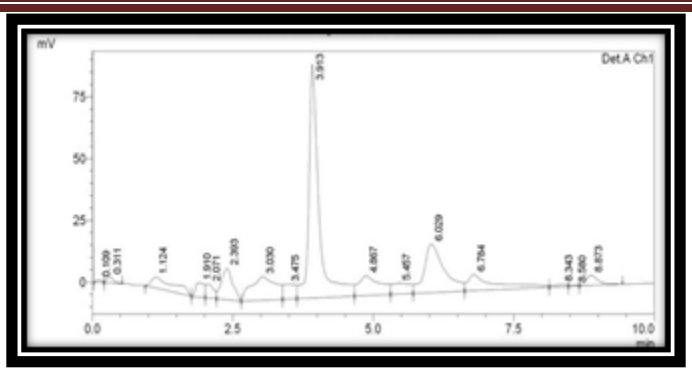

Fig. 5: Chromatogram of AMT and PRP for base degradation after 1st day

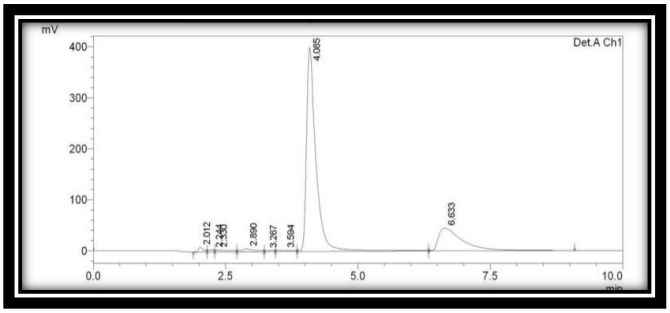

Fig. 6: Chromatogram of AMT and PRP for base degradation after 3rd day

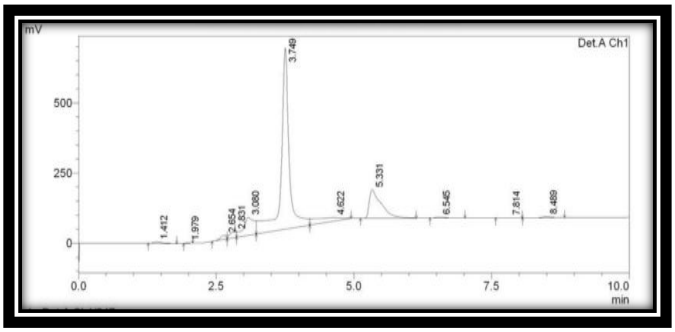

Fig. 7: Chromatogram of AMT and PRP for acid degradation after 1st day

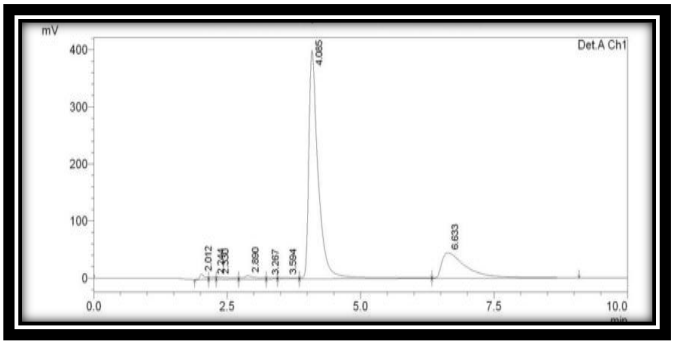

Fig. 8: Chromatogram of AMT and PRP for acid degradation after 3rd day

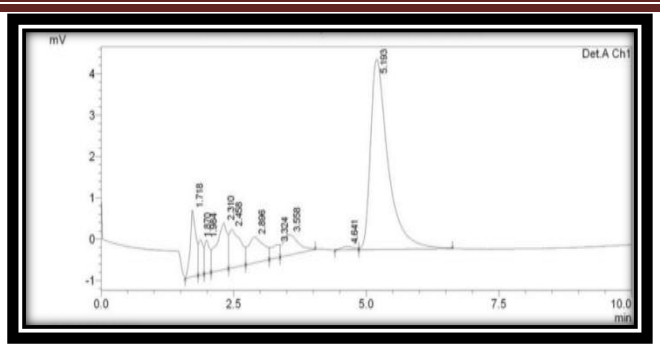

Fig. 9: Chromatogram of AMT and PRP for oxidation degradation after 1st day

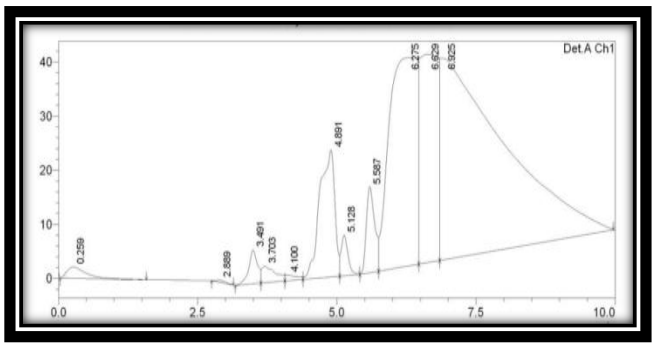

Fig. 10: Chromatogram of PRP for thermal degradation after 1st day

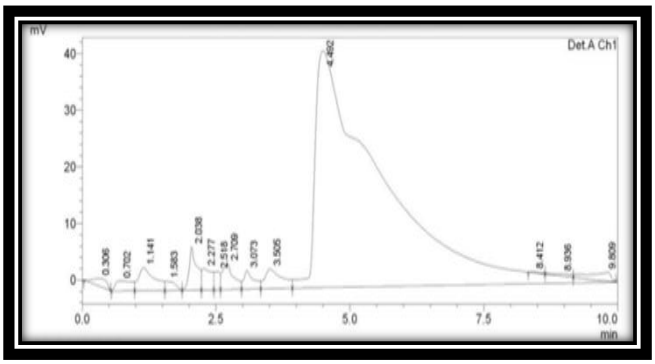

Fig. 11: Chromatogram of and AMT for thermal degradation after 1st day

Table No. 3: Linearity Results of AMT & PRP

S.No	Concentration (µg/mL)	Peak Area of PRP	Peak Area of AMT
1.	10	3702035	2533048
2.	30	3906653	4119924
3.	50	5758783	5767890
4.	70	8822717	7599144
5.	100	8325702	8239848
Correlation Coefficient		**0.9912**	**0.9995**

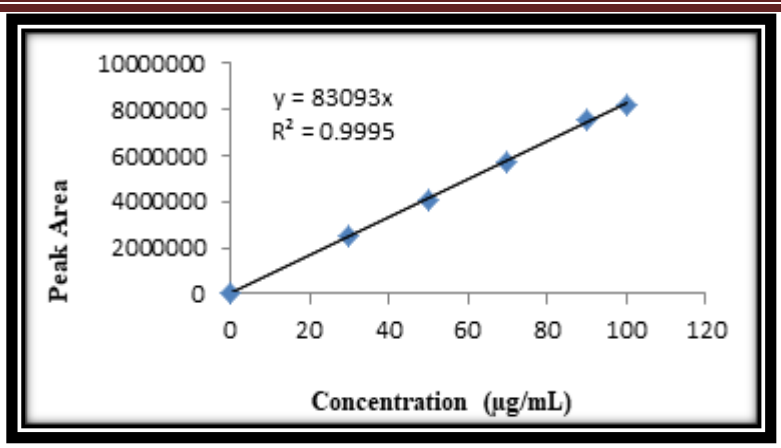

Fig. 12: Calibration curve of AMT

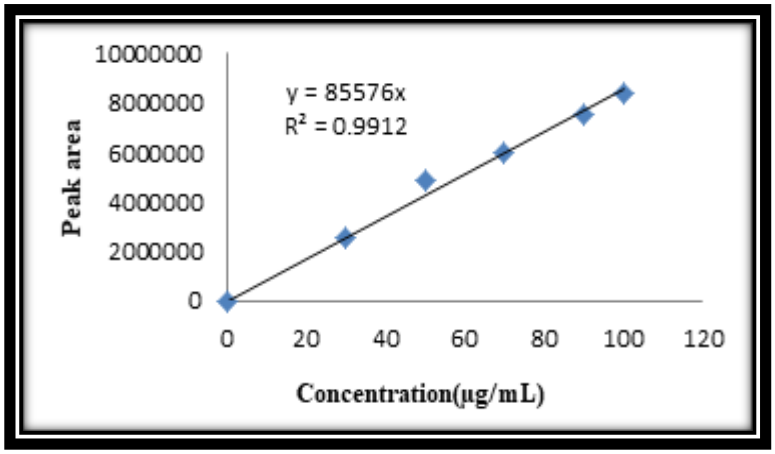

Fig. 13: Calibration curve of PRP

Table No. 4: Results of Intra-Day precision for AMT&PRP

S. No	Peak Area Of Perphenazine	Peak Area Of Amitriptyline Hydrochloride
1.	3702035	2533048
2.	3761642	1454845
3.	3260752	2337776
4.	3678196	3549831
5.	4201422	2541573
Average	3720809	2483414.6
S.D	101188.72	6028.08
% R.S.D	2.71	0.24

Table No. 5: Limit of detection and Limit of quantification

Drug	LOD	LOQ
Perphenazine	0.39 µg/mL	1.18 µg/mL
Amitriptyline HCl	0.23 µg/mL	0.72 µg/mL

Table No. 6: Accuracy Data of AMT & PRP

Concentration	%Recovery AMT	%Recovery PRP
50%	103%±2	102%±2
100%	102%±3	102%±4
150%	102%±2	102%±3

Table No. 7: Robustness results of AMT & PRP

S. no.	Mobile phase (%v/v)	Flow rate mL/min	λ	% RSD of AMT	%RSD of PRP
1.	90:10	1.1	247	0.10	0.28
2.	90:10	1.2	247	0.06	0.19
3.	88:12	1	247	0.80	0.19
4.	92:8	1	247	0.49	0.16

Table No. 8: Results for Forced degradation study of AMT and PRP

Degradation type	Degradation (days)	
	AMT	PRP
Acid dgradation	1 day	3rd day
Base degradation	1 day	3rdday
Oxidation stress	1 day	1 day
Thermal degradation	1 day	1 day

CONCLUSION

A simple, fast, precise, accurate, robust, economic and stability-indicating reversed phase high performance liquid chromatographic method was developed and validated according to ICH guidelines for the estimation of Amitriptyline HCl and Perphenazine in bulk.

Forced degradation of Amitriptyline Hydrochloride and Perphenazine in various conditions like alkaline, acidic, oxidative and thermal degradation was performed. Drugs degradation was confirmed by observing the improper and multiple peaks formation in chromatogram of Amitriptyline Hydrochloride and Perphenazine. Amitriptyline Hydrochloride is very sensitive drug it was not stable in any condition for more than a day. Perphenazine was stable in acidic, alkaline and oxidative than thermal condition.

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