International Journal of Multidisciplinary Research and Growth Evaluation.

Analytical method development and validation of simultaneous estimation of perphenazine and amitriptyline by Reverse-Phase high-performance liquid chromatography

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Article Info

ISSN (online): 2582-7138
Volume: 04
Issue: 01
January-February 2023
Received: 02-01-2023;
Accepted: 19-01-2023
Page No: 338-346

Abstract
A new, simple, precise, accurate and reproducible RP-HPLC method for Simultaneous estimation of Perphenazine and Amitriptyline in bulk and pharmaceutical formulations. Separation of Perphenazine and Amitriptyline was successfully achieved on a Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d. or equivalent in an isocratic mode utilizing Phosphate Buffer (0.2 M, pH=2): Acetonitrile in the ratio of 64:36% v/v at a flow rate of 1.0mL/min and eluates was monitored at 265nm, with a retention time of 2.131 and 2.816 minutes for Perphenazine and Amitriptyline respectively. The method was validated and the response was found to be linear in the drug concentration range of 6µg/mL to 14µg/mL for Perphenazine and 18µg/mL to 42µg/mL for Amitriptyline. The LOD and LOQ for Perphenazine were found to be 0.4µg/mL and 0.12µg/mL respectively. The LOD and LOQ for Amitriptyline were found to be 0.07µg/mL and 0.21µg/mL respectively. This method was found to be good %recovery for Perphenazine and Amitriptyline was found to be 100.415 and 100.264 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analytes in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for Linearity, Range, Accuracy, Precision, Specificity and Robustness.

Keywords: Perphenazine and Amitriptyline, HPLC, Method Development, Validation

Introduction

An antipsychotic phenothiazine derivative with actions and uses similar to those of chlorpromazine. Perphenazine [1] is a phenothiazine derivative and a dopamine antagonist with antiemetic and antipsychotic properties. Perphenazine blocks postsynaptic dopamine 2(D2) receptors in the mesolimbic and medullary chemoreceptor trigger zone (CTZ), thereby preventing the excess of dopamine in the brain. This leads to reduction in psychotic symptoms, such as hallucinations and delusions. Perphenazine [2] appears to exert its antiemetic activity by blocking the dopamine and histamine-1 receptor in the CTZ thereby relieving nausea and vomiting in the brain. In addition, Perphenazine binds to alpha-adrenergic receptors. Perphenazine is a piperazinyl phenothiazine, acts on the central nervous system, and has a greater behavioral potency than other phenothiazine derivatives whose side chains do not contain a piperazine moiety. It is a member of a class of drugs called phenothiazines, which are dopamine D1/D2 receptor antagonists. Perphenazine [3] is 10 to 15 times as potent as chlorpromazine; that means Perphenazine is a highly potent antipsychotic. In equivalent doses it has approximately the same frequency and severity of early and late extrapypramidal side-effects compared to Haloperidol. IUPAC Name of Perphenazine is 2-[4-[3-(2-chlorophenothiazin-10-yl) propyl] piperazin-1-yl] ethanol. The Chemical Structure of Perphenazine is.

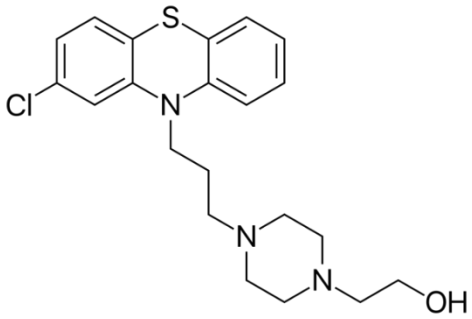

Fig 1: Chemical Structure of Perphenazine

Amitriptyline is a derivative of dibenzocycloheptadiene and a tricyclic antidepressant. Amitriptyline inhibits the re-uptake of norepinephrine and serotonin by the presynaptic neuronal membrane in the central nervous system (CNS), thereby increasing the synaptic concentration of norepinephrine and serotonin. Due to constant stimulation to these receptors, amitriptyline [4] may produce a down regulation of adrenergic and serotonin receptors, which may contribute to the antidepressant activity. Amitriptyline is a tricyclic antidepressant that is widely used in the therapy of depression. Amitriptyline [5] can cause mild and transient serum enzyme elevations and is rare cause of clinically apparent acute cholestatic liver injury. Effects in pain and depression: Amitriptyline is a tricyclic antidepressant and an analgesic. It has anticholinergic and sedative properties. Clinical studies have shown that oral amitriptyline achieves, at a minimum, good to moderate response in up to 2/3 of patients diagnosed with post-herpetic neuralgia and 3/4 of patients diagnosed with diabetic neuropathic pain, and neurogenic pain syndromes that are frequently unresponsive to narcotic analgesics. Amitriptyline [6] has also shown efficacy in diverse groups of patients with chronic non-malignant pain. There have also been some studies showing efficacy in managing fibromyalgia (an off-label use of this drug). The IUPAC Name of Amitriptyline is N, N-dimethyl-3-(2-tricyclo [9.4.0.03, 8] pentadeca-1(15), 3, 5, 7, 11, 13-hexa enylidene) propan-1-amine. The Chemical Structure of Amitriptyline is following.

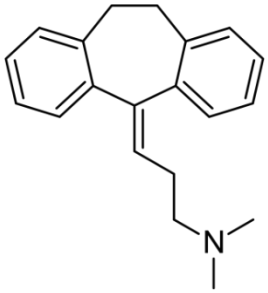

Fig 2: Chemical Structure of Amitriptyline

Literature survey [36-40] reveals that few analytical methods are available for simultaneous estimation of Perphenazine and Amitriptyline. But there is no analytical method for the determination of Perphenazine and Amitriptyline from its pharmaceutical dosage form. Due to lack of published chromatographic method and existing methods are time consuming and complex, the present work was aimed to develop a rapid, new, economical, precise and accurate method for the simultaneous determination of Perphenazine and Amitriptyline from its pharmaceutical dosage form.

Experimental

Table 1: List of Instrument used

S. No.	Instruments/Equipment/Apparatus
1.	HPLC with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	T60-LAB INDIA UV – Vis spectrophotometer
3.	Electronic Balance (SHIMADZU ATY224)
4.	Ultra Sonicator(Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.
7.	pH Analyzer (ELICO)
8.	Vacuum filtration kit (BOROSIL)

Table 2: List of Chemicals used

S.N.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	HPLC Grade Water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
3.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
4.	Hydrochloric Acid	99.9	A.R.	Sd fine-Chem ltd; Mumbai
5.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
6.	Sodium Hydroxide	99.9	A.R.	Sd fine-Chem ltd; Mumbai

Selection of Wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. It is scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Perphenazine & Amitriptyline, so that the same wave number can be utilized in HPLC UV detector [7] for estimating the Perphenazine & Amitriptyline. The scanned UV spectrum is attached in the following page.

Preparation of Mobile Phase

The mobile phase was prepared with the combination of Phosphate Buffer (0.2 M, pH=2) and Acetonitrile at the volume of 1000ml. 640ml of Phosphate Buffer and 360ml of Acetonitrile were mixed well and degassed [8] in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

Preparation of Standard Solutions

10 mg of Perphenazine & Amitriptyline was weighed accurately and transferred into 100 ml volumetric flask. About 10 ml mobile phase was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 10µg/ml and 10µg/ml of Perphenazine & Amitriptyline respectively.

Optimization of Chromatographic Conditions

The chromatographic conditions [9] were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

Table 3: Different Chromatographic used and their Optimizations

S. No.	Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
1	Symmetry C_{18}, 5µm, 25cmx4.6mm i.d.	ACN : Water = 70 : 30	0.8ml/min	265nm	Peaks did not separate	Method rejected
2	Waters C_{18}, 5µm, 25cmx4.6mm i.d.	Methanol: ACN = 40 :60	1.0 ml/min	265nm	Early elution of peak	Method rejected
3	Waters C_{18}, 5µm, 25cmx4.6mm i.d.	ACN: Phosphate buffer (0.02M) = 70:30	1.0 ml/min	265nm	Low resolution peak	Method rejected
4	Develosil ODS HG-5 RP C_{18}, 5µm,15cmx4.6mm i.d.	Phosphate buffer :Acetonitrile (0.01M) = 50:50	1.0 ml/ min	265nm	Resolution increases but Peak shapes not good	Method rejected
5	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.	Phosphate Buffer (0.2 M, pH=2) : Acetonitrile = 64:36	1.0 ml/min	265nm	Nice resolution & good peaks	Method Accepted

Method Validation

Analytical method validation [10-13] is the process of demonstrating that analytical procedures are suitable for their intended use. More specifically, analytical method validation is a matter of establishing documented evidence that the specified method will consistently provide accurate test results that evaluate a product against its defined specification and quality attributes. The method should be validatable, transferable, robust, reliable, accurate and precise for day to-day activities in the Quality Control environment. The method should not enter the validation phase unless it is fully developed. Validation experiments must be properly documented and performed on qualified and calibrated instrumentation and equipment.

System Suitability

System suitability is defined by ICH [35] as "the checking of a system, before or during the analysis of unknowns, to ensure system performance." System suitability criteria may include such factors as plate count, tailing, retention, and/or resolution. System suitability [14] criteria should also include a determination of reproducibility (%RSD) when a system suitability "sample" (a mixture of main components and expected by-products/interferences) is run.

Specificity

Definition: The ability [15] to assess unequivocally the analyte in the presence of components that may be expected to present, such as impurities, degradation products and matrix components, etc.

Accuracy

Definition: It is the closeness of agreement between the actual value and measured value. Accuracy [16] is calculated as the percentage of recovery by the assay of the known added amount of the analyte in the sample or the difference between the mean and accepted true value together with confidence intervals.

The ICH guidance [17] recommended to take a minimum of 3 concentration levels covering the specified range and 3 replicates of each concentration are analyzed (totally 3 * 3 = 9 determination).

Precision

Definition: The closeness of agreement between a series of measurements multiple samplings of the same homogeneous sample under prescribed condition. The precision [18] of test method is usually expressed as the standard deviation or relative standard deviation of a series of measurements.

Precision may be considered at three levels: Repeatability, Intermediate Precision and reproducibility.

Linearity and Range

Linearity

The linearity [19] of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Range

The range [20] of analytical procedure is the interval between the upper and lower concentrations of analyte in the analytical procedure has a suitable level of precision, accuracy, and linearity.

Detection Limit

Definition: It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated [21] under the stated experimental conditions.

Quantitation Limit

Definition: It is lowest amount of analyte in a sample, which can be quantitatively [22] determined with acceptable accuracy and precision.

Results and Discussion

Development of Method

Selection of Wavelength

While scanning the Perphenazine solution we observed the maxima at 275nm and for the Amitriptyline solution we observed the maxima at 248nm. The isobestic point [23] for the drugs was found at 265nm. The UV spectrum has been recorded on T60-LAB INDIA make UV-Vis spectrophotometer model UV-2450.

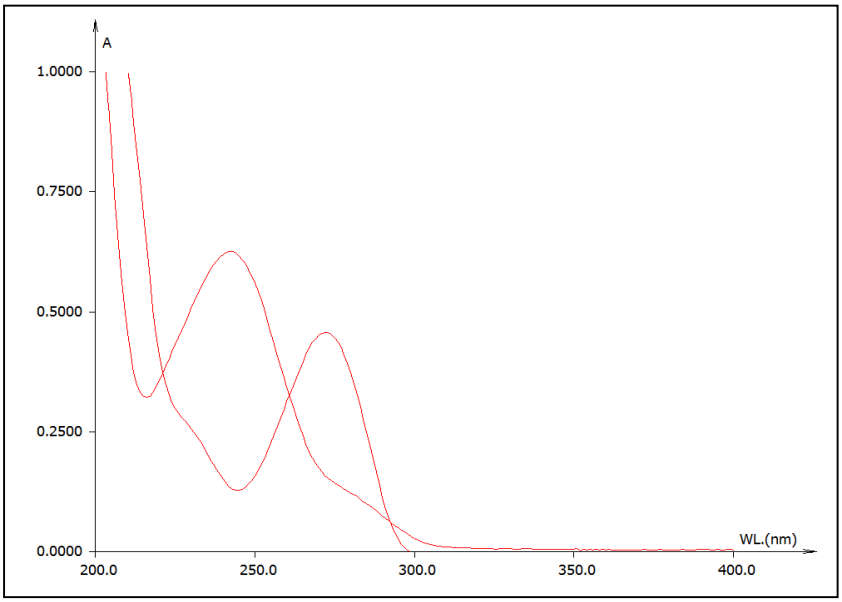

Fig 3: Isobestic point Perphenazine & Amitriptyline

Summary of optimized chromatographic conditions

The Optimum conditions [24] obtained from experiments can be summarized as below:

Table 4: Summary of Optimised Chromatographic Conditions

Mobile phase	**Phosphate Buffer (0.2 M, pH=2): Acetonitrile = 64:36% v/v**
Column	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.
Column Temperature	Ambient
Detection Wavelength	265 nm
Flow rate	1.0 ml/ min.
Run time	10 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	10µl
Type of Elution	Isocratic

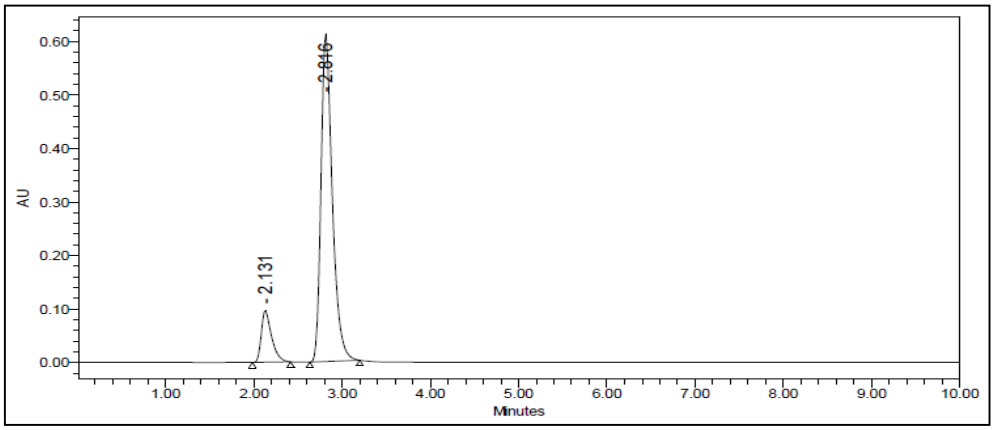

Fig 4: Chromatogram for Optimized Chromatographic Condition

Method Validation

1. Linearity and Range

Method: To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 6-14µg/ml for Perphenazine and concentration ranging from 12-28µg/ml for Amitriptyline. The prepared solutions were filtered through Whatman filter paper (No.41). From these solutions, 10µl injections of each concentration were injected into the HPLC system [25] and chromatographed under the optimized conditions. Calibration curve was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

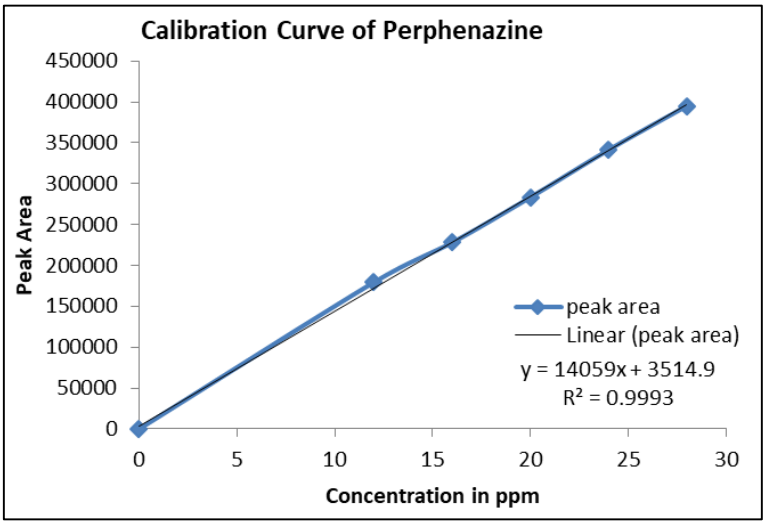

Fig 5: Standard Curve for Perphenazine

Table 5: Linearity Results for Perphenazine

CONC. (µg/ml)	AUC (n=6)
0	0
6	119571
8	167873
10	211264
12	255428
14	299987

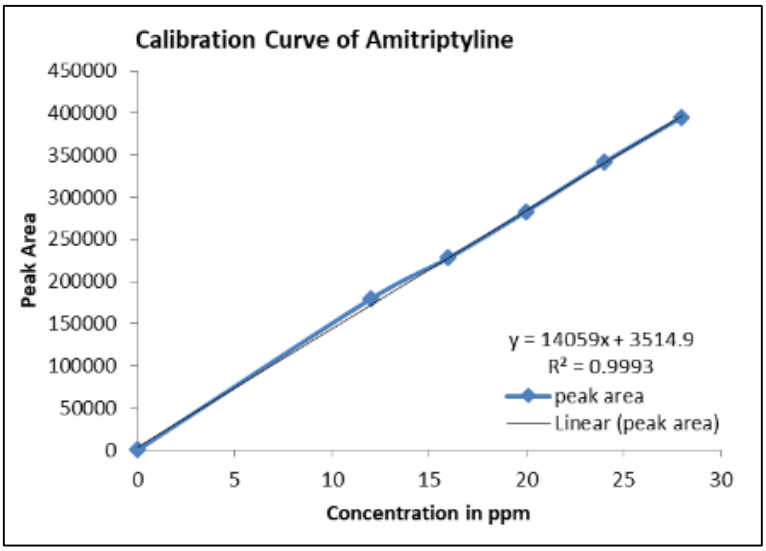

Fig 6: Standard Curve for Amitriptyline

Table 6: Linearity Results for Amitriptyline

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
12	179371
16	227893
20	283264
24	341428
28	394987

Results & Discussion

Linearity range was found to be 6-14 µg/ml for Perphenazine. The correlation coefficient was found to be 0.999, the slope was found to be 14059 and intercept was found to be 3514 for Perphenazine.

Linearity range [26] was found to be 12-28 µg/ml for Amitriptyline. The correlation coefficient was found to be 0.999, the slope was found to be 14059 and intercept was found to be 3514 for Amitriptyline.

2. Accuracy

Recovery study: For Perphenazine

To determine the accuracy of the proposed method, recovery studies [27] were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Perphenazine were taken and added to the pre-analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The results were shown in table-7.

Table 7: Accuracy Readings for Perphenazine

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	8	7.997368	115949	99.9671	Mean= 100.7003% S.D. = 0.6884036 % R.S.D.= 0.683616%
S_2 : 80 %	8	8.106622	117485	101.3328	
S_3 : 80 %	8	8.064087	116887	100.8011	
S_4 : 100 %	10	9.904901	142767	99.04901	Mean= 100.36157% S.D. = 1.346221 R.S.D.= 1.3413706%
S_5 : 100 %	10	10.02966	144521	100.2966	
S_6 : 100 %	10	10.17391	146549	101.7391	
S_7 : 120 %	12	12.01807	172476	100.1506	Mean= 100.183756% S.D. = 1.19411 % R.S.D. = 1.19191%
S_8 : 120 %	12	11.88079	170546	99.00657	
S_9 : 120 %	12	12.16729	174574	101.3941	

Observation : From the Accuracy Method, we observed that the mean %Recovery of the drug are 100.7003%, 100.36157% and 100.183756% which is within the range of 98-102% and %RSD is within the range <2 i.e. 0.683616%, 1.3413706% and 1.19191% respectively.

Recovery study: Amitriptyline

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Amitriptyline were taken and added to the pre-analysed formulation of concentration 50µg/ml. From that percentage recovery values were calculated. The results were shown in table-8.

Table 8: Accuracy Results for Amitriptyline

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	16	16.08685	229679	100.5428	Mean= 100.54488% S.D. = 0.97847% R.S.D.= 0.9731%
S_2 : 80 %	16	15.93079	227485	99.56745	
S_3 : 80 %	16	16.2439	231887	101.5244	
S_4 : 100 %	20	20.07632	285767	100.3816	Mean= 99.97095% S.D. = 0.395406 % R.S.D.= 0.39552%
S_5 : 100 %	20	19.98769	284521	99.93847	
S_6 : 100 %	20	19.91856	283549	99.59279	
S_7 : 120 %	24	23.75432	337476	98.97634	Mean= 100.27718% S.D. = 1.21262 % R.S.D. = 1.20927%
S_8 : 120 %	24	24.11494	342546	100.4789	
S_9 : 120 %	24	24.33032	345574	101.3763	

Observation : From the Accuracy Method, we observed that the mean %Recovery of the drug are 100.54488%, 99.97095% and 100.27718% which is within the range of 98-102% and %RSD is within the range <2 i.e. 0.9731%, 0.39552% and 1.20927% respectively.

3. Precision

Repeatability: The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug Perphenazine & Amitriptyline (API). The percent relative standard deviation [28] was calculated for Perphenazine & Amitriptyline are presented in the table-9.

Table 9: Data showing repeatability analysis for Perphenazine & Amitriptyline

HPLC Injection Replicates	AUC for Perphenazine	AUC for Amitriptyline
Replicate – 1	113568	241022
Replicate – 2	113241	240137
Replicate – 3	115408	242911
Replicate – 4	117412	245245
Replicate – 5	112541	241941
Replicate – 6	112546	240444
Average	**114119.3333**	**241356.6667**
Standard Deviation	**1925.83838**	**1416.95812**
% RSD	**1.68756**	**0.58708**

Result & Discussion: The repeatability study which was conducted on the solution having the concentration of about 10µg/ml for Perphenazine and 20µg/ml for Amitriptyline (n =6) showed a RSD of 1.68756% for Perphenazine and 0.58708% for Amitriptyline. It was concluded that the analytical technique showed good repeatability.

Intermediate precision

The Intermediate Precision [29] consists of two methods:-

Intra Day: In Intra Day process, the 80%, 100% and 120% concentration are injected at different intervals of time in same day.

Inter Day: In Inter Day process, the 80%, 100% and 120% concentration are injected at same intervals of time in different days.

Table 10: Data for Perphenazine analysis

Conc. of Perphenazine (API) (µg/ml)	Observed Conc. of Perphenazine (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=3)	% RSD	Mean (n=3)	% RSD
8	8.17	0.35	8.28	0.48
10	10.19	0.56	10.66	0.65
12	12.26	0.76	12.56	0.46

Table 11: Data for Amitriptyline analysis

Conc. Of Amitriptyline (API) (µg/ml)	Observed Conc. of Amitriptyline (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=3)	% RSD	Mean (n=3)	% RSD
16	16.33	0.24	16.56	0.33
20	20.56	0.48	20.76	0.67
24	24.23	0.63	24.63	0.43

Observations: The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Perphenazine and Amitriptyline revealed that the proposed method is precise.

4. Limit of detection and limit of quantification

The LOD was found to be 0.04µg/ml and LOQ was found to be 0.12µg/ml for Perphenazine respectively which represents that sensitivity of the method is high.

The LOD was found to be 0.07µg/ml and LOQ was found to be 0.21µg/ml for Amitriptyline respectively which represents that sensitivity of the method is high.

5. Method Robustness

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Wavelength of detection (±2nm) & organic phase content in mobile phase (±2%) studied to determine the robustness [30] of the method are also in favour of (Table-12, % RSD < 2%) the developed RP-HPLC method for the analysis of Perphenazine (API).

Table 12: Result of Method Robustness Test for Perphenazine

Change in parameter	% RSD
Flow (0.8 ml/min)	0.23
Flow (1.2 ml/min)	0.39
More Organic	0.83
Less Organic	0.76
Wavelength of Detection (277 nm)	0.56
Wavelength of detection (273 nm)	0.43

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Wavelength of detection (±2nm) & organic phase content in mobile phase [31] (±2%) studied to determine the robustness of the method are also in favour of (Table-13, % RSD < 2%) the developed RP-HPLC method for the analysis of Amitriptyline (API).

Table 13: Result of Method Robustness Test for Amitriptyline

Change in parameter	% RSD
Flow (0.8 ml/min)	0.37
Flow (1.2 ml/min)	0.57
More Organic	0.76
Less Organic	0.53
Wavelength of Detection (250 nm)	1.21
Wavelength of detection (246 nm)	0.39

6. System Suitability Parameter

System suitableness testing [32-33] is associate degree integral a part of several analytical procedures. The tests are supported the idea that the instrumentality, physics, associate degree analytical operations and samples to be analyzed represent an integral system which will be evaluated intrinsically. Following system suitableness take a look at parameters were established. The information is shown in Table-14.

Table 14: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs> 2	2.57
2	Asymmetry	T ≤ 2	Perphenazine = 0.46 Amitriptyline = 0.77
3	Theoretical plate	N > 2000	Perphenazine = 2946 Amitriptyline = 3076

7. Estimation of Perphenazine and Amitriptyline in Pharmaceutical Dosage Form

Twenty Tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 25 mg of drugs were transferred to 25 ml volumetric flask, make and solution was sonicated for 15 minutes, there after volume was made up to 25 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with mobile phase. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. The solution prepared was injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-15.

Assay

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak Area of drug obtained with test preparation
AS = Peak Area of drug obtained with standard preparation
WS = Weight of working standard taken in mg
WT = Weight of sample taken in mg
DS = Dilution of Standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard

Table 15: Recovery Data for estimation Perphenazine and Amitriptyline

Brand name of Perphenazine and Amitriptyline	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Perphenazine and Amitriptyline USP Tablets	2mg/10mg	1.956 (±0.422) / 9.878 (± 0.372)	99.5 (±0.576) / 99.4 ± 0.822)

Result & Discussion: The assay [34] of Perphenazine and Amitriptyline USP Tablets containing 2mg of Perphenazine & 10mg of Amitriptyline was found to be 99.5% and 99.4% respectively.

Stability Studies

Results of Degradation Studies

The results of the forced degradation studies indicated the specificity of the developed method that has been developed. Perphenazine and Amitriptyline were stable only in acidic, basic and thermal stress conditions and photolytic stress conditions. The results of stability studies are given in the following Table-16.

Table 16: Results of Force Degradation Studies of Perphenazine and Amitriptyline API

Stress Condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	95.62	4.38	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	97.13	2.87	100.00
Thermal Degradation (60 ^{0}C)	24Hrs.	96.24	3.76	100.00
UV (254nm)	24Hrs.	95.43	4.57	100.00
3% Hydrogen peroxide	24Hrs.	96.16	3.84	100.00

Summary and Conclusion

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Perphenazine and Amitriptyline, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution.

In case of RP-HPLC various columns are available, but here Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good. Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Perphenazine and Amitriptyline it is evident that most of the HPLC work can be accomplished in the wavelength range of 200-300 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 10µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay which can help in the analysis Perphenazine and Amitriptyline in different formulations.

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Perphenazine & Amitriptyline in bulk and pharmaceutical dosage form. Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Perphenazine & Amitriptyline indicated that the developed method is specific for the simultaneous estimation of Perphenazine & Amitriptyline in the bulk and pharmaceutical dosage forms. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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