RESEARCH ARTICLE

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Method Development and Validation for the Simultaneous Determination of Perphenazine and Amitriptyline in Pure and Marketed Pharmaceutical Dosage Form by using RP-HPLC

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ABSTRACT

An uncomplicated, precise, rapid, selective, and stable reversed-phase high-performance liquid chromatographic (RP-HPLC) technique has been developed and validated for the concurrent quantification of Perphenazine (PPZ) and Amitriptyline (AMT) in pure and its pharmaceutical dosage form. The method is based on Phenomenex Gemini C18 (4.6×250mm) 5µ column. Separation is accomplished by pumping a 65:35% v/v mixture of methanol (MeOH) and TEA buffer at a rate of 1.0 mL/min, followed by UV detection at 230 nm. The study is performed with the column set at 40 °C. The total run time is about 6 min. According to the ICH guidelines, the approach has been verified for accuracy, specificity, precision, robustness, linearity, ruggedness, the limit of detection (LoD), the limit of quantification (LoQ), and system suitability. The technique showed accuracy and linearity for determining PPZ and AMT between 10–50 µg/mL and 20–100 µg/mL, respectively. The average %recovery (100.37% for PPZ and 100.34% for AMT), ruggedness (<2%), and robustness are proven to provide good outcomes. This technique's benefits include strong resolution with distinct peaks and adequate precision. The outcomes show that the approach is appropriate for regular quality control analysis of commercial pharmaceutical formulations.

Keywords: Perphenazine, Amitriptyline, RP-HPLC, ICH Guidelines, Accuracy, Precision.

1. INTRODUCTION

Reversed-phase HPLC (RP-HPLC) is based on a stationary and mobile phase. In the process of RP-HPLC, compounds are separated as per their hydrophobic nature.[1] The solute particle from the solvent system binds to the adsorbed hydrophobic ligands attached to the sorbent in a hydrophobic manner, causing their separation.[2,3] In the presence of water-based buffers, the dissolved solute is initially put onto the stationary phase; the solutes are then extracted by adding a solvent to the mobile phase. Either gradient setting, in which the level of the organic phase is raised progressively over time, or isocratic elution, in which the quantity of organic phase is constant, can be used to elute.[4-6]

Lastly, the proposed methodology needs to be as uncomplicated as possible and should permit the utilization of complex tools like computational modeling. A good method development plan must only involve as many experimental trials as are required to obtain the intended end outcome.[7] To produce a valid quantitative method, several crucial considerations must be made, including thorough sample preparation and sampling, selecting the right column, etc.[8,9]

Perphenazine (PPZ) is a type of phenothiazine and is employed as an antipsychotic drug, still infrequently used in medical practice.[10-12] PPZ could occasionally cause temporary serum enzyme increase and rarely results in medically evident acute and chronic cholestatic hepatotoxicity. It exhibits activities and uses comparable to those of other antipsychotics.[13] Amitriptyline hydrochloride, also marketed under the brand name Elavil, is an antidepressant with antinociceptive effects that are frequently employed for treating depression and nociceptive pain.[14–16] Sandoz developed the drug, which received FDA approval in 1977.

The present study was aimed at method development and validation of RP-HPLC for concurrent quantitation of Perphenazine and Amitriptyline in pure and marketed forms.

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2. MATERIALS AND METHODS

2.1 Materials

Perphenazine and amitriptyline were obtained from Sura labs, Hyderabad, Telangana. Water, methanol, and acetonitrile for HPLC were obtained from MERCK, USA.

Apparatus used: HPLC (WATERS, software: Empower 2, Alliance 2695 separation module. 996 PDA detector), Weighing machine (Sartorius), pH meter (Lab India), Volumetric flasks, pipettes, burettes, and beakers (Borosil), Digital ultra sonicator (Labman).

2.2 Methodology

2.2.1 *Method Development*

Standard solution-preparation

10 mg of PPZ and AMT working standards each were taken in separate volumetric flasks of 10 mL, and to each of them, 7 mL of methanol (MeOH) was added, followed by sonication to mix the components and eliminate gas bubbles thoroughly. The volume was increased up to the mark by further adding MeOH 0.3 mL and 0.6 mL of above prepared PPZ and AMT solutions, respectively, were diluted to 10 mL by addition of MeOH.

Procedure

The prepared solutions of PPZ and AMT were incorporated into the HPLC column under different chromatographic settings, and their respective chromatograms were obtained. The chromatographic conditions under which better peaks were obtained were set as optimized conditions and used for further validation of the developed chromatographic technique, following ICH standards.

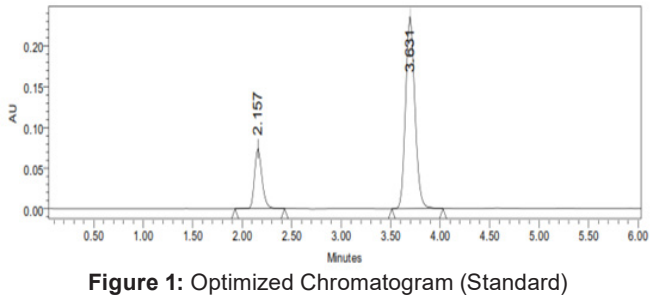

Figure 1: Optimized Chromatogram (Standard)

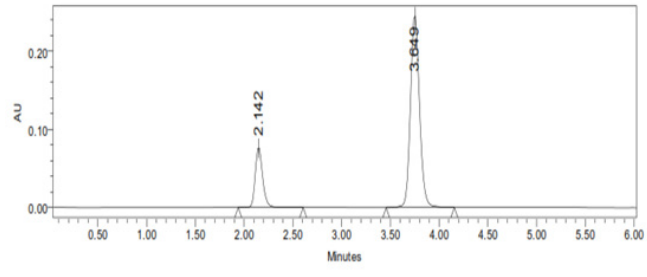

Figure 2: Optimized Chromatogram (Sample)

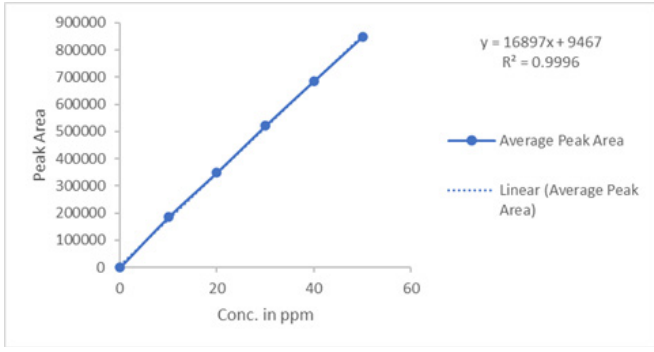

Figure 3: Calibration Curve of PPZ

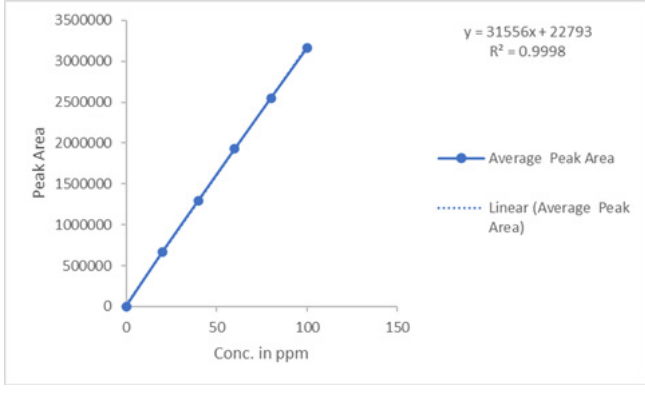

Figure 4: Calibration Curve of AMT

Mobile Phase Optimization

Different compositions with MeOH, buffer, water, and ACN were used to make up the mobile phase, and the one that showed the best results was selected as the final mobile phase composition. Triethylamine (TEA): MeOH in a 65:35 ratio was selected as the optimized mobile phase.

Optimization of Column

The technique was conducted by employing different C18 columns like Symmetry, X terra, and ODS column. Phenomenex Gemini C18 (4.6×250mm) 5µ showed better resolution as well as peaks, hence was selected as the optimized column for the technique.

2.2.2 *Method Validation*

Preparation of Mobile Phase and Stock Solutions

Preparation of TEA buffer (pH-4.0)

6 mL of TEA was added to a 1000 mL volumetric flask followed by the addition of 750 mL of water. The volume was brought up to the mark by further adding water and the pH was set to 4 by the addition of orthophosphoric acid. The solution was filtered and sonicated.

Preparation of mobile phase

350 mL (35%) of TEA buffer and 650 mL of HPLC methanol (65%) were taken in a flask, stirred, and sonicated to

eliminate gas bubbles with the help of an ultrasonicator. The solution was passed through a 0.45 µ membrane filter.

Diluent Preparation

The optimized mobile phase served as a diluent (dilutant).

Preparation of Standard Solution

10 mg of PPZ and AMT working standards each were taken in separate volumetric flasks of 10 mL. To each of them, 7 mL of MeOH was added, followed by sonication to mix the components and eliminate gas bubbles thoroughly. The volume was increased up to the mark by further adding MeOH

0.3 mL and 0.6 mL of above prepared PPZ and AMT solutions, respectively, were diluted to 10 mL by the addition of MeOH.

Preparation of Sample Solution

One tablet each of PPZ and AMT (of equal dose) was crushed in a mortar. From this powder, 10 mg equivalent weight of PPZ and AMT were taken into separate volumetric flasks followed by the addition of 7 mL of diluent and sonicated until it dissolves fully. The same solvent was added to bring the volume to the mark. The solution was filtered with the help of a 0.45 µ pore-sized filter.

0.3 mL and 0.6 mL of above prepared PPZ and AMT solutions, respectively, were diluted to 10 mL by the addition of MeOH.

2.2.3 *Validation Parameters*

2.2.3.1. System Suitability

The previously prepared standard solution was incorporated into the column 5 times & the area obtained was noted. The %RSD obtained for the five values of the area should not exceed the specified range.

2.2.3.2. Specificity Study of Drug:

Three injections of sample and standard solutions each were run into the column and the % assay was computed with the help of below formula:

$$\%Assay = \frac{Sample\ area}{standard\ area} \times \frac{Weight\ of\ standard}{Dilution\ of\ standard} \times \frac{Dilution\ of\ sample}{Weight\ of\ sample} \times \frac{Purity}{100} \times \frac{Weight\ of\ tablet}{Label\ claim} \times 100$$

2.2.3.3. Linearity

Preparation of Drug Solutions for Linearity

Level – I (10 ppm of perphenazine and 20 ppm of amitriptyline)

0.1 mL and 0.2 mL of PPZ and AMT, respectively, were taken in a flask and diluted to 10 mL with the help of a dilutant and sonicate for air entrapment.

Level – II (20 ppm of perphenazine and 40 ppm of amitriptyline)

0.2 mL and 0.4 mL of PPZ and AMT, respectively, were taken in a flask and diluted to 10 mL with the help of a dilutant and sonicate for air entrapment.

Level – III (30 ppm of perphenazine and 60 ppm of amitriptyline)

0.3 mL and 0.6 mL of PPZ and AMT, respectively, were taken in a flask and diluted to 10 mL with the help of a dilutant and sonicate for air entrapment.

Level – IV (40 ppm of perphenazine and 80 ppm of amitriptyline)

0.4 mL and 0.8 mL of PPZ and AMT, respectively, were taken in a flask and diluted to 10 mL with the help of a dilutant and sonicate for air entrapment.

Level – V (50 ppm of perphenazine and 100 ppm of amitriptyline)

0.5 mL and 1 mL of PPZ and AMT, respectively, were taken in a flask and diluted to 10 mL with the help of a dilutant and sonicate for air entrapment.

Procedure

Each of the above-prepared solutions was injected into the column, and the peak areas obtained were noted. A graph with peak area (Y-axis) was plotted against concentration (X-axis), and the correlation coefficient was calculated from the graph.

2.2.3.4. Precision

The previously prepared standard solution was injected into the column 5 times, and the area obtained was noted.

Table 1: Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	TF	TP	Resolution
1	PPZ	2.157	526541	78564	1.62	5859	
2	AMT	3.631	1645875	265842	1.48	7965	9.9

Table 2: Optimized Chromatogram (Sample)

S.No.	Name	Rt	Area	Height	TF	TP	Resolution
1	PPZ	2.142	538954	79658	1.63	5986	
2	AMT	3.649	1658745	275854	1.49	8056	10.1

Table 3: System suitability for PPZ

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	TP	TF
1	PPZ	2.152	526856	78569	1.63	5856
2	PPZ	2.157	528794	78545	1.63	5874
3	PPZ	2.141	526598	78954	1.62	5869
4	PPZ	2.133	524875	78224	1.63	5897
5	PPZ	2.166	526584	78965	1.62	5829
Mean			526741.4			
Std. Dev.			1392.398			
% RSD			0.264342			

Table 4: System suitability for amitriptyline

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	TP	TF	Resolution
1	AMT	3.674	1645985	268542	5869	1.48	10.01
2	AMT	3.631	1648579	267854	5874	1.49	10.01
3	AMT	3.625	1645739	268598	5864	1.48	9.99
4	AMT	3.692	1645285	268745	5826	1.49	10.01
5	AMT	3.629	1648598	268598	5824	1.48	10.02
Mean			1646837				
Std. Dev.			1618.325				
% RSD			0.098269				

Table 5: Results for PPZ standard solution

S.No	Name	RT	Area	Height	TF	TP	Injection
1	PPZ	2.152	526595	78569	1.63	5896	1
2	PPZ	2.198	524658	78496	1.63	5879	2
3	PPZ	2.179	528476	78459	1.62	5895	3

Table 6: Results for AMT standard solution

S.No	Name	RT	Area	Height	TF	TP	Injection
1	AMT	3.646	1648546	265845	1.48	8012	1
2	AMT	3.604	1648598	265418	1.49	7955	2
3	AMT	3.610	1648574	265365	1.48	7989	3

Table 7: Results for PPZ sample solution

S.No	Name	RT	Area	Height	TF	TP	Injection
1	PPZ	2.152	536598	79856	1.64	5969	1
2	PPZ	2.150	536589	79265	1.65	5997	2
3	PPZ	2.187	534658	79898	1.65	5986	3

Table 8: Results for AMT sample solution

S.No	Name	RT	Area	Height	TF	TP	Injection
1	AMT	3.646	1658952	278598	1.49	8016	1
2	AMT	3.651	1658954	276984	1.48	8041	2
3	AMT	3.601	1653659	275849	1.49	8079	3

Table 9: Peak areas for PPZ

Conc. µg/mL	Average Peak Area
10	185689
20	349852
30	521541
40	685986
50	848265

Table 10: Peak areas for AMT

Conc. µg/ml	Average Peak Area
20	665985
40	1298698
60	1927852
80	2548545
100	3162468

Table 11: Repeatability for PPZ

S. No.	Peak name	RT	Area (µV*sec)	Height (µV)	TP	TF
1	PPZ	2.157	526854	78569	5869	1.62
2	PPZ	2.159	523659	78469	5874	1.63
3	PPZ	2.186	523856	78525	5896	1.63
4	PPZ	2.160	523485	78548	5818	1.62
5	PPZ	2.170	523485	78594	5879	1.63
Mean			524267.8			
Std.dev			1453.805			
%RSD			0.277302			

Table 12: Repeatability for Amitriptyline

S. No.	Peak name	RT	Area (µV*sec)	Height (µV)	TP	TF
1	AMT	3.603	1645879	265845	7985	5869
2	AMT	3.608	1648578	265487	7964	5849
3	AMT	3.600	1645985	265982	7915	5879
4	AMT	3.696	1648759	265478	7928	5874
5	AMT	3.629	1648572	265422	7964	5829
Mean			1647555			
Std.dev			1483.603			
%RSD			0.090049			

Table 13: Intermediate precision (Day 1) for PPZ

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	PPZ	2.198	536598	79584	5963	1.64
2	PPZ	2.196	536985	79685	5978	1.65
3	PPZ	2.160	534587	79654	5947	1.64
4	PPZ	2.160	536985	79845	5982	1.65
5	PPZ	2.160	536985	79864	5971	1.65
6	PPZ	2.186	538568	79685	5968	1.64
Mean			536784.7			
Std. Dev.			1277.909			
% RSD			0.238067			

Table 14: Intermediate precision (Day 1) for amitriptyline

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	AMT	3.623	1658254	266598	8036	1.50	10.06
2	AMT	3.611	1659872	266473	8045	1.51	10.04
3	AMT	3.696	1653589	266958	8075	1.50	10.05
4	AMT	3.696	1658458	266451	8049	1.50	10.06
5	AMT	3.696	1653652	266352	8069	1.50	10.05
6	AMT	3.642	1652395	266954	8024	1.51	10.06
Mean			1656037				
Std. Dev.			3175.804				
% RSD			0.191771				

The %RSD obtained for the five values of the area should not exceed the specified limits.

2.2.3.5. Ruggedness

To assess the ruggedness, precision was carried out on different days under the same chromatographic conditions. The previously prepared standard solution was incorporated into the column 6 times and the area obtained was noted. The procedure was repeated for two consecutive days. The %RSD obtained for the six values of the area should not exceed the specified limits.

Table 15: Intermediate precision Day 2 for PPZ

S.No	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USPPlate count	USPTailing
1	PPZ	2.198	519689	77859	5749	1.61
2	PPZ	2.196	518957	77985	5792	1.60
3	PPZ	2.178	519856	77854	5746	1.60
4	PPZ	2.142	519857	77869	5749	1.61
5	PPZ	2.177	519869	77935	5718	1.61
6	PPZ	2.177	519687	77954	5795	1.60
Mean			519652.5			
Std. Dev.			351.0976			
% RSD			0.067564			

Table 16: Intermediate precision Day 2 for AMT

S.No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	TP	TF	Resolution
1	AMT	3.611	1638598	256985	7968	1.47	9.90
2	AMT	3.623	1637849	257589	7952	1.46	9.91
3	AMT	3.684	1635982	256985	7934	1.46	9.90
4	AMT	3.697	1636598	254613	7986	1.47	9.90
5	AMT	3.684	1635874	258487	7924	1.46	9.91
6	AMT	3.684	1635984	259861	7915	1.47	9.91
Mean			1636814				
Std. Dev.			1145.885				
% RSD			0.070007				

Table 17: Accuracy of PPZ

%Conc.(at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	263572	15	15.038	100.253%	
100%	518870.3	30	30.147	100.490%	100.37%
150%	772572.3	45	45.162	100.360%	

Table 18: Accuracy for AMT

%Conc.(at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	972935.7	30	30.109	100.363%	
100%	1919319	60	60.100	100.166%	100.34%
150%	2877020	90	90.449	100.498%	

Table 19: Robustness of PPZ

Variation in chromatographic settings	Peak Area	RT	TP	TF
Flow at 1.0 mL/min	526541	2.157	5859	1.62
Flow at 0.9 mL/min	589564	2.210	5635	1.61
Flow at 1.1 mL/min	515246	2.184	5569	1.64
Less organic phase	502659	2.200	5154	1.63
More Organic phase	526485	2.172	5365	1.62

Table 20: Robustness of AMT

Variation in chromatographic settings	Peak Area	RT	TP	TF
Flow at 1.0 mL/min	1645875	3.643	7965	1.48
Flow at 0.9 mL/min	1635985	4.498	7856	1.46
Flow at 1.1 mL/min	1624587	3.505	7425	1.43
Less organic phase	1652834	4.504	7621	1.45
More organic phase	1625548	3.512	7582	1.42

2.2.3.6. Accuracy

Preparation of stock solutions for accuracy assay

50% standard stock solution

0.15 mL and 0.3 mL of PPZ and AMT, respectively, were taken from the previously prepared standard solutions diluted to 10 mL by adding a dilutant.

100% standard stock solution

0.3 ml and 0.6 mL of PPZ and AMT, respectively, were taken from the previously prepared standard solutions diluted to 10 mL by adding a dilutant.

150% standard stock solution

0.45ml and 0.9 mL of PPZ and AMT were taken from the prepared standard solutions diluted to 10 mL by adding a dilutant.

Procedure

Each standard concentration solution (50%, 100%, and 150%) was injected into the column three times while maintaining optimized settings. Chromatograms were produced and peak areas were analyzed. The amount of drug found/added was computed and mean recovery values were obtained from recovery values obtained after each run.

2.2.3.7. Robustness

To determine the degree of test result variability, the analysis was carried out under various chromatographic conditions. The influence of flow rate and mobile phase composition modification on outcomes was observed.

Change in flow rate

While maintaining all the other chromatographic settings optimized, the flow rate was changed to 0.9 mL/min and 1.1 mL/min. 10 µL of the sample was inserted into the column 2 times, and the chromatograms obtained were recorded.

Change in mobile phase composition:

While maintaining all the other chromatographic settings optimized, mobile phase composition was changed to MeOH: buffer in ratios of 60:40 and 70:30, and a chromatographic procedure was performed. 10 µL of the sample was inserted into the column 2 times, and chromatograms were obtained.

3. RESULTS

3.1 Optimized Chromatographic Conditions

Instrument used: Waters Alliance 2695 HPLC with PDA Detector 996 model.

Temperature	:	40ºC
Column	:	Phenomenex Gemini C18 (4.6×250mm) 5µ
Mobile phase	:	Methanol: TEA Buffer (65:35 v/v)
Flow rate	:	1-mL/min
Wavelength	:	230nm
Injection volume	:	10 µL
Run time	:	6 minutes

The chromatogram obtained for standard solutions of PPZ and AMT under optimized conditions is shown in Figure 1. The results for optimized chromatograms of PPZ and AMT standard solutions are tabulated in Table 1.

Observation

The above chromatogram revealed that peaks of PPZ and AMT are distinct, exhibiting appropriate retention period, sharpness, tailing factor (TF), and theoretical plate (TP).

The chromatogram obtained for sample solutions of PPZ and AMT under optimized conditions is shown in Figure 2. The results for optimized chromatograms of PPZ and AMT sample solutions are tabulated in Table 2.

Acceptance Criteria

- There should be at least 2 resolutions between any two medications.
- There should be at least 2000 TP.
- The TF must range between 0.9 and 2 but cannot be greater.

Based on the aforementioned criteria, it was determined that the proposed technique's system suitability characteristics were all within the acceptable range.

3.2 Method Validation

3.2.1 System Suitability

The results obtained for system suitability studies of PPZ are tabulated in Table 3 and those of AMT in Table 4.

Acceptance criteria

- The average % RSD of five sample solutions must not exceed 2.

Since the average %RSD falls within the acceptable range, the approach can be considered suitable.

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3.2.2 Specificity

Peak results for assay standards of PPZ and AMT are shown in Tables 5 and 6, respectively. Peak results for assay samples of PPZ and AMT are shown in Tables 7 and 8, respectively.

$$\%Assay = \frac{Sample\ area}{Standard\ area} \times \frac{Weight\ of\ standard}{Dilution\ of\ standard} \times \frac{Dilution\ of\ sample}{weight\ of\ sample} \times \frac{Purity}{100} \times \frac{Weight\ of\ tablet}{Label\ claim} \times 100$$

The % purity of PPZ and AMT in their pharmaceutical formulations was reported to be 99.63%

3.2.3 Linearity

The data obtained for the linearity study of PPZ and AMT are shown in Tables 9 and 10, respectively.

Linearity Plot

The calibration curve of PPZ is shown in Figure 3. The graph of PPZ with a concentration on X-axis and average peak area on Y-axis is a linear function.

Y = mx + c

Slope (m) =16897

Intercept (c) = 9467

Correlation Coefficient (r^2) = 0.99

The intercept is 9467 while r^2 is 0.99. Therefore, the validation criteria are met by these figures.

Linearity Plot

The calibration curve of PPZ is shown in Figure 4. The graph of AMT with a concentration on the X-axis and average peak area on the Y-axis is a linear function..

Y = mx + c

Slope (m) = 31556

Intercept (c) = 22793

Correlation Coefficient (r) = 0.99

The intercept is 22793, while r^2 is 0.99. Therefore, the validation criteria are met by these figures.

3.2.4 Repeatability

The findings obtained for repeatability studies of PPZ and AMT are tabulated in Tables 11 and 12, respectively.

Acceptance Criteria

- The %RSD must not exceed 2.

The standard solution's %RSD is less than 1, which is acceptable and indicates that the approach is repeatable.

3.2.5 Ruggedness

Results obtained for ruggedness (intermediate precision) on Day 1 for PPZ and AMT are shown in Tables 13 and 14. Tables 15 and 16 show the results obtained for the ruggedness of PPZ and AMT on the next day (Day 2), respectively.

Acceptance criteria

- The average %RSD of five sample solutions must not exceed 2.

%RSD was reported to be 0.238067, which is acceptable and confirms the ruggedness of the technique.

Acceptance criteria

- The average %RSD of five sample solutions must not exceed 2.

%RSD was reported to be 0.191771, which is acceptable and confirms the ruggedness of the technique.

Acceptance criteria

- The average %RSD of five sample solutions must not exceed 2.

Acceptance criteria

- The average %RSD of different samples must not exceed 2.

3.2.6 Accuracy

Tables 17 and 18 show results for accuracy studies of PPZ and AMT, respectively.

Acceptance Criteria

- The mean recovery was reported to fall within the acceptable range (98-102%).

The mean recovery was reported to fall within the acceptable range (98-102%), confirming the accuracy of the technique

3.2.7 Limit Of Detection

The minimum quantity of analyte present in a given sample, which is detectable, however, not always quantitated as an accurate value is the limit of detection (LOD) of a specific analytical method.

$$LOD = 3.3 \times \sigma / s$$

Where σ = Standard deviation of the response and S = Slope of the calibration curve

PPZ = 0.9μg/ml

AMT = 1.2μg/ml

3.2.8 Limit of Quantitation

The minimum quantity of analyte present in a sample capable of being quantitatively calculated is known as LoQ of a specific analytical technique.

$$LoQ = 10 \times \sigma / S$$

Where σ = Standard deviation of the response and S = Slope of the calibration curve

PPZ =2.7 μg/mL

AMT =3.6 μg/mL

3.2.9 Robustness

Tables 19 and 20 show the data obtained for robustness studies of PPZ and AMT, respectively.

Acceptance criteria

- TF must not exceed 2
- TP must not fall below 2000.

Under all conditions, TF and TP were within the acceptable range, confirming that the technique is robust for PPZ.

Acceptance criteria

- TF must not exceed 2
- TP must not fall below 2000.

Under all conditions, TF and TP were within the acceptable range, confirming that the technique is robust for AMT.

4. DISCUSSION

The chromatographic settings in the current study were adjusted to produce the best clarity and peak morphologies for PPZ and AMT. The suggested approach is favorable since it demonstrated good peak symmetry resolution, repeatability, effectiveness, and separation of target drugs. Additionally, no peaks of other excipients were discovered to interact with the peak of the drug compound, confirming no interference. Additionally, high-performance thin layer chromatography (HPTLC) is not as accurate as HPLC for chemical quantification.[17] In comparison to other approaches, this technique is significant in terms of selectivity and sensitivity.[18] The technique's lengthy run time is its major flaw. Given the benefits, this technique may be used to evaluate such drug molecules simultaneously, aiding in the standardization of polyherbal compositions as well as for usage in academic and industrial applications.

5. CONCLUSION

In the current paper, the RP-HPLC technique was developed for the concurrent determination of Perphenazine and Amitriptyline in pure and marketed forms. The RP-HPLC technique's findings, which were presented in tables, were acceptable. Comparing the RP-HPLC technique to spectroscopic approaches, the RP-HPLC technique seems to provide more sensitivity, accuracy, and precision. This technique may be employed to routinely determine the presence of perphenazine and amitriptyline in pharmaceutical dosage forms and bulk medications.

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