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METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF MIRTAZAPINE IN BULK FORM AND MARKETED PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC

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ABSTRACT : In the present work simple and precise Reversed Phase - High Performance Liquid Chromatography method have been developed for the estimation of Mirtazapine in bulk form and Marketed Pharmaceutical dosage form.The method developed for the determination of Mirtazapine in Mirtazapine in bulk form and Marketed Pharmaceutical dosage form involved using RP-HPLC which incorporated a Phenomenex Luna C18 (4.6×150mm, 5µm), in isocratic mode, with mobile phase comprising of Water: Methanol in the ratio of 55:45 (v/v). The flow rate was 0.9 mL/min and the detection was monitored at 225 nm. The total run time was 6.0 min and the column was maintained at 40ºC temperature.Mirtazapine was eluted in the given mobile phase with a retention time (tr) of 2.958 min. The linearity for the quantification of Mirtazapine was 5.0 – 25.0 µg/mL (R2 =0.9773, Y= 237972x + 521600) with coefficients of variation (based on mean peak area for six replicate injections) in the range 7.5 to 22.5. The limits of detection and of quantification were 1.4 and 4.3 µg/mL, respectively. Recovery of the method was found to be 99.8 % whiles the relative standard deviation (RSD) of intra-day and inter-day precision was 0.616 and 0.847, respectively. Method precision was found to be 0.194%, respectively. The proposed RP-HPLC method is simple, sensitive, rapid, cost-effective and accurate for the determination of Mirtazapine in both bulk materials and marketed pharmaceutical dosage forms.
Key Words: Mirtazapine, RP-HPLC, Accuracy, Precision, Linearity, ICH Guidelines.

1.INTRODUCTION

DRUG PROFILE

Drug: Mirtazapine [1-2]

Description: Mirtazapine is an antidepressant introduced by Organon International in 1996 used for the treatment of moderate to severe depression. Mirtazapine has a tetracyclic chemical structure and is classified as a noradrenergic and specific serotonergic antidepressant (NaSSA). It is the only tetracyclic antidepressant that has been approved by the Food and Drug Administration to treat depression.

Structure:

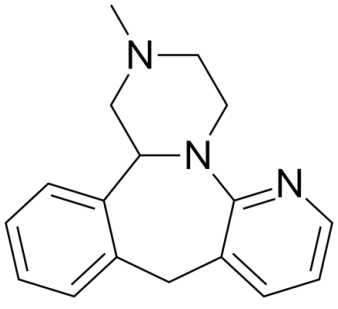


Fig-1.: Structure of Mirtazapine

IUPAC Name:5-methyl-2,5,19-triazatetracyclo [13.4.0.02,7.08.13] nonadeca-1(15),8,10,12,16,18-hexaene

2.AIM AND OBJECTIVE & PLAN OF WORK

AIM

The main aim of the present study is development of accurate, precise, sensitive, selective, reproducible and rapid analytical technique for cost effective estimation of Mirtazapine.Review of literature for Mirtazapine gave information regarding its physical and chemical properties, various analytical methods that were conducted alone and in combination with other drugs.

✓ Literature survey reveals that certain chromatographic methods were reported for simultaneous estimation of Mirtazapine.

✓ Validation is a necessary and important step in both framing and documenting the capabilities of the developed method.

✓ The utility of the developed method to determine the content of drug in commercial formulation was also demonstrated. Validation of the method was done in accordance with USP and ICH guideline for the assay of active ingredient.

✓ The method was validated for parameters like system suitability, linearity, precision, accuracy, specificity, ruggedness and robustness, limit of detection and limit of quantification. This method provides means to quantify the component. This proposed method was suitable for the analysis of Pharmaceutical dosage forms.

OBJECTIVES:

Following are the objectives of present work**:**

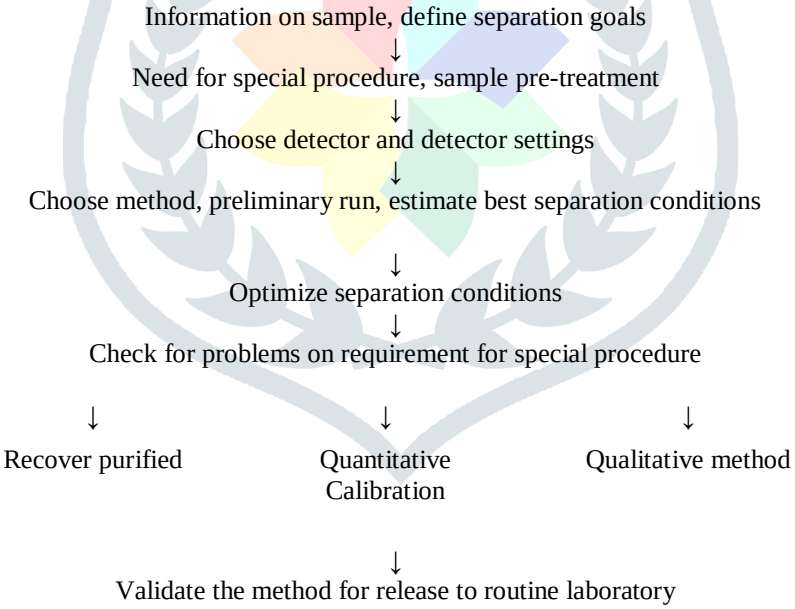
➢ To develop analytical method

- Selecting the HPLC separation mode
- Selecting/ optimizing the mobile phase
- Selecting column for analysis
- Selecting the appropriate detector system
- Selecting appropriate gradient/ isocratic medium
- Selecting appropriate flow rate, temperature and pH

➢ To validate different parameters.

PLAN OF WORK

Strategy for Method Development:



3.EXPERIMENTAL WORK

HPLC METHOD DEVELOPMENT:

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Mirtazapine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.Further pipette 0.15ml of the above Mirtazapine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

PREPARATION OF MOBILE PHASE:

Preparation of mobile phase:

Accurately measured 550ml (55%) of HPLC Water and 450ml of Methanol (45%) were mixed and degassed by sonication for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

SYSTEM SUITABILITY

Accurately weigh and transfer 10 mg of Mirtazapine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.15ml of the above Mirtazapine stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

3.1.1 SPECIFICITY STUDY OF DRUG:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Mirtazapine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.15ml of the above Mirtazapine stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution:

Take average weight of one Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight ofMirtazapine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.15ml of Mirtazapine above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

PREPARATION OF DRUGSOLUTIONS FOR LINEARITY:

Accurately weigh and transfer 10 mg of Mirtazapine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (5ppm of Mirtazapine):

Take 0.05ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – II (10 ppm of Mirtazapine):

Take 0.1ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – III (15ppm of Mirtazapine):

Take 0.15ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – IV (20ppm of Mirtazapine):

Take 0.2ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – V (25ppm of Mirtazapine):

Take 0.25ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Procedure:
Inject each level into the chromatographic system and measure the peak area.
Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION

REPEATABILITY

Preparation of Mirtazapine Product Solution for Precision:

Accurately weigh and transfer 10 mg of Mirtazapine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.15ml of the above Mirtazapine stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION:
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

DAY 1:
The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

DAY 2:
The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

ACCURACY:

For preparation of 50% Standard stock solution:
Accurately weigh and transfer 10 mg of Mirtazapine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.075ml of the above Mirtazapine stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% Standard stock solution:
Accurately weigh and transfer 10 mg of Mirtazapine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.15ml of the above Mirtazapine stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% Standard stock solution:
Accurately weigh and transfer 10 mg of Mirtazapine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.225ml of the above Mirtazapine stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:
Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Mirtazapine and calculate the individual recovery and mean recovery values.

ROBUSTNESS:
The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution:
Accurately weigh and transfer 10 mg of Mirtazapine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.15ml of the above Mirtazapine stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of flow conditions:
The sample was analyzed at 0.8ml/min and 1.0ml/min instead of 0.9ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded

Effect of Variation of mobile phase organic composition:
The sample was analyzed by variation of mobile phase i.e. Methanol: Water was taken in the ratio and 40:60, 50:50 instead of 45:55, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

4..RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Column : Phenomenex Luna C18 (4.6×150mm, 5µm)

Column temperature : 40ºC

Wavelength : 225nm

Mobile phase ratio : Water: Methanol (55:45%v/v) Flow rate : 0.9ml/min
Injection volume : 10 µl

Run time : 6min

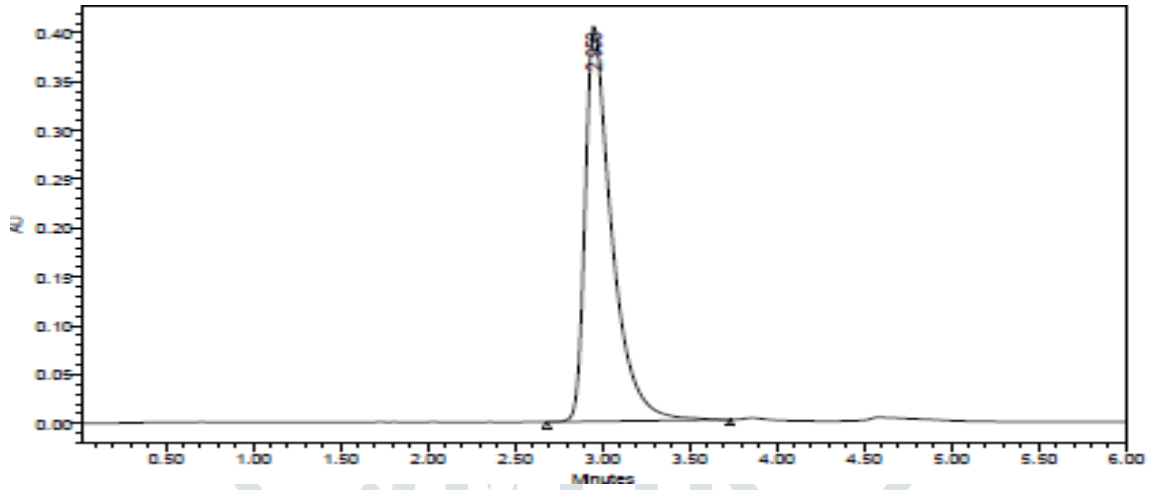


Fig-2: Optimized Chromatogram (Standard)

Table-1: Optimized Chromatogram (Standard)

S.No.	Name	RT	Area	Height	USPTailing	USPPlate Count
1	Mirtazapine	2.958	381656	38964	1.14	8947

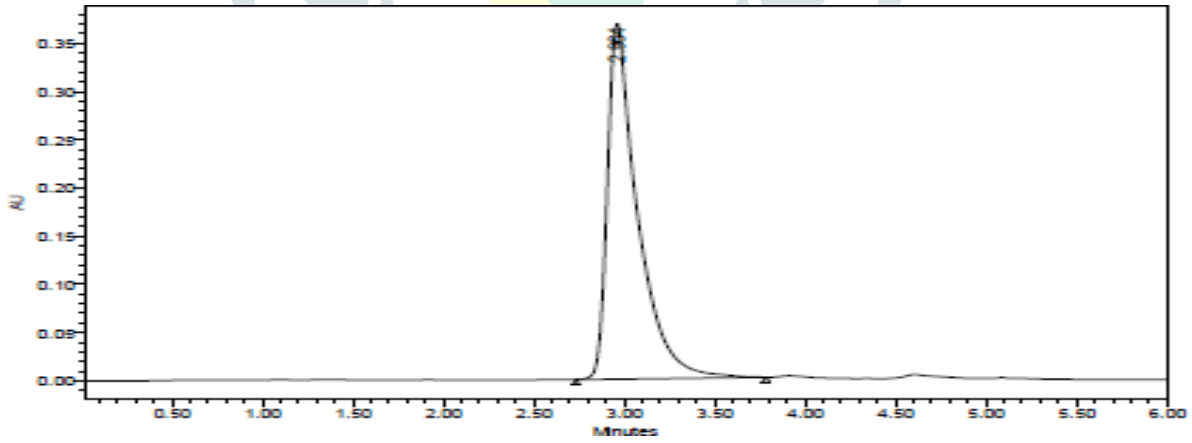


Fig-3: Optimized Chromatogram (Sample)

Table-2: Optimized Chromatogram (Sample)

S.No.	Name	RT	Area	Height	USP Tailing	USPPlate Count
1	Mirtazapine	2.964	4262917	41635	1.2	8846

Acceptance Criteria:

- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

METHOD VALIDATION Blank:

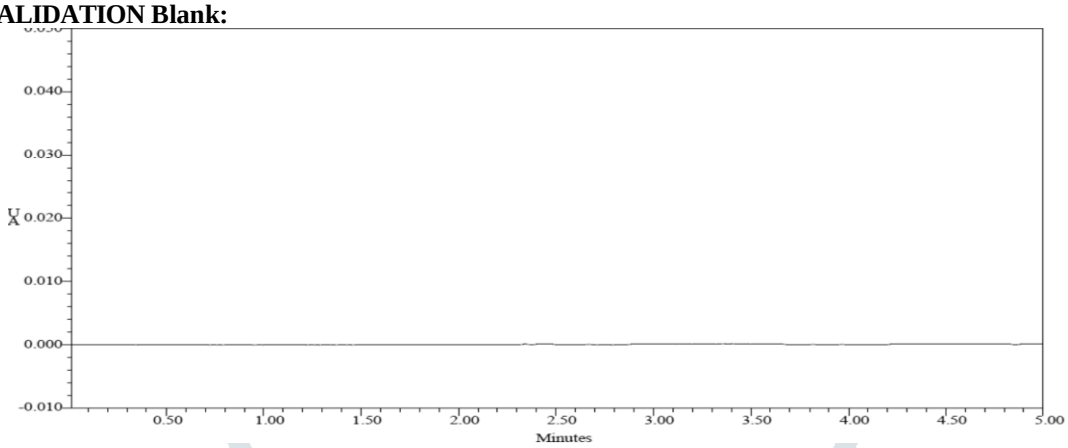


Fig-4: Chromatogram showing blank (mobile phase preparation)

Table-3: Results of system suitability for Mirtazapine

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Mirtazapine	2.269	1187187	159416	6622.7	1.4
2	Mirtazapine	2.264	1188125	161793	6758.1	1.5
3	Mirtazapine	2.267	1189202	161854	7700.8	1.4
4	Mirtazapine	2.270	1191196	159246	8619.9	1.5
5	Mirtazapine	2.262	1192867	162665s	5652.7	1.4
Mean			1189715			
Std.Dev.			2258.166			
%RSD			0.19401			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitates Mirtazapine in drug product.

Assay (Standard)

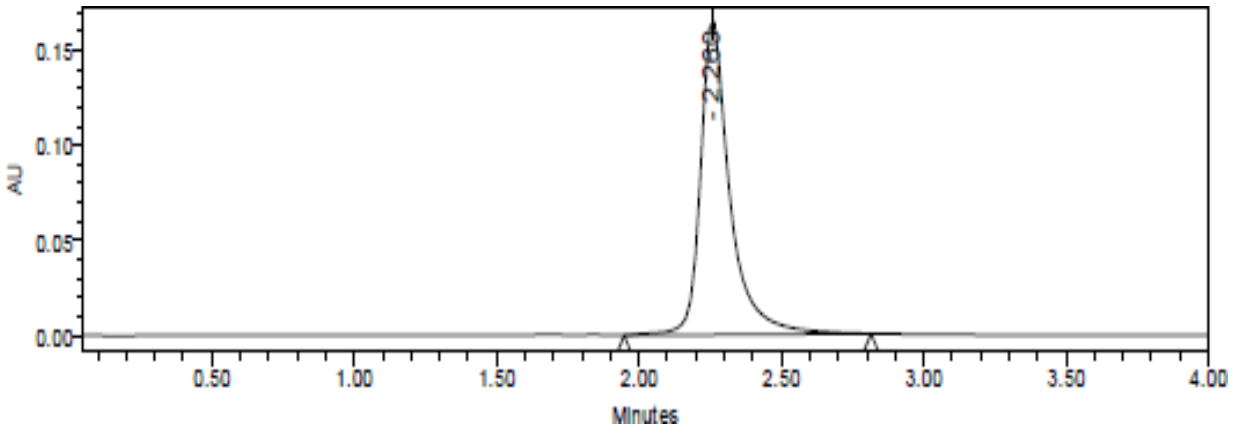


Fig-5: Chromatogram showing assay of standard injection

Table-4: Peak results for assay standard

S.No.	Name	RT	Area	Height	USPTailing	USPPlateCount	Injection
1	Mirtazapine	2.260	1194945	164458	1.4	8762.1	1
2	Mirtazapine	2.262	1199561	166111	1.4	7848.8	2
3	Mirtazapine	2.269	1194945	164458	1.4	7762.1	3

Assay (Sample):

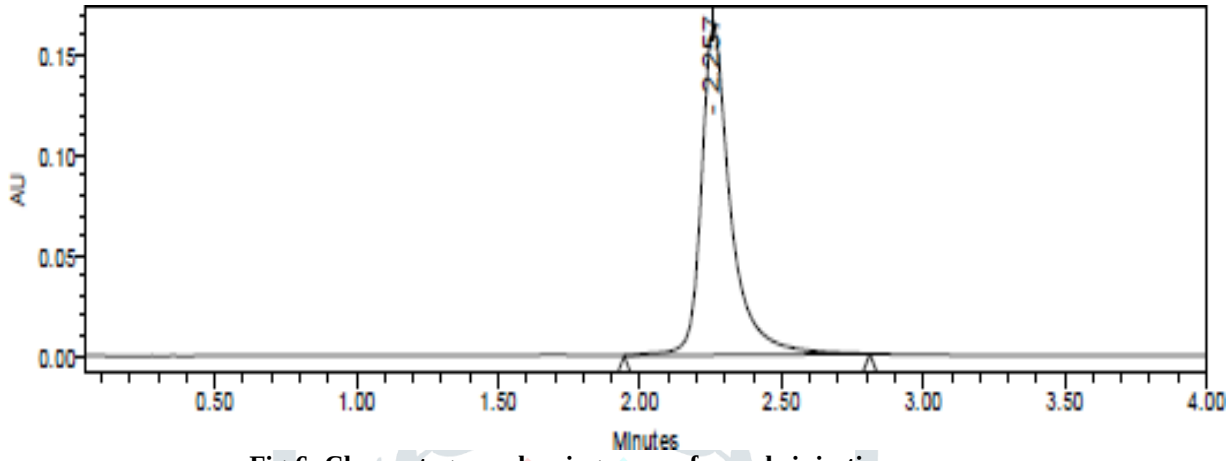


Fig-6: Chromatogram showing assay of sample injection

Table-5: Peak results for Assay sample

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Mirtazapine	2.257	1192479	166858	1.5	2807.6	1
2	Mirtazapine	2.262	1193260	165060	1.4	2833.0	2
3	Mirtazapine	2.264	1188125	161793	1.5	2758.1	3

The % purity of Mirtazapine in pharmaceutical dosage form was found to be 99.5%.

LINEARITY

Table-6: Linearity Data for Mirtazapine

Concentration Level (%)	**Concentration µg/ml**	**Average Peak Area**
60	5	1334772
80	10	2627131
100	15	3950788
120	20	5199091
140	25	6465680

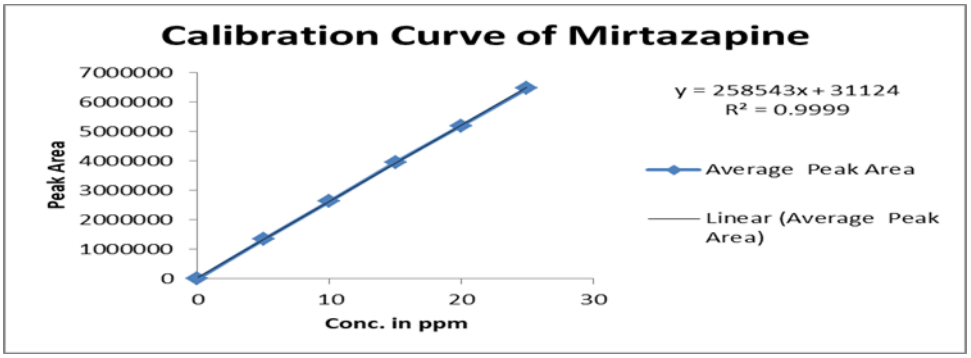


Fig-7: Calibration Curve of Mirtazapine

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Mirtazapine is a straight line.

Y = mx + c

Slope (m) = 258543 Intercept (c) = 31124

Correlation Coefficient (r) = 0.9999

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.9999, and the intercept is 31124. These values meet the validation criteria.

PRECISION:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-7: Results of Repeatability for Mirtazapine:

S. No.	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Mirtazapine	2.958	4278645	319736	6933	1.1
2	Mirtazapine	2.964	4262917	313772	7748	1.2
3	Mirtazapine	2.966	4268626	319741	9018	1.4
4	Mirtazapine	2.964	4284172	319747	7926	1.1
5	Mirtazapine	2.966	4272561	310061	8846	1.2
Mean			4273384			
Std.dev			8320.653			
%RSD			0.194709			

Acceptance Criteria:

- %RSD for sample should be not more then

The %RSD for the standard solution is below 1,

- which is within the limits hence method is precise.

Intermediate Precision:

Table-8: Results of Intermediate precision Day1 for Mirtazapine

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP PlateCount	USP Tailing
1	Mirtazapine	3.014	4257343	391742	6881	1.1
2	Mirtazapine	3.018	4286479	399167	9971	1.2
3	Mirtazapine	3.022	4215267	399765	70001	1.2
4	Mirtazapine	3.024	4279556	391664	8910	1.18
5	Mirtazapine	3.033	4281071	391048	9927	1.1
6	Mirtazapine	2.989	4263309	390017	7785	1.3
Mean			4263838			
Std.Dev.			26299.25			
%RSD			0.616798			

Acceptance Criteria:

- %RSD of Six different sample solutions should not more than 2

Day 2:

Table-9: Results of Intermediate precision Day 2 for Mirtazapine

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP PlateCount	USP Tailing
1	Mirtazapine	2.958	4278645	391773	5573	1.1
2	Mirtazapine	2.964	4262917	390018	9166	1.2
3	Mirtazapine	2.966	4268626	397564	6926	1.17

4	Mirtazapine	2.964	4284172	381081	7782	1.45
5	Mirtazapine	2.966	4272561	390885	9926	1.17
6	Mirtazapine	2.957	4186847	397715	8856	1.39
Mean			4258961			
Std.Dev.			36104.03			
%RSD			0.847719			

Acceptance Criteria: %RSD of six different sample solutions should not more than 2

ACCURACY:

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Table-10: Results of Accuracy for concentration-50%

S.No.	Name	RT	Area	Height	USPTailing	USPPlateCount	Injection
1	Mirtazapine	2.984	3107434	391841	1.21	8846	1
2	Mirtazapine	2.982	3115659	399471	1.2	9174	2
3	Mirtazapine	2.984	3134220	391741	1.1	8972	3

Table-11: Results of Accuracy for concentration-100%

S.No.	Name	RT	Area	Height	USPTailing	USPPlateCount	Injection
1	Mirtazapine	2.989	6275692	399622	1.1	8474	1
2	Mirtazapine	2.997	6278256	391645	1.2	9917	2
3	Mirtazapine	2.996	6229187	399171	1.1	8917	3

Table-12: Results of Accuracy for concentration-150%

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Mirtazapine	3.009	9409506	391746	1.14	8864	1
2	Mirtazapine	3.025	9468961	377611	1.2	9742	2
3	Mirtazapine	3.018	9461768	399463	1.1	7827	3

Table-13: The accuracy results for Mirtazapine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	3184623	7.5	7.49	99.8	99.8%
100%	6294735	15	14.86	99.0	
150%	9474618	22.5	22.47	99.8	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

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

Result:

=3.3× 11050/25169

=1.4µg/ml

QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

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

Result:

=10× 11050/25169

=4.3µg/ml

ROBUSTNESS

The robustness was performed for the flow rate variations from 0.7 ml/min to 0.9 ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Mirtazapine. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Mirtazapine were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table-14: Results for Robustness of Mirtazapine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9 mL/min	381656	2.958	5857	1.12
Less Flow rate of 0.8mL/min	381641	3.526	7673	1.25
More Flow rate of 1mL/min	381645	2.035	3947	1.5
Less organic phase (about 5 % decrease in organic phase)	385663	3.528	9947	1.1
More organic phase (about 5 % Increase in organic phase)	389467	2.004	8576	1.6

Acceptance Criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

5.SUMMARY & CONCLUSION

The analytical method was developed by studying different parameters.

First of all, maximum absorbance was found to be at 225nm and the peak purity was excellent.

Injection volume was selected to be 10µl which gave a good peak area.

The column used for study was Phenomenex Luna C18 (4.6×150mm, 5µm) because it was giving good peak.

40 º C temperatures was found to be suitable for the nature of drug solution. The flow rate was fixed at 0.9ml/min because of good peak area and satisfactory retention time.

Mobile phase is Methanol: Water (45:55 v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study.

Methanol: Water was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 6min because analyze gave peak around 2.9 and also to reduce the total run time.

The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range.

The analytical method was found linearity over the range of 5-15µg/ml of the Mirtazapine target concentration.

The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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