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Development and Validation of Assay Method for Determination of Quetiapine Fumarate by RP-HPLC from Bulk and Pharmaceutical Dosage Form

Nikita Garhewal; Archana Dubey; Dr. P.K Dubey
Swami Vivekanand College of Pharmacy, Indore
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ABSTRACT: A simple, sensitive, rapid, robust and reproducible method for the determination of Quetiapine fumarate in bulk and pharmaceutical formulation (Tablets) was developed using reverse phase high performance liquid chromatographic method (RP-HPLC). The RP-HPLC analysis was performed The analyte was monitored with UV detector at 290nm. The developed method Quetiapine fumarate elutes at a run time of 10 min. The proposed method is having linearity in the concentration range from 40 to 80 µg/mL of Quetiapine fumarate. The present method was validated with respect to system suitability, linearity, precision, limit of detection (LOD) and limit of quantification (LOQ), accuracy (recovery), ruggedness, and robustness. The proposed method can be readily utilized for bulk drug and pharmaceutical formulation.

1. INTRODUCTION:

Analytical chemistry is defined as the science and technique that determines the composition of objects at the time of objects or compounds contained in them. Analytical chemistry plays a very important role in the construction of science and involves: classifying, identifying and determining the corresponding number of objects in a matter sample. Analysis of two types of material; it is a quality and quantitative analysis. Quality analysis reveals the chemical identity of the species in the sample and the quantitative analysis establishes one or more estimated amounts of these ingredients or word analysis.

Drug analysis uses a wide range of materials, dosage forms and recent biological samples that support biopharmaceuticals and pharmacokinetic studies. Drug limitations from bulks, complex formulations and biological fluids of many chemicals, Physicochemicals, apparent radioactivity, methods -X-ray fluorescence, separation methods etc. are often used.

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2. MATERIALS AND METHOD

1. MATERIALS

2. Quetiapine Fumarate: - Working standard and its claimed purity was 98.20% (gift sample from Medgel Pharmaceuticals Ltd, Pithampur, M.P).
3. Quetiapine Fumarate Sustained Release Tablet (Quetipine 200 mg) and placebo from Medgel Pharmaceuticals Ltd, Pithampur, M.P **&** Acetonitrile: -HPLC grade, Rankem, India.
4. Methanol: - HPLC grade, Rankem, India.
5. Milli-Q water: - It was purified by Millipore Corporation's system.
6. Acetic acid: - Reagent Grade, Merck, India.

2. EXPERIEMENTALS

2. Selection of chromatographic parameters

Selection of chromatographic mode

The reverse phase HPLC was selected for separation because it is convenient and rugged than other forms of the liquid chromatography and is more likely to result in a satisfactory final separation.

Selection of stationary phase

On the basis of reversed phase HPLC mode and number of carbon present in molecule (analyte) stationary phase with C_{18} bonded phase i.e. Zorbax XDB C-18, 150 mm x 4.6 mm, 5.0 µm was selected.

Selection of mobile phase

The selection was made on the basis of literature survey. After assessing the solubility of drug in different solvents as well in mobile phases; ACN:Methanol:Buffer (275:275:450) was selected as mobile phase

Mobile phase Preparation

Mix 275 ml of Acetonitrile, 275 ml of Methanol and 450ml of buffer solution, sonicate and filter through 0.45µ membrane filter and degas.

Buffer Preparation

Weigh accurately 3.4 gm of potassium dihydrogen phosphate and 1.77 gm of 1-pentane sulphonic acid sodium salt in 1000 ml of water. Adjust the pH to 6.6(±0.1) with 20% KOH solution. Sonicate it and filter through 0.45µ membrane filter.

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Selection of solvent

Water and acenonitrile (20:80) was used as diluent.

Selection of detector and detection wavelength : Photo Diode Array detector was selected, as it is reliable and easy to set at the correct wavelength. From the spectra of drug, 250 nm wavelength was selected as detection wavelength.

Optimization of chromatographic parameters : Optimization in HPLC is the process of finding a set of conditions that adequately separate and enable the quantification of the analytes from the endogenous material with acceptable accuracy, precision, sensitivity, specificity, cost, ease and speed.

Preparation of standard stock solution : Standard stock solution was prepared by dissolving 50 mg of Quetiapine Fumarate reference standard to a 50 mL volumetric flask and dissolve and dilute up to the mark with diluent. Pipette out 5 ml of above solution in a 50 ml volumetric flask and make up the volume with diluents.

Chromatograms are shown in fig 1 and 2 and results are shown in table 1.

Preparation of test solution : Weigh accurately 20 tablet and triturate it take the powder equivalent to 50 mg of Quetiapine Fumarate and transfer it into 50 ml volumetric flask add about 10 mL of diluent, sonicate at for about 10 min with intermittent shaking, keep to achieve room temperature make up the volume with diluent. Pipette out 5 mL of the above solution and transfer to 50 mL volumetric flask and make up the volume with diluent.

Chromatograms are shown in fig 3 and 4 and results are shown in table 2 and 3.

System Suitability Test : Inject Blank preparation in single injection, standard preparation in five replicate, record the chromatogram and calculate the system suitability parameters as given below:

Procedure: Injection sequence:-

Sr. No	Description	No of Injection
1	Blank	01
2	Standard solution	05
3	Test solution 1	02
4	Test solution 2	02
5	Bracketing standard	01

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Calculations

$$\%\text{Assay} = \frac{AT}{AS} \times \frac{WS}{WT} \times \frac{\text{average wt}}{\text{label claim}} \times P$$

Where,

AT= average area due to quetiapine fumarate in test solution.

AS= average area of peak response of quetiapine fumarate in standard solution.

WS=weight of standard in mg.

WT=weight of sample in mg.

P =purity of standard.

Chromatograms

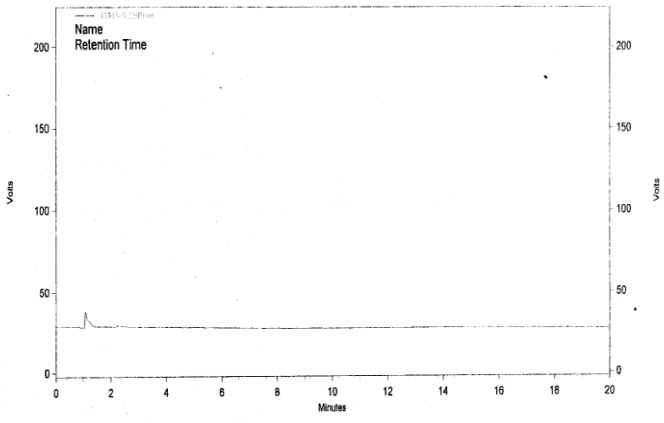

Fig.1. Chromatogram of blank

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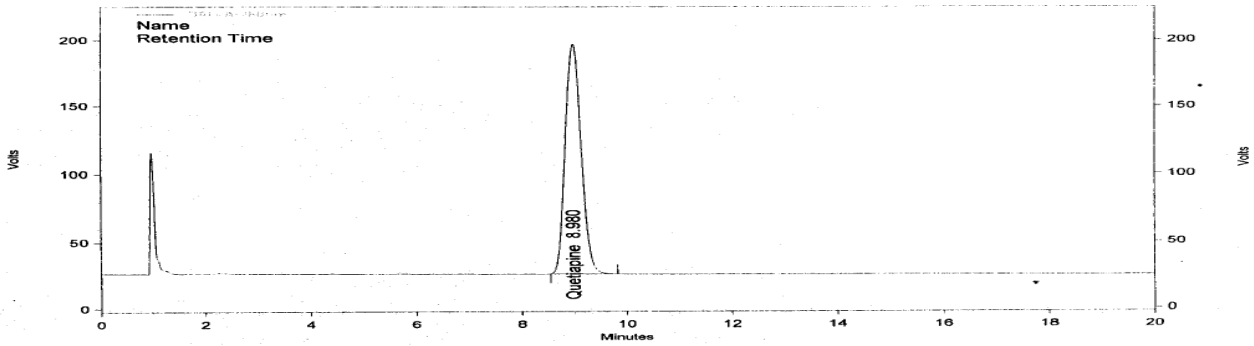

Fig.2. Chromatogram of Sample Solution

Table.1. Area for Standard Preparation

Sr. No	Concentration µg/ml	Area for standard injection
1	100	3652958
2	100	3648373
3	100	3642707
4	100	3645430
5	100	3643674
6	Mean	3646628
7	S.D	4142.7
8	%R.S.D	0.11

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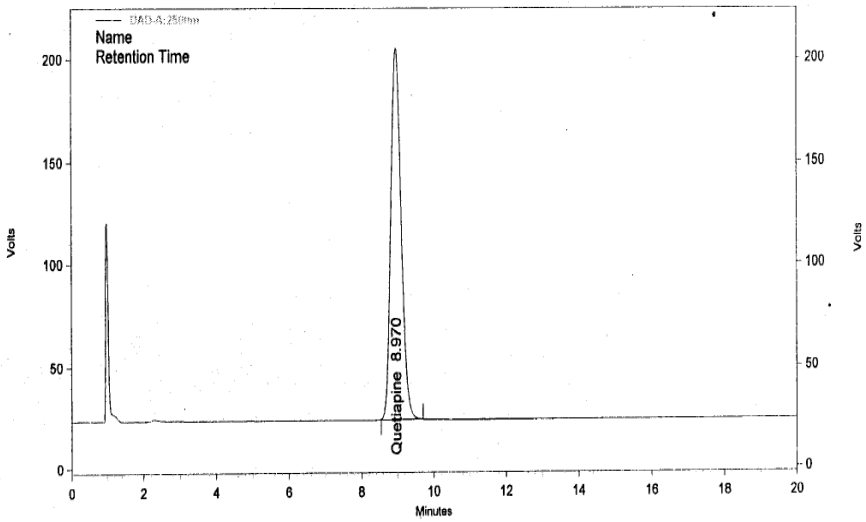

Fig.3. Chromatogram of Test Solution I

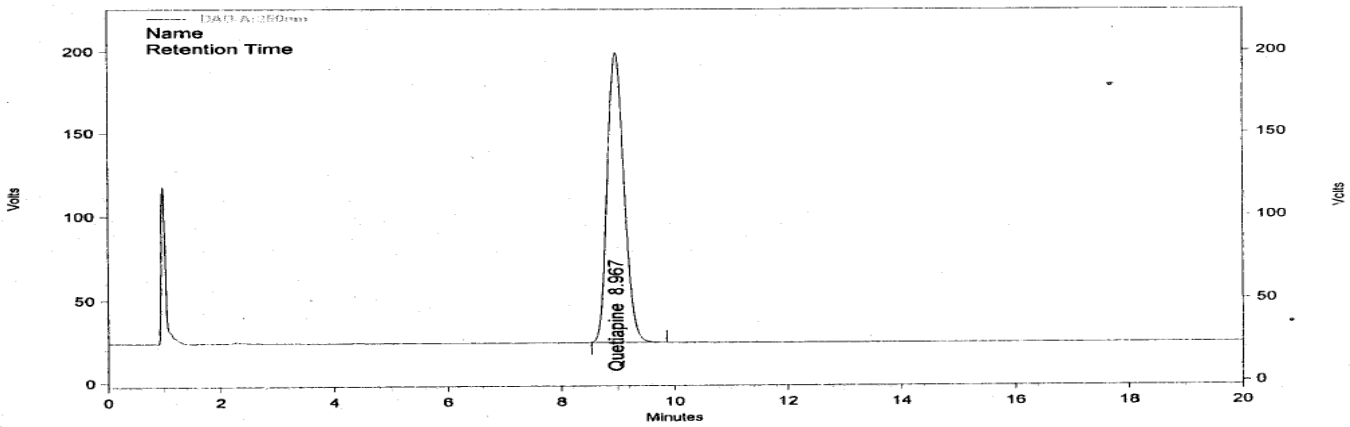

Fig.4. Chromatogram of Test Solution II

Table.2. Results for Test Solution I

Sr.No	Label Claimed(mg)	Area of Test Solution	% Assay
1	200	3639432	98.01
2	200	3641342	98.06

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Table.3. Results for Test Solution II

Sr.No	Label Claimed(mg)	Area of Test Solution	% Assay
1	200	3640453	98.03
2	200	3599123	96.92

Method Validation

❖ **Specificity**

Procedure

Prepare blank preparation, prepared placebo preparation, standard preparation, and sample preparation for 200mg tablet as per the method.

Placebo preparation

Weighed accurately 254 mg of placebo and transferred to a 50 mL volumetric flask add about 35 mL of mobile phase , sonicate at for about 15 min with intermittent shaking, keep to achieve room temperature make up to volume with mobile phase.

❖ **Linearity**

The linearity was determined at five levels over the range of 50% to 150% of sample concentration. Quetiapine Fumarate standard stock solutions were prepared and from this solution dilution was done with solvent mixture to obtain standard calibration solutions of Quetiapine Fumarate having concentration in the range of 50-150 µg/ml.

Preparation of linearity solution

Stock solution:

Weigh and transfer accurately about 50 mg Quetiapine Fumarate working standard into a 50 ml volumetric flask, Sonicate to dissolve and dilute up to the mark with diluent. Further dilute this stock solution as describe below for linearity.

Sno	Linearity Level In %	Volume of stock solution in (ml)	Diluted to volume (ml)	Concentration in ppm
1	50%	2.5	50	50
2	80%	4	50	80
3	100%	5	50	100

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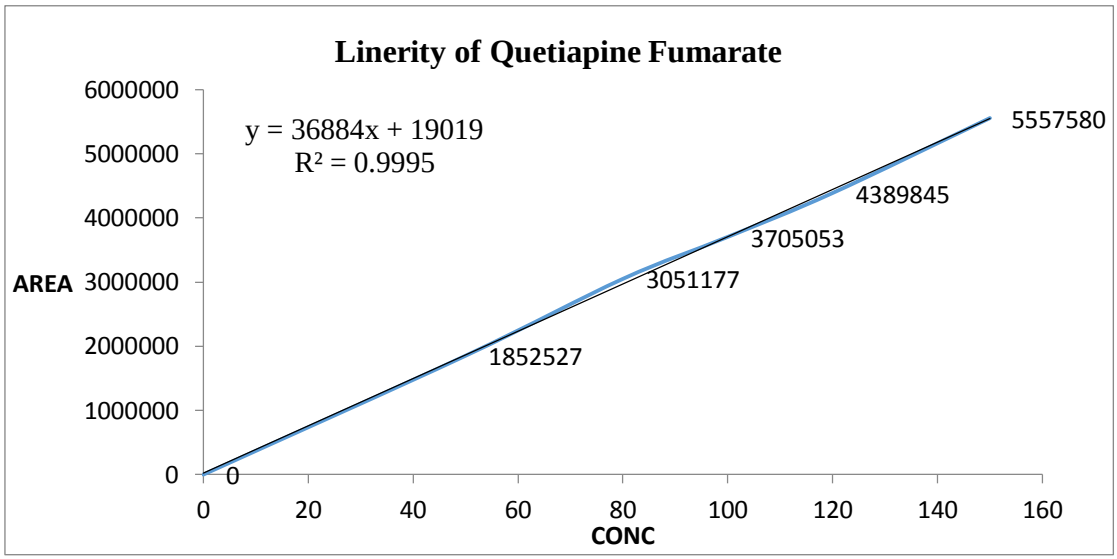
4	120%	6	50	120
5	150%	7.5	50	150

Calculate correlation co-efficient (r), y-intercept, and slope of regression line.

Table 4: Result for Linearity

Linearity Level	Standard concentration	Concentration of Quetiapine Fumarate (ppm)	Mean area (n = 3)	Regression coefficient (R^2)
Level – 1	50%	50	**1852527**	0.9995
Level – 2	80%	80	**3051177**	
Level – 3	100%	100	**3705053**	
Level – 4	120%	120	**4389845**	
Level – 5	150%	150	**5557580**	

Fig 5. Linearity of Quetiapine Fumarate



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❖ **Accuracy (Recovery)**

Accuracy was determined over the range 50% to 150% of the sample concentration. Calculated amount of Quetiapine Fumarate from standard stock solution was added in placebo to attain 50%, 100% and 150% of sample concentration. Each sample was prepared in triplicate at each level and injected each preparation in duplicate. Blank and standard preparations were injected and the chromatograms were recorded

Level-1 (50%) Weighed accurately 254 mg of placebo and 25 mg of Quetiapine Fumarate transfer to a 50 mL volumetric flask add about 35 ml of mobile phase, sonicate for about 15 min with intermittent shaking, keep to achieve room temperature and make the volume up to mark with mobile phase and mix, pipette out 5 ml of above solution and transfer it to 50 ml volumetric flask and make up the volume with mobile phase.

Level-2 (100%)

Weighed accurately 254 mg of placebo and 50 mg of Quetiapine Fumarate transfer to a 50 mL volumetric flask add about 35 ml of mobile phase, sonicate for about 15 min with intermittent shaking, keep to achieve room temperature and make the volume up to mark with mobile phase and mix, pipette out 5 ml of above solution and transfer it to 50 ml volumetric flask and make up the volume with mobile phase.

Level-3 (150%)

Weighed accurately 254 mg of placebo and 75 mg of Quetiapine Fumarate transfer to a 50 mL volumetric flask add about 35 ml of mobile phase, sonicate for about 15 min with intermittent shaking, keep to achieve room temperature and make the volume up to mark with mobile phase and mix, pipette out 5 ml of above solution and transfer it to 50 ml volumetric flask and make up the volume with mobile phase.

Table 5 Result for Recovery studies

Level	Preparation	Amount added (mg)	Amount found (mg)	% Recovery	Mean % Recovery	% RSD
50 % Level	Preparation-1	24.97	25.01	100.2	100.0	0.15
	Preparation-2	25.01	25.00	100.0		
	Preparation-3	25.05	25.03	99.9		
100 % Level	Preparation-1	50.12	48.53	96.8	97.0	0.26
	Preparation-2	50.01	48.66	97.3		
	Preparation-3	49.86	48.38	97.0		

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150 % Level	Preparation-1	75.07	73.52	97.9	98.1	0.75
	Preparation-2	74.98	74.13	98.9		
	Preparation-3	75.02	73.08	97.4		

❖ **Precision**

a. Method Precision (Repeatability)

Procedure

Method precision should be established by analyzing six sample preparations under same conditions as per test procedure for Assay using same lot of sample. Individual assay value, mean assay value, 95% Confidence Interval and %RSD shall be calculated for the results obtained and recorded.

Table 6. Result for Method Precision

Sample Preparation	**% Assay of Quetiapine Fumarate**
Test solution -1	99.67
Test solution -2	99.59
Test solution -3	99.55
Test solution -4	99.38
Test solution -5	99.48
Test solution -6	99.69
Mean	**99.56**
Standard Deviation	**0.12**
Relative Standard Deviation (%)	**0.12**

Intermediate Precision (Ruggedness)

Procedure : The procedure followed for method precision shall be repeated on a different day by different analyst, using a different HPLC system and different column of same make using same lot of sample. Individual assay value, mean assay value, 95% Confidence Interval and %RSD shall be calculated. The mean assay value shall be compared with the average assay value obtained in method precision study. The difference in the mean assay values shall be calculated and recorded.

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Table 7. Result for Intermediate Precision

Analysis performed during method precision study Analyst: Analyst-I HPLC ID No.: EAR040 Make : Zorbax XDB C-18, 150 mm x 4.6 mm, 5.0 µm Column serial number. : 0402471K	
Sr. No.	**% Assay of Quetiapine Fumarate**
Test solution-1	99.67
Test solution-2	99.59
Test solution-3	99.55
Test solution-4	99.38
Test solution-5	99.48
Test solution-6	99.69
Analysis performed during intermediate precision study Analyst: Analyst-II HPLC ID No.: EAR039 Make : Zorbax XDB C-18, 150 mm x 4.6 mm, 5.0 µm Column serial number : 0502481L	
Test solution-1	99.44
Test solution-2	99.56
Test solution-3	100.05
Test solution-4	99.42
Test solution-5	99.64
Test solution-6	99.83
Mean of twelve samples	**99.66**

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Standard Deviation	**0.24**
Relative Standard Deviation (%)	**0.24**

❖ **Robustness**

Change following parameters one by one and observe their effect on system suitability.

1) Change in flow rate of mobile phase by ± 10% [use flow rate 1.08 ml/min and 1.32 ml/min].

Table 8. Result for Flow rate 1.08 ml/min.

Parameter	Test solution	%Assay for Quetiapine Fumarate
Method precision	1	99.67
	2	99.59
	3	99.55
	4	99.38
	5	99.48
	6	99.69
Change in flow rate 1.08 mL/ min.	1	99.65
	2	99.38
Mean		**99.55**
Standard deviation		**0.12**
Relative standard deviation (%)		**0.12**

Table 9. Result for Flow rate 1.32 ml/min.

Parameter	Test solution	%Assay for Quetiapine Fumarate
Method precision	**1**	99.67
	2	99.59
	3	99.55
	4	99.38
	5	99.48

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	6	99.69
Change in flow rate 1.32 mL/ min.	**1**	99.21
	2	98.99
Mean		**99.44**
Standard deviation		**0.24**
Relative standard deviation (%)		**0.24**

2) Change in wavelength by ± 2 nm[use wavelength 248 nm and 252 nm].

Table 10. Result for wavelength 248 nm

Parameter	**Test solution**	**%Assay for Quetiapine Fumarate**
Method precision	**1**	99.67
	2	99.59
	3	99.55
	4	99.38
	5	99.48
	6	99.69
Change in wavelength 248 nm.	**1**	98.47
	2	97.65
Mean		**99.18**
Standard deviation		**0.69**
Relative standard deviation (%)		**0.69**

Table 11.Result for wavelength 252 nm.

Parameter	**Test solution**	**%Assay for Quetiapine Fumarate**
Method precision	**1**	99.67
	2	99.59
	3	99.55

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	4	99.38
	5	99.48
	6	99.69
Change in wavelength 252 nm.	**1**	98.13
	2	98.01
Mean		**99.18**
Standard deviation		**0.65**
Relative standard deviation (%)		**0.66**

Table 12.System suitability parameters

Parameter			**Theoretical Plates**	**Tailing Factor**	**%RSD**
Limits			Not less than 2500	Not more than 2.0	Not more than 2.0%
1	**Specificity**				
	1.1	Specificity-Part A	8313	1.05	0.15
2	**Linearity and Range**		8204	1.02	0.18
3	**Accuracy study (Recovery)**		8135	1.02	0.15, 0.26 and 0.75
4	**Precision**				
	4.1	Method precision (Repeatability)	8526	1.04	0.12
	4.2	Intermediate Precision (Ruggedness)	8431	1.06	0.24

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5	**Robustness**				
	5.1	Change flow rate by ± 10% (1.08 ml/minute and 1.32 ml/minute).	7952	1.09	0.12 and 0.24
	5.2	Change in wavelength by ± 2 nm(248 nm and 252 nm)	7952	1.08	0.69 and 0.66

Method II

Quantitative Estimation of Hydralazine HCl by RP-HPLC in Pharmaceutical dosage form.

1.Experimental requirements

1.1. Instrument used

- High Performance Liquid chromatography system (HPLC): Waters Liquid Chromatography.
- Chromatographic software:– Empower Pro
- Analytical Balance: - AD 265S, Mettler Toledo, Schwerzenland.
- pH Meter: - Labindia, India.
- Sonicator: - 5510, Branson Ultrasonics Corporation, Danbury, CT, USA.
- Hot air oven: - Labline, India.

1.2. Materials

- Hydralazine HCl: - Working standard and its claimed purity was 99.20% (gift sample from Medgel Pharmaceuticals Ltd, Pithampur, M.P).
- Hydralazine HCl Sustained Release Tablet (label claim Apresolin 50 mg) and placebo, which was gift sample from Medgel Pharmaceuticals Ltd, Pithampur, M.P
- **1.3. Reagent and chemicals**
- Acetonitrile: -HPLC grade, Rankem, India.
- Milli-Q water: - It was purified by Millipore Corporation's system.

2. Selection of chromatographic parameters

2.1. Selection of chromatographic mode

The reverse phase HPLC was selected for separation because it is convenient and rugged than other forms of the liquid chromatography and is more likely to result in a satisfactory final separation.

2.2. Selection of stationary phase

On the basis of reversed phase HPLC mode Inertsil ODS-3, 250 mm x 4.6 mm, 5.0 µm column was selected.

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2.3. Selection of mobile phase
The selection was made on the basis of literature survey. After assessing the solubility of drug in different solvents as well in mobile phases; ACN:Buffer (200:800) was selected as mobile phase

Mobile phase Preparation

Mix 200 ml of Acetonitrile, and 800 ml of buffer solution, sonicate and filter through 0.45µ membrane filter and degas.

Buffer Preparation

Weigh accurately 6.8 gm of potassium dihydrogen orthophosphate and 15 gm of 1-octane sulphonic acid sodium salt in 1000 ml of water. Adjust the pH to 3.0(±0.1) with OPA solution. Sonicate it and filter through 0.45µ membrane filter.

2.4 Selection of solvent

Mobile phase was used as solvent.

2.5. Selection of detector and detection wavelength: Photo Diode Array detector was selected, as it is reliable and easy to set at the correct wavelength. From the spectra of drug, 230 nm wavelength was selected as detection wavelength.

3. Optimization of chromatographic parameters

Optimization in HPLC is the process of finding a set of conditions that adequately separate and enable the quantification of the analytes from the endogenous material with acceptable accuracy, precision, sensitivity, specificity, cost, ease and speed.

Optimized HPLC Parameters:

Instrument	: Waters Liquid Chromatography
Column	: Inertsil ODS-3, 250 mm x 4.6 mm, 5.0 µm
Flow Rate	: 1.0 mL/min
Injection volume	: 20 µL
Column temperature	: Ambient
Sample cooler Temperature	: Ambient
Detection	: 230 nm
Run time	: 10 minutes

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Preparation of standard stock solution

Standard stock solution was prepared by dissolving 25 mg of Hydralazine HCL reference standard to a 25 mL volumetric flask and dissolve and dilute up to the mark with diluent. Pipette out 1 ml of above solution in a 10 ml volumetric flask and make up the volume with diluents.

Chromatograms are shown in fig 6 and 7 and results are shown in table 13.

Preparation of test solution

Weigh accurately 20 tablet and triturate it take the powder equivalent to 25 mg of Hydralazine HCL and transfer it into 25 ml volumetric flask add about 10 mL of diluent, sonicate at for about 10 min with intermittent shaking, keep to achieve room temperature make up the volume with diluent. Pipette out 1 mL of the above solution and transfer to 10 mL volumetric flask and make up the volume with diluent.

Chromatograms are shown in fig 8 and 9 and results are shown in table 14 and 15.

System Suitability Test

Inject Blank preparation in single injection, standard preparation in five replicate, record the chromatogram and calculate the system suitability parameters as given below:

Procedure: Injection sequence:-

Sr. No	Description	No of Injection
1	Blank	01
2	Standard solution	05
3	Test solution 1	02
4	Test solution 2	02
5	Bracketing standard	01

Calculations

$$\%\text{Assay} = \frac{AT}{AS} \times \frac{WS}{WT} \times \frac{\text{average wt}}{\text{label claim}} \times P$$

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Where,

AT= average area due to Hydralazine HCl in test solution.

AS= average area of peak response of Hydralazine HCl in standard solution.

WS=weight of standard in mg.

WT=weight of sample in mg.

P =purity of standard.

Chromatograms

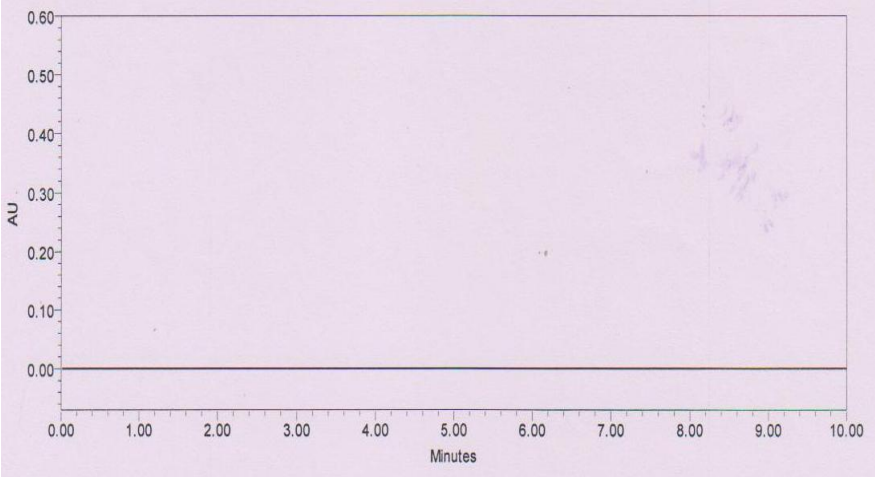

Fig.6. Chromatogram of blank

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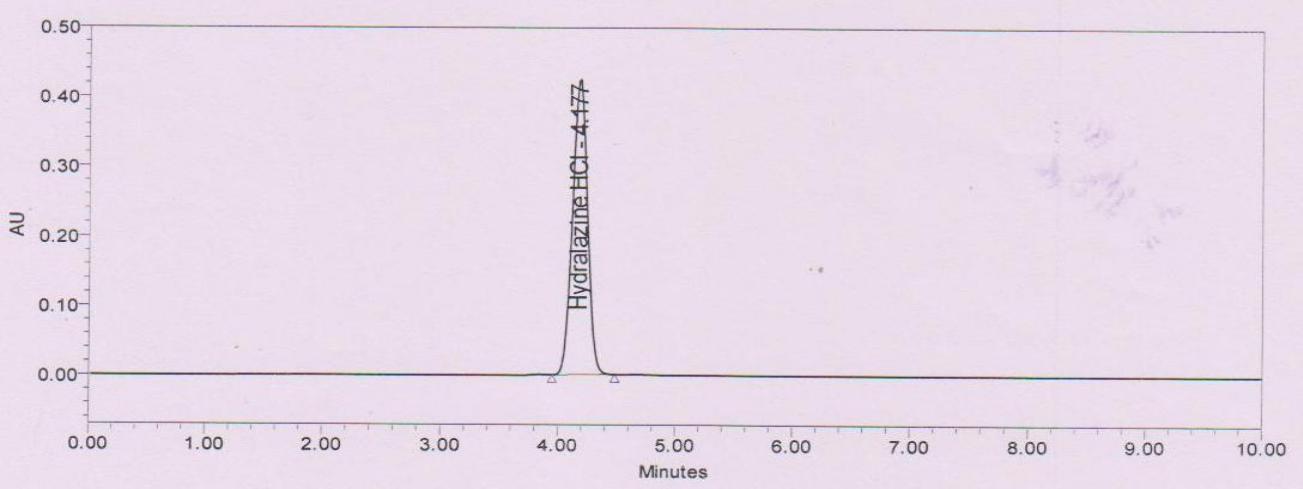

Fig.7. Chromatogram of Sample Solution

Table.13. Area for Standard Preparation

Sr. No	Concentration µg/ml	Area for standard injection
1	100	3753564
2	100	3755658
3	100	3751234
4	100	3756456
5	100	3754345
6	Mean	3754251
7	S.D	2026.37
8	%R.S.D	0.05

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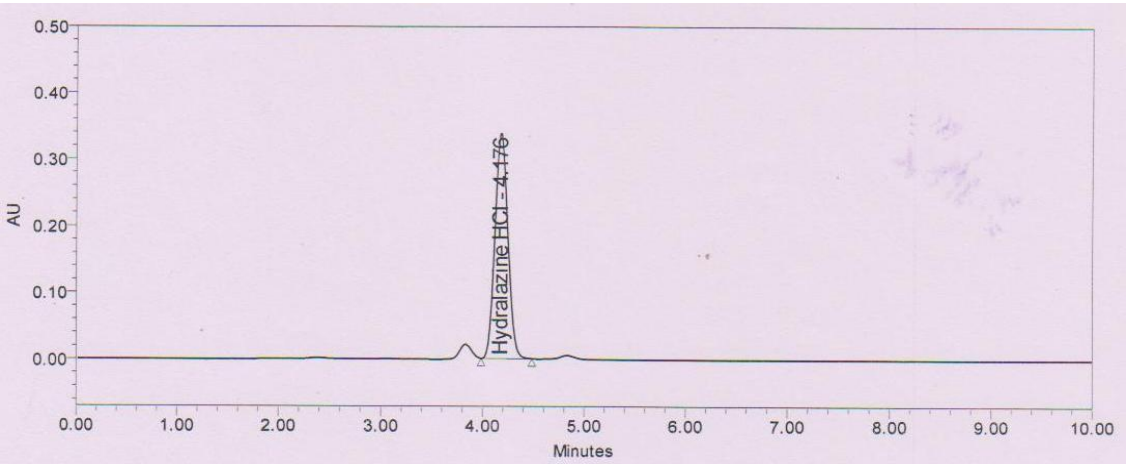

Fig.8. Chromatogram of Test Solution I

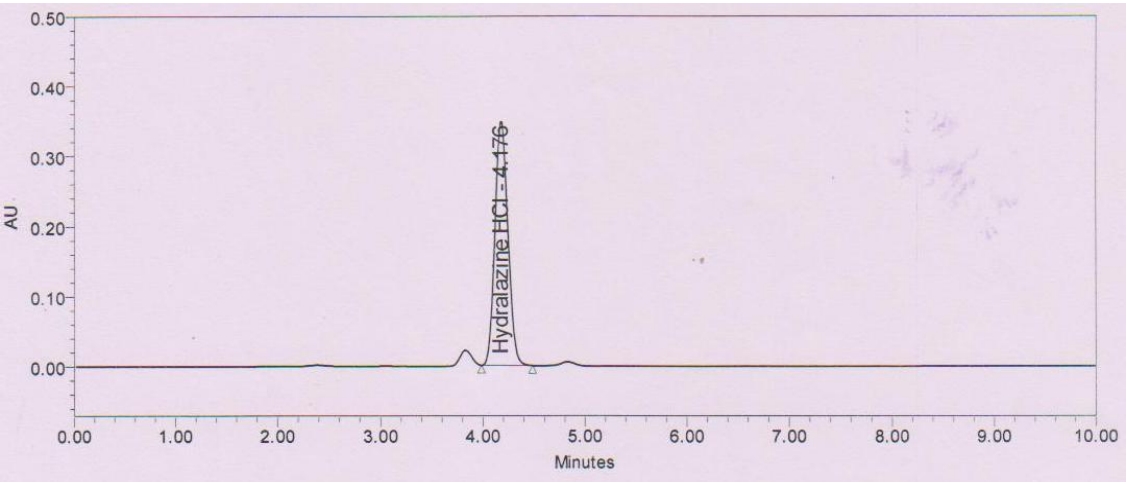

Fig.9. Chromatogram of Test Solution II

Table.14. Results for Test Solution I

Sr.No	Label Claimed(mg)	Area of Test Solution	% Assay
1	50	3595667	95.55
2	50	3609876	95.96

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Table.15. Results for Test Solution II

Sr.No	Label Claimed(mg)	Area of Test Solution	% Assay
1	50	3678544	97.90
2	50	3779572	100.51

Method Validation

❖ **Specificity**

Prepare blank preparation, prepared placebo preparation, standard preparation, and sample preparation for 50 mg tablet as per the method.

Placebo preparation

Weighed accurately 267 mg of placebo and transferred to a 25 mL volumetric flask add about 35 mL of mobile phase , sonicate at for about 15 min with intermittent shaking, keep to achieve room temperature make up to volume with mobile phase.

❖ **Linearity**

The linearity was determined at five levels over the range of 50% to 150% of sample concentration. Hydralazine HCl standard stock solutions were prepared and from this solution dilution was done with solvent mixture to obtain standard calibration solutions of Hydralazine HCl having concentration in the range of 50-150 µg/ml.

Preparation of linearity solution

Stock solution:

Weigh and transfer accurately about 25 mg Hydralazine HCl working standard into a 25 ml volumetric flask, Sonicate to dissolve and dilute up to the mark with diluent. Further dilute this stock solution as describe below for linearity.

Sno	Linearity Level In %	Volume of stock solution in (ml)	Diluted to volume (ml)	Concentration in ppm
1	50%	0.5	10	50
2	80%	0.8	10	80
3	100%	1.0	10	100

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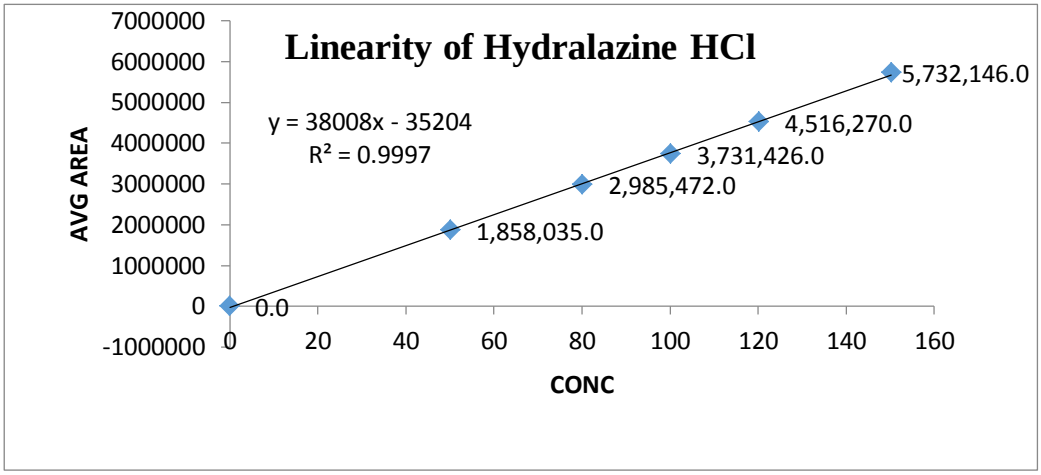
4	120%	1.2	10	120
5	150%	1.5	10	150

Calculate correlation co-efficient (r), y-intercept, and slope of regression line.

Table 16: Result for Linearity

Linearity Level	Standard concentration	Concentration of Hydralazine HCl (ppm)	Mean area (n = 3)	Regression coefficient (R^2)
Level – 1	50%	50	**1858035**	0.9997
Level – 2	80%	80	**2985472**	
Level – 3	100%	100	**3731426**	
Level – 4	120%	120	**4516270**	
Level – 5	150%	150	**5732146**	

Fig 10. Linearity of Hydralazine HCl



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❖ **Accuracy (Recovery)**

Accuracy was determined over the range 50% to 150% of the sample concentration. Calculated amount of Hydralazine HCl from standard stock solution was added in placebo to attain 50%, 100% and 150% of sample concentration. Each sample was prepared in triplicate at each level and injected each preparation in duplicate. Blank and standard preparations were injected and the chromatograms were recorded

Level-1 (50%)

Weighed accurately 270 mg of placebo and 12.5 mg of Hydralazine HCl transfer to a 25 mL volumetric flask add about 15 ml of mobile phase, sonicate for about 15 min with intermittent shaking, keep to achieve room temperature and make the volume up to mark with mobile phase and mix, pipette out 1 ml of above solution and transfer it to 10 ml volumetric flask and make up the volume with mobile phase.

Level-2 (100%)

Weighed accurately 270 mg of placebo and 25 mg of Hydralazine HCl transfer to a 25 mL volumetric flask add about 15 ml of mobile phase, sonicate for about 15 min with intermittent shaking, keep to achieve room temperature and make the volume up to mark with mobile phase and mix, pipette out 1 ml of above solution and transfer it to 10 ml volumetric flask and make up the volume with mobile phase.

Level-3 (150%)

Weighed accurately 270 mg of placebo and 37.5 mg of Hydralazine HCl transfer to a 25 mL volumetric flask add about 15 ml of mobile phase, sonicate for about 15 min with intermittent shaking, keep to achieve room temperature and make the volume up to mark with mobile phase and mix, pipette out 1 ml of above solution and transfer it to 10 ml volumetric flask and make up the volume with mobile phase.

Table 17 Result for Recovery studies

Level	Preparation	Amount added (mg)	Amount found (mg)	% Recovery	Mean % Recovery	% RSD
50 % Level	Preparation-1	12.49	11.99	96.0	95.6	0.58
	Preparation-2	12.51	12.00	95.9		
	Preparation-3	12.55	11.92	95.0		
100 % Level	Preparation-1	25.08	24.39	97.2	97.3	0.73
	Preparation-2	24.97	24.50	98.1		
	Preparation-3	25.18	24.36	96.2		
150 % Level	Preparation-1	37.52	36.90	98.4	98.3	0.32
	Preparation-2	37.59	37.07	98.6		
	Preparation-3	37.38	36.63	98.0		

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❖ **Precision**

b. Method Precision (Repeatability)

Procedure

Method precision should be established by analyzing six sample preparations under same conditions as per test procedure for Assay using same lot of sample. Individual assay value, mean assay value, 95% Confidence Interval and %RSD shall be calculated for the results obtained and recorded.

Table 18. Result for Method Precision

Sample Preparation	**% Assay of Hydralazine HCl**
Test solution -1	98.46
Test solution -2	95.69
Test solution -3	95.79
Test solution -4	96.04
Test solution -5	95.67
Test solution -6	96.15
Mean	**96.30**
Standard Deviation	**1.08**
Relative Standard Deviation (%)	**1.12**

c. Intermediate Precision (Ruggedness)

Procedure

The procedure followed for method precision shall be repeated on a different day by different analyst, using a different HPLC system and different column of same make using same lot of sample. Individual assay value, mean assay value, 95% Confidence Interval and %RSD shall be calculated. The mean assay value shall be compared with the average assay value obtained in method precision study. The difference in the mean assay values shall be calculated and recorded.

Table 19. Result for Intermediate Precision

Analysis performed during method precision study Analyst: Analyst-I HPLC ID No.: EAR040 Make : Inertsil ODS-3, 250 mm x 4.6 mm, 5.0 µm Column serial number. : 030677K	

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Sr. No.	% Assay of Hydralazine HCl
Test solution-1	98.46
Test solution-2	95.69
Test solution-3	95.79
Test solution-4	96.04
Test solution-5	95.67
Test solution-6	96.15
Analysis performed during intermediate precision study Analyst: Analyst-II HPLC ID No.: EAR039 Make : Inertsil ODS-3, 250 mm x 4.6 mm, 5.0 µm Column serial number : 0450481L	
Test solution-1	96.65
Test solution-2	99.61
Test solution-3	97.83
Test solution-4	99.76
Test solution-5	96.44
Test solution-6	97.01
Mean of twelve samples	**97.09**
Standard Deviation	**1.48**
Relative Standard Deviation (%)	**1.52**

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❖ **Robustness**

Change following parameters one by one and observe their effect on system suitability.

3) Change in flow rate of mobile phase by ± 10% [use flow rate 0.9ml/min and 1.10 ml/min].

Table 20. Result for Flow rate 0.9 ml/min.

Parameter	Test solution	%Assay for Hydralazine HCl
Method precision	1	98.46
	2	95.69
	3	95.79
	4	96.04
	5	95.67
	6	96.15
Change in flow rate 0.9 mL/ min.	1	95.36
	2	95.27
Mean		**96.05**
Standard deviation		**1.02**
Relative standard deviation (%)		**1.06**

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Table 21. Result for wavelength 228 nm

Sample Preparation	Area	Mean	% Assay	% Assay	Cumulative mean	SD	Cumulative % RSD
FreshTest solution	3776654	3776654	101.50	101.50	NA	--	--
24 th Hr Test solution	3707896	3707896	99.17	99.17	100.34	1.65	**1.64**
48 th Hr Test solution	3693456	3693456	98.95	98.95	99.87	1.41	**1.41**
72 th Hr Test solution	3656475	3656475	97.99	97.99	99.40	1.49	**1.50**

22.Result for wavelength 232 nm.

Parameter	Test solution	%Assay for Hydralazine HCl
Method precision	**1**	98.46
	2	95.69
	3	95.79
	4	96.04
	5	95.67
	6	96.15
Change in wavelength 232 nm.	**1**	97.05
	2	97.08
Mean		**96.49**
Standard deviation		**0.98**
Relative standard deviation (%)		**1.02**

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❖ **Solution Stability**

In solution stability freshly prepared solution and solution kept for 24 hours, 48 hours and 72 hours are taken and chromatograms are recorded and result is calculated as per assay method

❖ **Filter Validation**

In filter validation unfiltered solution and filtered solution which is filtered from 0.45µm membrane filter are prepared as per the method and chromatograms are recorded and results are calculated as per assay method.

Table 23 Result for Filter Validation.

Sample Preparation	Area of Injection-1	% Assay of Hydralazine	Absolute difference w.r.t Unfiltered test
Unfiltered Test solution	3734330	99.44	–
Test solution-1	3701673	98.58	0.86
Test solution-2	3702285	98.58	0.86
Test solution-3	3695500	98.43	1.01
Test solution-4	3700216	98.50	0.94
Test solution-5	3707741	98.72	0.72
	Mean	**98.71**	
	SD	**0.37**	
	%RSD	**0.37**	

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Table 26. System suitability parameters

Parameter			Theoretical Plates	Tailing Factor	%RSD
Limits			Not less than 2500	Not more than 2.0	Not more than 2.0%
1	**Specificity**				
	1.1	Specificity-Part A	4824	1.34	1.02
2	**Linearity and Range**		4960	1.08	0.58
3	**Accuracy study (Recovery)**		4960	1.08	0.58, 0.73 and 0.32
4	**Precision**				
	4.1	Method precision (Repeatability)	4847	1.08	1.12
	4.2	Intermediate Precision (Ruggedness)	5561	1.47	1.50
5	**Robustness**				
	5.1	Change flow rate by ± 10% (0.9 ml/minute Low Flow)	5365	1.09	1.06
	5.2	Change flow rate by ± 10% (1.10 ml/minute High Flow)	4762	1.09	0.94
	5.3	Change in wavelength by ± 2 nm(228 nm)	5314	1.10	1
	5.4	Change in wavelength by ± 2 nm(232 nm)	5477	1.10	1.02
6	**Solution Stability**		5543	1.11	0.45
	24 hours				1.64

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	48 hours			1.41
	72 hours			1.50
7	**Filter Validation**	4847	1.08	0.37

3. RESULTS AND DISCUSSION:

In the research work done, a successful attempt for estimation of few drugs using RP-HPLC was made by experimentation based on through literature survey.

The chromatographic methods developed are:

Method I :-

Development and Validation of RP-HPLC Method for the Determination of Quetiapine fumarate from Bulk and Tablets

Method II :-

Development and Validation of RP-HPLC Method for the Determination of Hydralazine HCl from Bulk and Tablets

The simplicity, rapidity, reproducibility, and repeatability of the proposed methods completely fulfill the objective of the research work of estimation of this drug molecule.

Waters HPLC equipped with Empower pro software and Agilent HPLC equipped with EZ chrome elite software were used for entire research work.

All the methods were validated.

It can be said that above methods can be used for routine laboratory analysis and are applicable for industrial use also with precision and accuracy.

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