RP-HPLC Method Development and Validation for Simultaneous Estimation of Cetirizine Hydrochloride and Griseofulvin Pharmaceutical Dosage Form.

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Abstract: A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of Cetirizine Hydrochloride and Griseofulvin in bulk and tablet dosage form. C18 C-18Agilent Zorbax Bonus –RP (250 x 4.6 mm, Particle size -5 Micron) was used in this method. The mobile phase comprises of : Acetonitrile : 0.1 % Trifluoroaceticacid (50:50, % v/v) with flow rate 1 mL/min (Photodiode array Detector). The retention time for Cetirizine Hydrochloride was 5.16 min and for Griseofulvin was 5.00 min. The detection concentration was linear over 4-6 µg/mL for Cetirizine Hydrochloride and 200-300 µg/mL for Griseofulvin . The regression equation of Cetirizine Hydrochloride and Griseofulvin were found to be y = 75272x – 6702 and y = 68965x - 17908 respectively with regression co-efficient of Cetirizine Hydrochloride and Griseofulvin were 1 and 0.999. so, the present work is aimed for Development of simple, reproducible chromatographic RP-HPLC method for simultaneous estimation of Cetirizine Hydrochloride and Griseofulvin. The developed method was successfully validated in accordance to ICH guideline. Hence the method can be conveniently adopted for the routine analysis in quality control laboratories.

Key words: -Cetirizine Hydrochloride, Griseofulvin, RP-HPLC, Method Development, Validation.

INTRODUCTION:-

Chemical name of cetirizine hydrochloride is 2-[2-]4-[(4-chlorophenly)-phenylmethyl]piperazin-1-yl]ethoxy]acetic acid. Cetirizine is a second generation antihistamine used to treat allergeicrhinitis , dermatitis and urticaria it is taken by mouth effects generally begin with in and hour and last for about a day .

It was patented in 1981 and came into medical use 1987 it is on the world health organisation listof essential medicines. In 2019,it was the 67th most commonly prescribed medication in united states with more than 11 million prescriptions (google ,wiki)

Griseofulvin is [7-chloro-2,4,6-trimethoxy-6-methylspiro(benzofuran-2(3H),1-(2)cyclohexen] 3,4-dion. an anti fungal medication used to treat a number of types of dermatophytoses (ringworm) this includes fungal infection of the nails and scalp, as well as skin when anti-fungal creams is not worked then taken by mouth. (goggle wiki .)

Griseofulvin is mycotoxic metabolic product of penicillium spp. It was first available oral agent for the treatment of dematophytoses and has now been usd for more than 40 years . griseofulvin is fungistatic with in -vitro activity against various species of microsporum Epidermophyton , and trichophyton. It has number no effect on bacteria or on other genera of fungi .

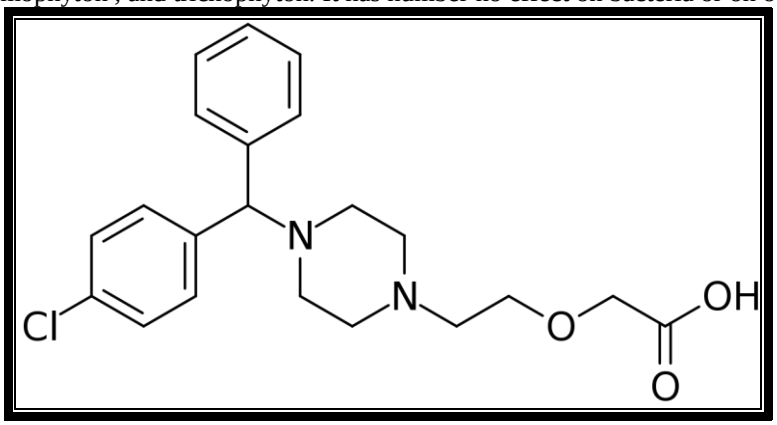


Fig.1-Structure of Cetirizine Hydrochloride

Molecular formula: -C21H27C13N203

Molecular Weight: -461.8 gm/mol**.**

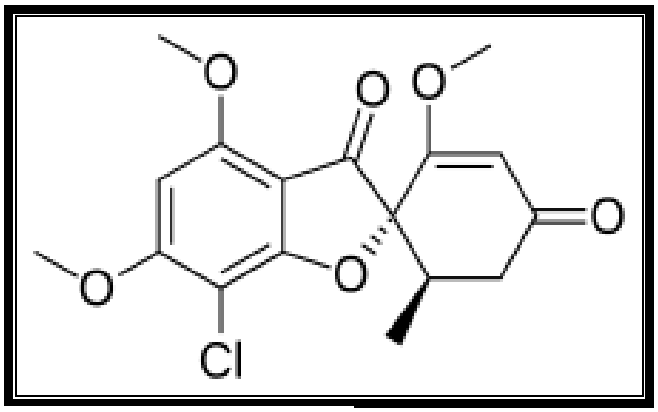


Fig.2- Structure of Griseofulvin.

Molecular Formula: C17H17ClO6
Molecular Weight: - 352.76 g/mol

Materials and Method:-

- Chemicals :-
  A pure drug in powder form Cetirizine Hydrochloride and Griseofulvin were received from Cipla Pvt, Ltd, kurkumbh and Danish health care (P)Ltd, Methanol (AR grade) and Trifluoracetic acid were utilize as diluent and received from Fine Chem laboratories, Mumbai . A commercial product manufactured by Sun life science Pvt.Ltd. "Grisozin" which contain both the Cetirizine Hydrochloride and Griseofulvin were Received from Market.
- Instrument:-
  Agilent 1260 Infinity Quaternary HPLC device equipped with Photodiode array detector. The output signal was checked and processed using openLabEZChrome software.
- Chromatographic condition :-
  1. Oven Temperature :- 30^0 C
  2. Flow Rate:-1mL/min.
  3. Run Time: - 10 min.
  4. Injection Volume: - 10µL.
  5. Wavelength: -232 nm.
  6. Column: - Agilent Zorbax Bonus-RP (250 x 4.6 mm, 5 µ)
  7. Diluent: - Acetonitrile : 0.1 % Trifluoroaceticacid (50:50, % v/v)

- **Standard Preparation:**
- **Standard Stock Solution-I (SSS-I):**
  i. Initially Prepare a Standard Stock Solution (SSS-I) of Griseofulvin by adding 10mg in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Griseofulvin = 1000µg/ml.
- **Standard Stock Solution-II (SSS-II):**
  ii. Then prepare a Standard Stock Solution (SSS-II) of Cetirizine by adding 5mg in 10 ml volumetric flask & add 5 ml Diluent, mix for 2 minutes and make the volume to 10 ml with Diluent.(Conc. of Cetirizine = 500µg/ml).

- **Standard Stock Solution-III (SSS-III)**
  iii. Then add 1 ml SSS-II in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent.(Conc. of Cetirizine = 50µg/ml).
- Further pipette out 2.5 ml of SSS-I and 1.0 ml of SSS-IIIin 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent.(Conc. of Griseofulvin = 250µg/ml &Cetirizine = 5 µg/ml).
-
- **Selection of Wavelength** :-
  The sample was scanned from 200-400 nm Photodiode array detectors. The wavelength selected for analysis chosen was 232 nm on basis of appropriate intensity of both the drug.

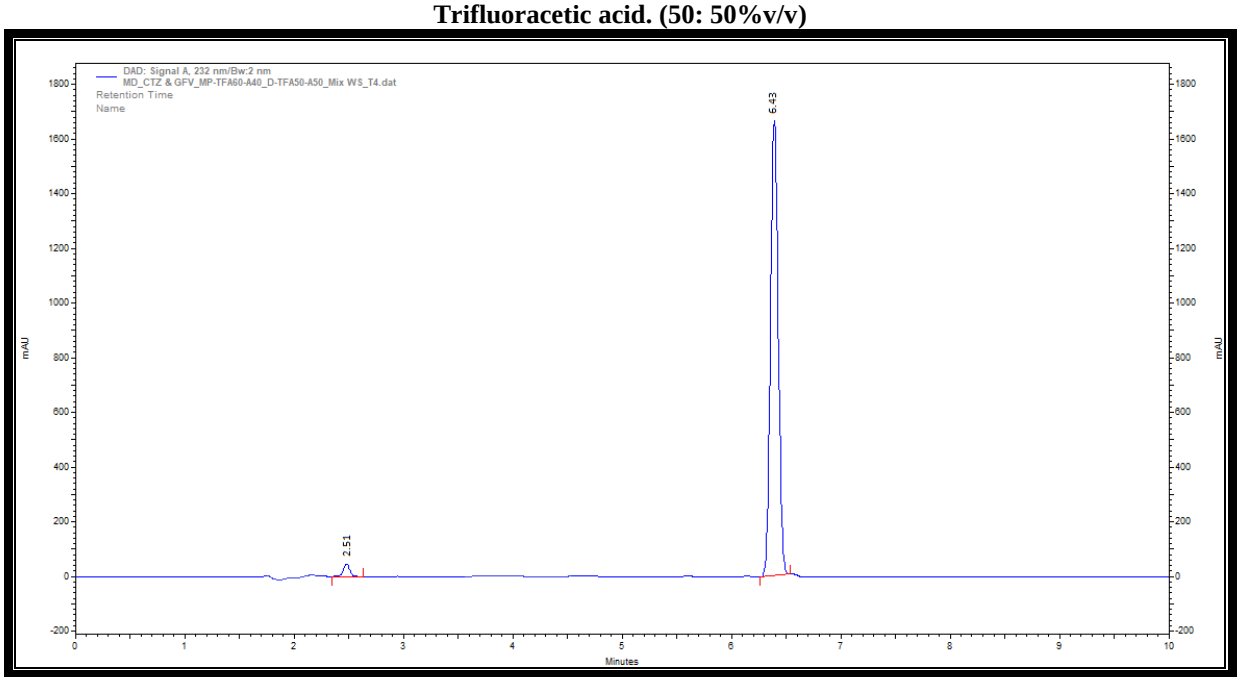
Analysis of marketed formulation: -

Take 5 tablets each tablet contains 85mg of Cetirizine Hydrochloride and 10 mg of Griseofulvin and weighed and powdered from that transfer 5mg of Cetirizine Hydrochloride and 10 mg of Griseofulvin in 10 mL of volumetric flask and make up volume up to 10 mL with help of diluent and further dilution would make final concentration of solution were 50µg/mL and 250 µg/mL respectively. Then solution was filtered by 0.45 µm nylon membrane filter by using vacuum filter. Tablet formulation analysis was carried out as mentioned under section tablet formulation analysis. Procedure was repeated for 5 times. Sample solution was injected and area was recorded for each drug concentration and percentage purity was determined as shown in table -1

Table-1: - Assay result with Cetirizine Hydrochloride & Griseofulvin.

Sr.no	Cetirizine Hydrochloride			Griseofulvin		
	Peak area	Amount recovered in µg/mL	% Recovery	Peak area	Amount recovered in µg/mL	% Recovery
1	369613	44	99.81	17084770	95	100.14
2	371456	43.99	100.46	17120634	96	99.18
3	372217	45	98.45	17364528	96	100.6
4	370389	44	100.77	17328947	96.5	99.87
5	373975	45	99.04	17406325	96.6	100.21
Mean	371530	44.398	99.706	17261041	96.02	100
% RSD	0.45	1.23	0.96	0.85	0.66	0.52

Fig no.3:- Chromatogram of Mixture of Cetirizine Hydrochloride and Griseofulvin using Acetonitrile: 0.1% Trifluoracetic acid. (50: 50%v/v)



Analysis is most important aspect of any drug development; a suitable method must be developed so as to ensure that any drug in dosage from. With help of method development ensure that the amount of particular drug can easily determine. The validation parameter confirms that the developed method is precise, accurate and reproducible and can be used for used for routine evaluation of Cetirizine Hydrochloride and Griseofulvin in combined dosage form.

In the present study suitable for RP-HPLC method was developed with the aim of making detection of Cetirizine Hydrochloride and Griseofulvin more accurate and precise with addition of validation parameters like Specificity, Linearity, Accuracy, and LOD&LOQ.

Method Validation: -

1. **Linearity** :-

   To get desired analyte concentrations the standard solution was created by adding 5 mg of Cetirine Hydrochloride and 10 mg of Griseofulvin in 10 mL of volumetric flask and make up volume up to 10 mL using diluent then further dilution was made to get 4-6µg/mL of Cetirizine Hydrochloride and Griseofulvin 200-300µg/mL respectively. The correlation coefficient for calibration curve Benidipine Hydrochloride and Chlorthalidonewas found to be 1 and 0.999 respectively. The obtained result are shown in Table no.-2&3

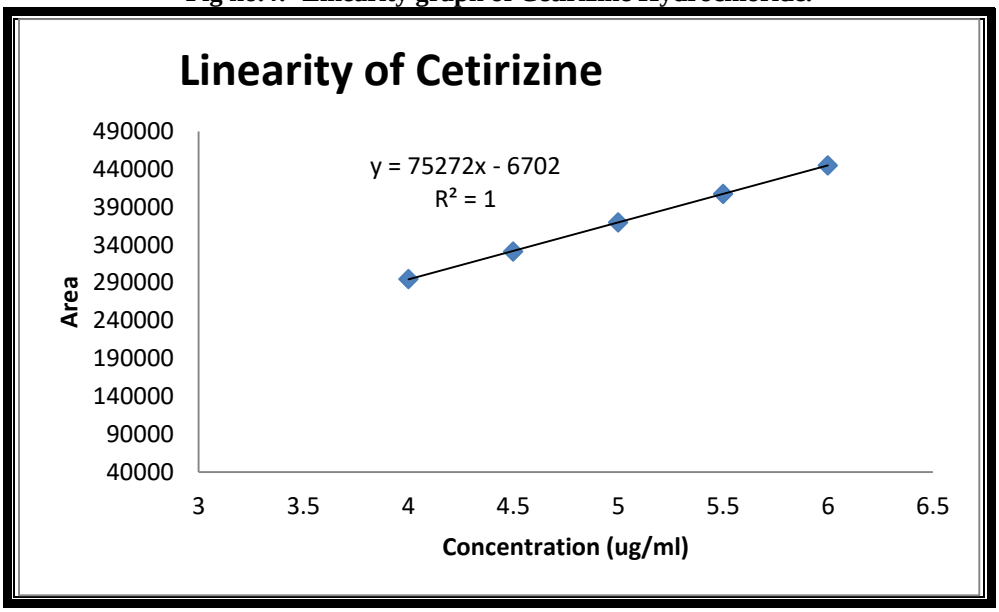
Table no.2: - Concentration and Area of Cetirizine Hydrochloride.

Cetirizine		
% Level	**Conc (ug/ml)**	**Area**
80	4	294827
90	4.5	331454
100	5	369613
110	5.5	407333
120	6	445068

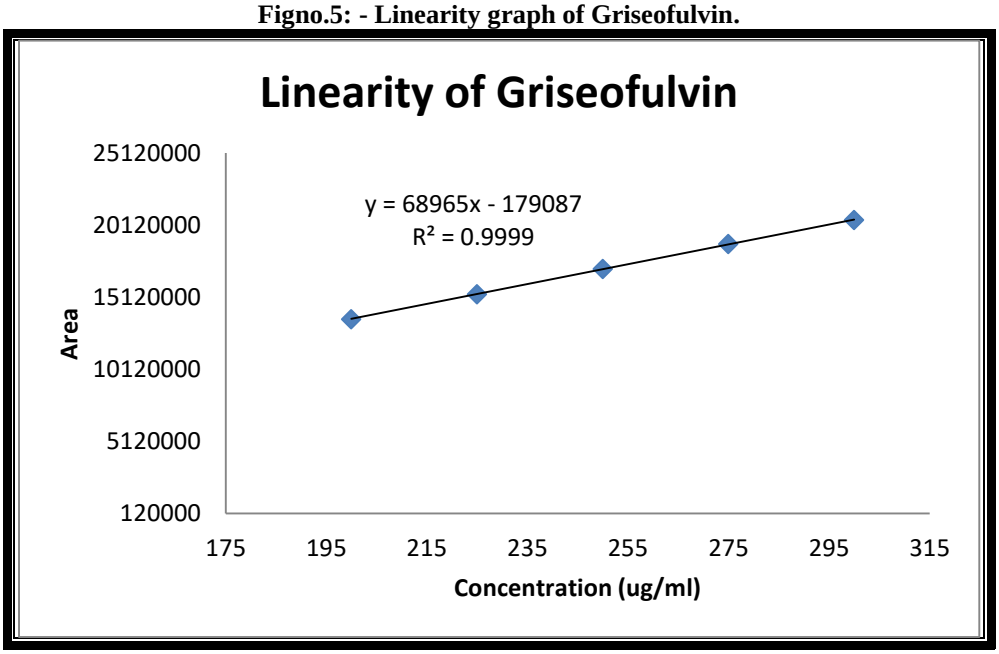
Table no.3: - Concentration and Area of Griseofulvin.

Griseofulvin		
% Level	**Conc (ug/ml)**	**Area**
80	200	13608390
90	225	15323686
100	250	17084770
110	275	18805318
120	300	20488149

Fig no.4:- Linearity graph of Cetirizine Hydrochloride.



Figno.5: - Linearity graph of Griseofulvin.



2 .Precision:-

Precision of an analytical procedure express the closeness of agreement between a series of measurement obtained from multiple sampling of same homologous sample under précised condition.

Intraday precision for Benidipine hydrochloride and Chlorthalidone are shown in Table no. (4&5). The %RSD for Cetirizine Hydrochloride and Griseofulvin was found to be 0.93and 0.15 respectively.

Table no.4: - Precision study of Cetirizine Hydrochloride

Sample ID	100% Rep 1	100% Rep 2	100% Rep 3	100% Rep 4	100% Rep 5	100% Rep 6	Average	STDEV	% RSD
Area	369613	365476	362452	369754	368445	362415	366359	3407.797	0.93

Table no.5: - Precision study of Griseofulvin.

Sample ID	100% Rep 1	100% Rep 2	100% Rep 3	100% Rep 4	100% Rep 5	100% Rep 6	Average	STDEV	% RSD
Area	17084770	17024142	17021463	17065422	17059786	17058864	17052408	24776.35	0.15

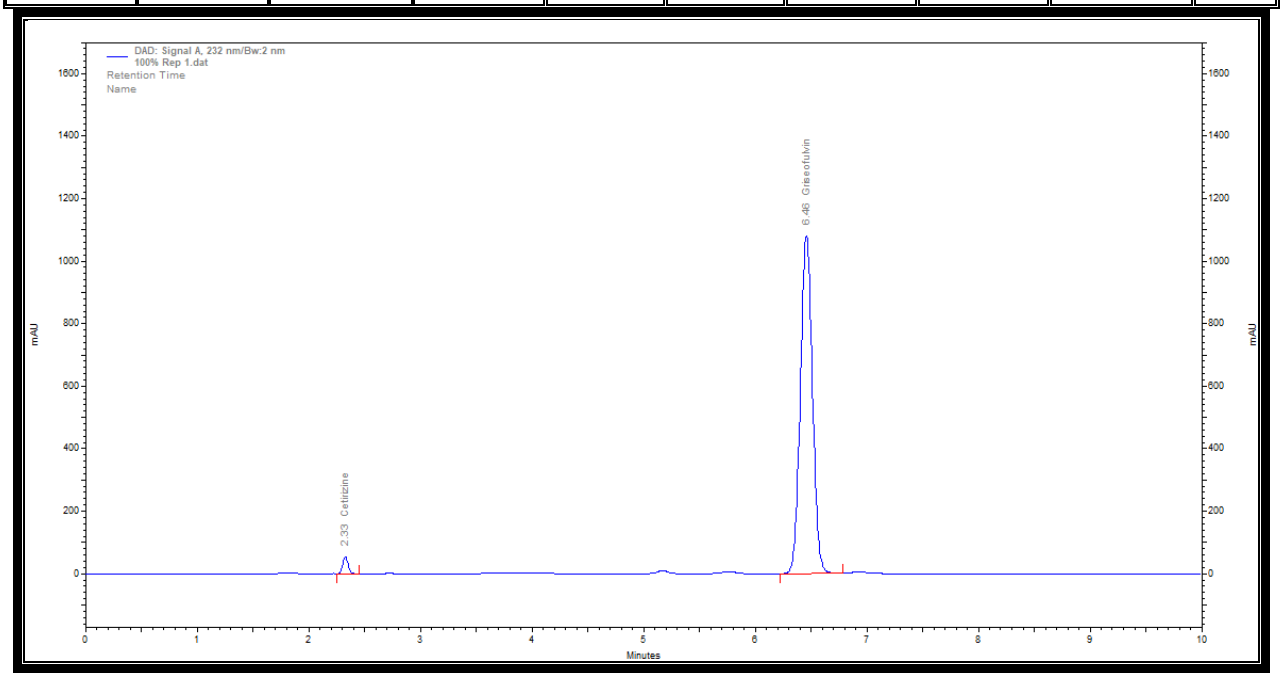


Fig no-6 Repeatability of 1 st Drugs at 100%

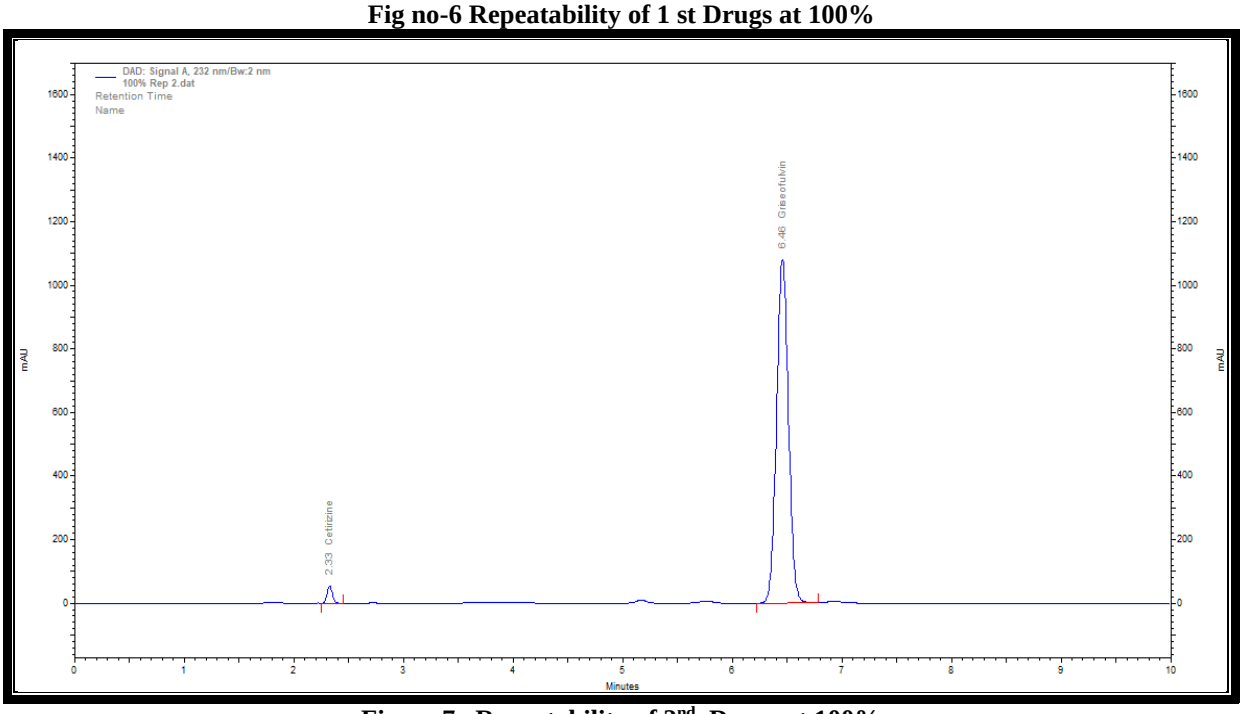


Fig no-7 Repeatability of 2nd Drugs at 100%

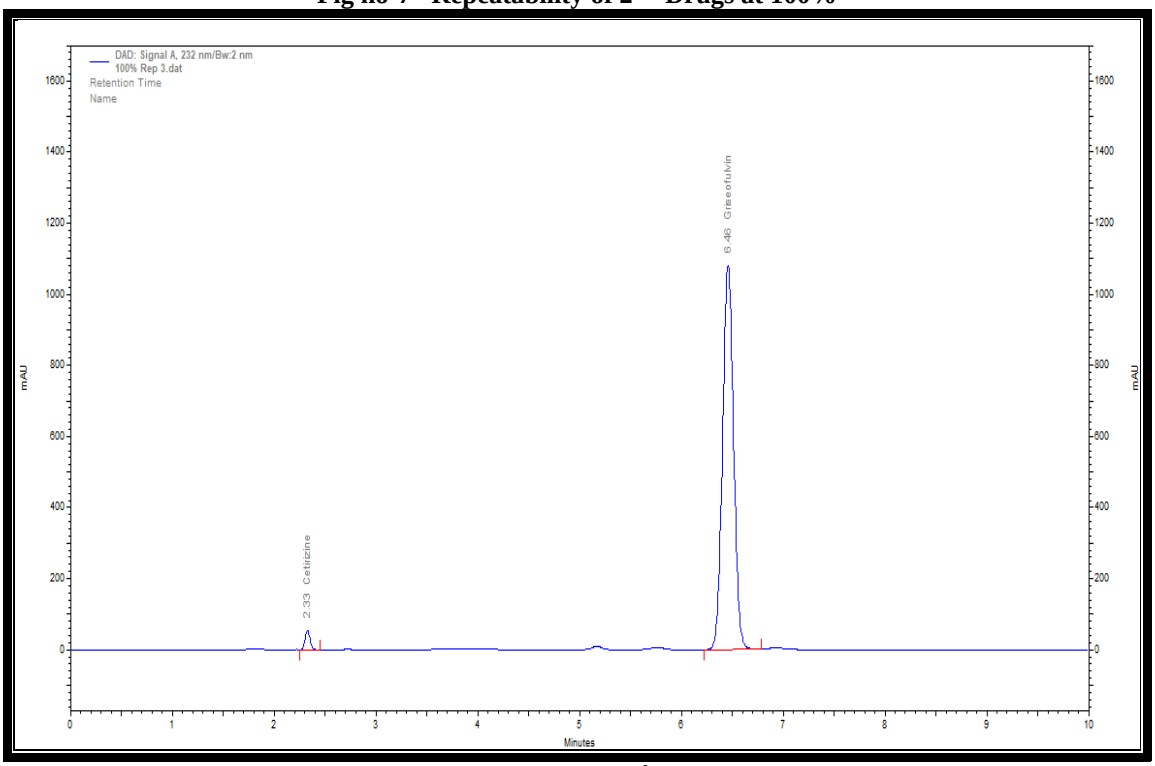


Fig no- 8 Repeatability of 3rd Drugs at 100%

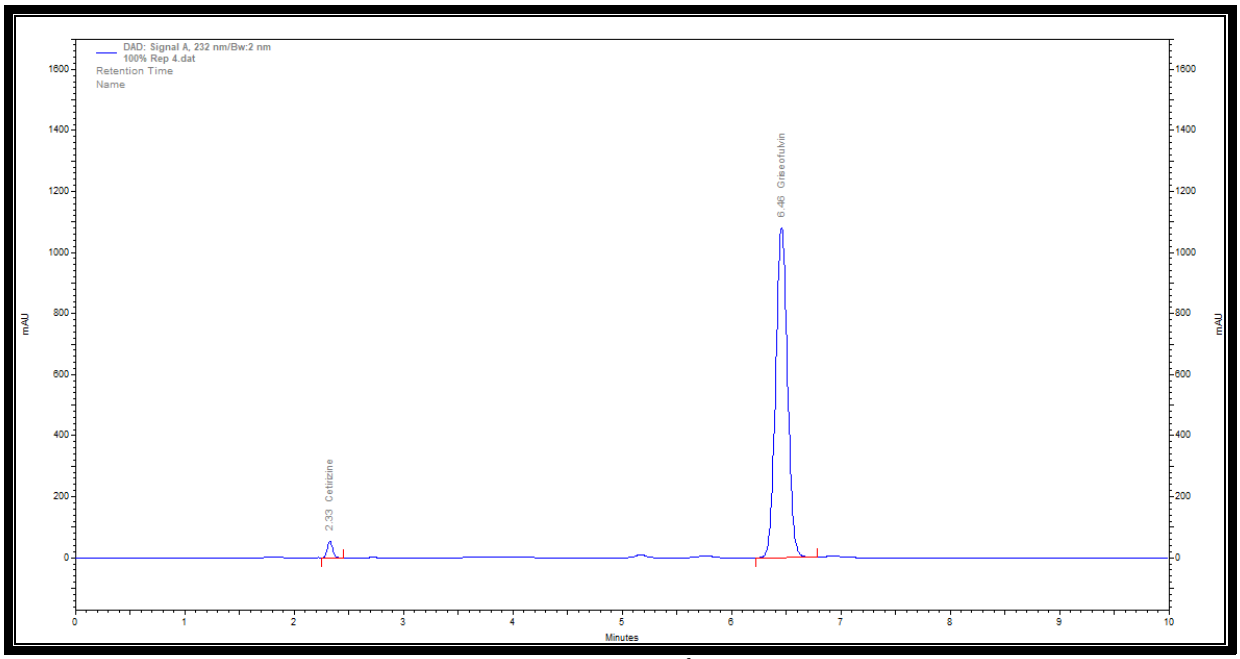


Fig no- 9 Repeatability of 4th Drugs at 100%

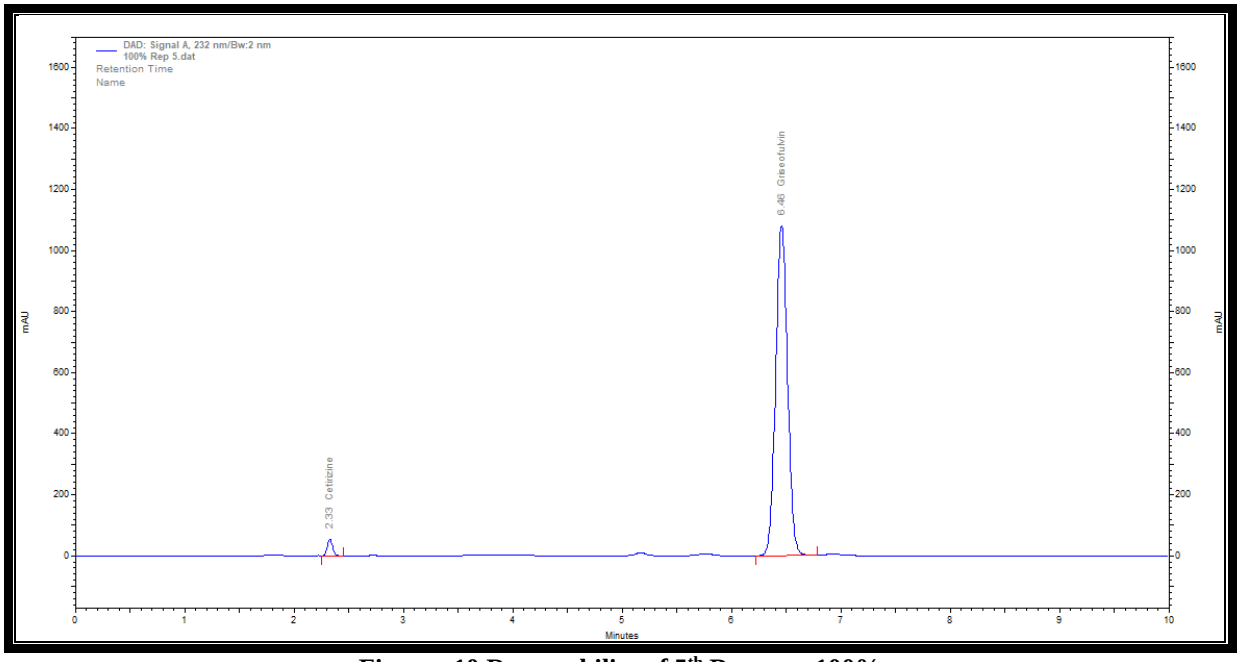


Fig no- 10 Repeatability of 5th Drugs at 100%

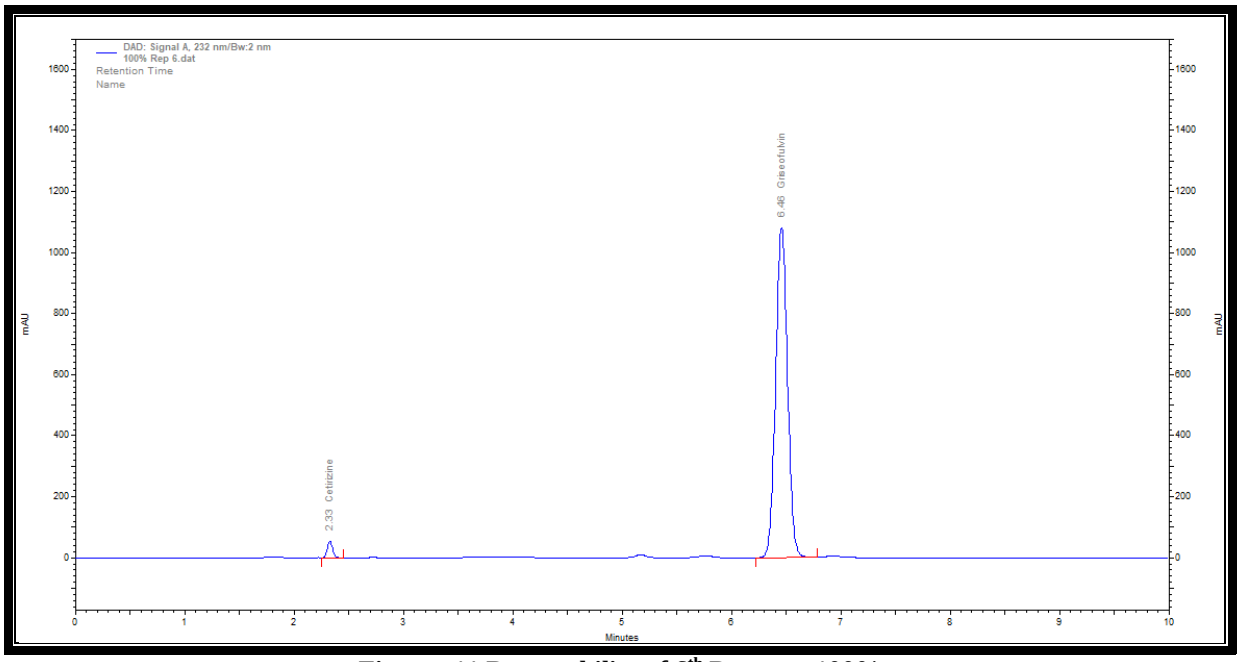


Fig no- 11 Repeatability of 6th Drugs at 100%

3. Accuracy:-

Accuracy is the closeness of agreement between the values found. The value accepted as the conventional true value or the accepted reference value. The accuracy of the method was confirmed by recovery study from the marketed formulation at three level of standard addition. The results are shown in table no. (6&7)

Table no.6- Recovery study of Cetirizine Hydrochloride

% Level	Reps	Spiked Conc (ug/ml)	Area	Amount Recovered (ug/ml)	% Recovery	% RSD
80	Rep 1	4.00	294827	4.02	100.59	0.39
	Rep 2	4.00	293214	4.00	100.04	
	Rep 3	4.00	295413	4.03	100.79	
100	Rep 1	5.00	369613	5.04	100.89	0.98
	Rep 2	5.00	365476	4.99	99.76	
	Rep 3	5.00	362452	4.95	98.93	
120	Rep 1	6.00	445068	6.07	101.24	0.47
	Rep 2	6.00	441012	6.02	100.31	
	Rep 3	6.00	442384	6.04	100.63	

Table no.7- Recovery study of Griseofulvin.

% Level	% Level	% Level	% Level	% Level	% Level	% Level
80	Rep 1	200.00	13608390	199.51	99.75	0.05
	Rep 2	200.00	13614547	199.60	99.80	
	Rep 3	200.00	13621145	199.70	99.85	
100	Rep 1	250.00	17084770	250.47	100.19	0.21
	Rep 2	250.00	17024142	249.59	99.83	
	Rep 3	250.00	17021463	249.55	99.82	
120	Rep 1	300.00	20488149	300.37	100.12	0.20
	Rep 2	300.00	20421478	299.39	99.80	
	Rep 3	300.00	20415587	299.31	99.77	

Fig no.:-6 Chromatogram of 80% Accuracy

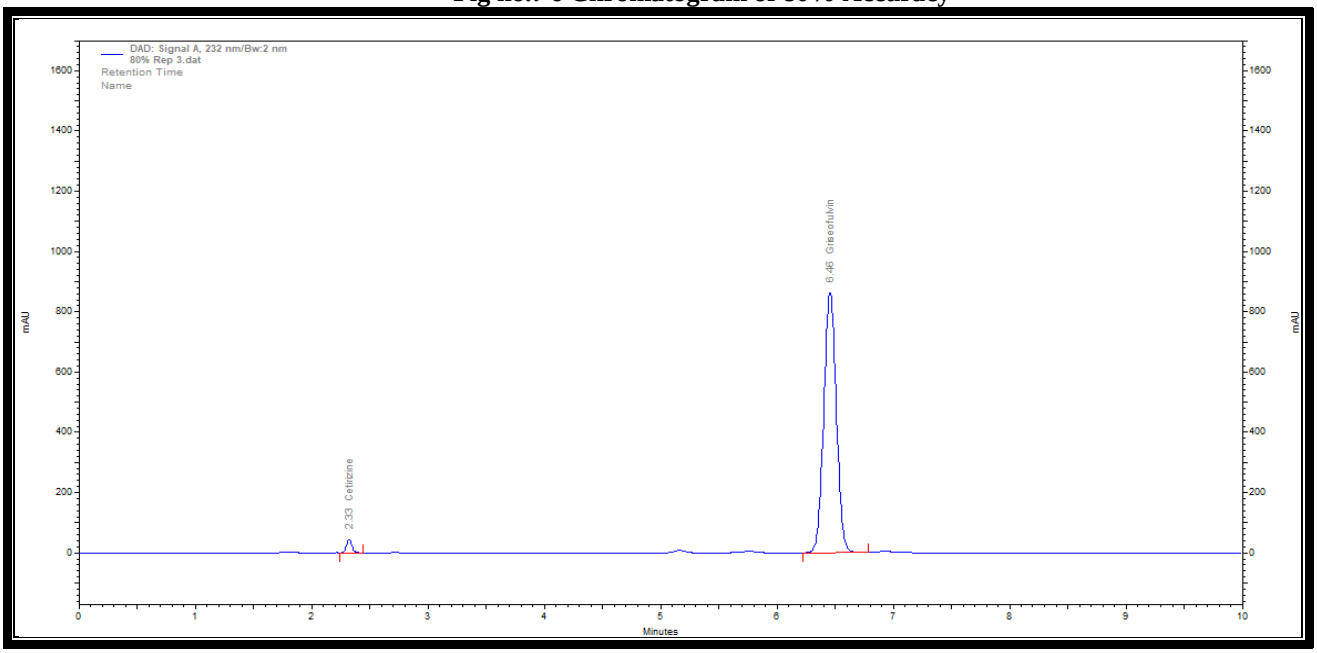


Fig no.:-7 Chromatogram of 100% Accuracy.

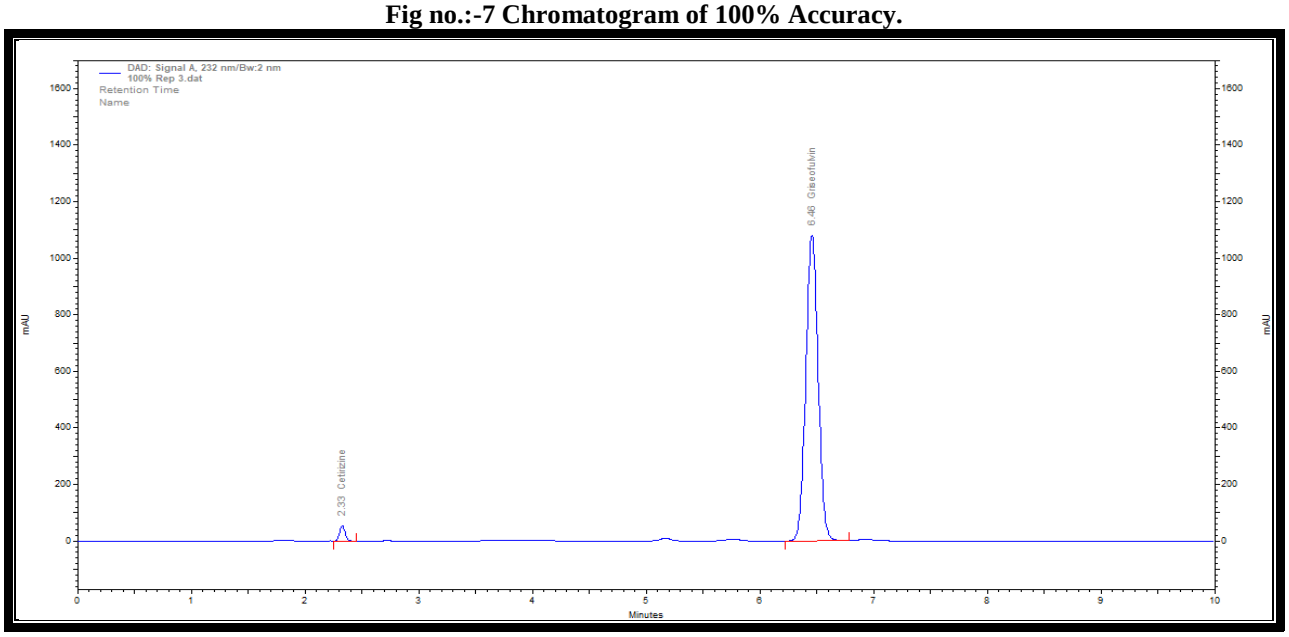
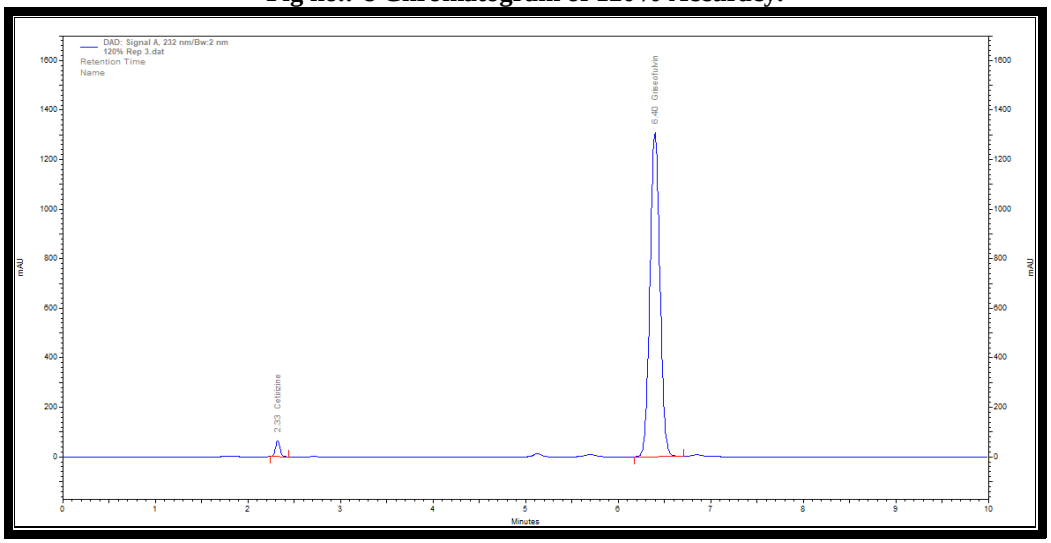


Fig no.:-8 Chromatogram of 120% Accuracy.



4. System suitability parameters: -

Table no 8 -Sysyem suitability parameters

Parameter	Cetirizine Hydrochloride	Griseofulvin
Retention time	2.32	6.41
Theoretical plates	10537	16683
Asymmetry (tailing factor)	1.05	1.02
Resolution	0.00	28.33

5. LOD&LOQ: -

Cetirizine Hydrochloride's LOD and LOQ values were determined to be 0.06 g/mL and 0.18 g/mL, while Griseofulvin's LOD and LOQ values were 3.54 g/mL and 10.71 g/mL, respectively.

Table no -9 LOD&LOQ: -

Sr.no	Name of drug	LOD (µg/mL)	LOQ(µg/mL)
1	Cetirizine Hydrochloride	0.06	0.18
2	Griseofulvin	3.54	10.71

Result and Discussion: -

In this development certain chromatographic conditions are favorable, the created method has been improved. We used methanol with 0.1% t Tifluoracetic acid in a 50:50 v/v ratio, a C-18 column, and obtained good resolution, crisp peak form, etc. The standard sample of Griseofulvin and Cetirizine hydrochloride was scanned using a PDA detector between 200 and 400 nm. Due to the substance Cetirizine Hydrochloride and Griseofulvin's highest absorbance at 232nm, this wavelength was chosen for analysis. HPLC was therefore performed at 232 nm at its absorptive point

For selection the mobile phase, Cetirizine Hydrochloride and Griseofulvin are tested using different mobile Phase like Methanol: Ethanol (50:50 v/v) , Ethyl acetate : Methanol (55:45 v/v), Acetonitrile : Toluene (70 :30), that Acetonitrile: 0.1% Trifluoracetic acid. (50 : 50%v/v), 0.1% Trifluroacetic acid : Ethanol (60:40 v/v) and from that Acetonitrile: 0.1% Trifluoracetic acid. (50: 50%v/v).was found to be satisfactory and well get good peaks for Cetirizine Hydrochloride and Griseofulvin. In this work of RP-HPLC method Agilent RP-HPLC was utilize with Agilent Zorbax Bonus (250 x 4.6 mm,5µ particle size) was used. Flow rate was 1.0 mL/min of mobile phase the sample were detected using PDA detector, and run time was selected up to 10 min. Agilent EZ Chrome software are used during analysis. Equation was used to determine the amount of medication in the commercial formulation (Grisozin). Cetirizine Hydrochloride and Griseofulvin were discovered to be present in amounts of 100.99 and 100.15%, respectively. This technique can be used to analyse Griseofulvin and Cetirizine Hydrochloride on a regular basis.

The co-relation coefficient (R) should not be greater than 1.00, and the Y-intercept limit should be no more than 2% away from the working level's corresponding Y-coordinate. Data indicate that the correlation coefficient and Y-intercept limit are both within acceptable bounds, indicating that the approach is linear.

Precision and repeatability are synonyms. System precision and method precision carried out the precise method. With % RSD less than 2, it was determined that Cetirizine Hydrochloride and Griseofulvin had good precision By using the conventional addition method to calculate the recovery values of Cetirizine hydrochloride and Griseofulvin, the accuracy of the method was assessed. Recovery studies were conducted at various levels of 80%, 100%, and 120%, and an average recovery rate was noted. By adding the same amount of Cetirizine Hydrochloride and Griseofulvin concentrations listed below in the table, samples were made up of 80%, 100%, and 120% concentrations. Samples were used twice to obtain the %RSD.

Cetirizine Hydrochloride's LOD and LOQ values were determined to be 0.06 g/mL and 0.18 g/mL, while Griseofulvin's LOD and LOQ values were 3.54 g/mL and 10.71 g/mL.

Conclusion: -

RP-HPLC METHOD:

Sr no.	Parameters	Cetirizine Hydrochloride	Griseofulvin
1	Linearity Range µg/ml	4-6	200-300
2	Regression Equation (y=mx + c)	75272x - 6702	68965x - 179087
3	Correlation coefficient (r2)	1	0.9999
4	LOD µg/ml	0.06	3.54
5	LOQ µg/ml	0.18	10.71
6	Analysis of Tablet (%Assay)	99.96	99.88
7	% Recovery	100.63%	99.77%
8	Precision (%RSD)	0.93	0.15

Table: RP-HPLC Method

4-6 g/ml and 200-300 g/ml, respectively, are the linearity ranges for Cetirizine hydrochloride and Griseofulvin. For the compounds cetirizine hydrochloride and Griseofulvin, the coefficients (r2) were determined to be 0.9999 and 1, respectively. The LOD and LOQ for Griseofulvin and Cetirizine hydrochloride, respectively, were determined to be 0.06 g/ml and 3.54 g/ml and 0.18 g/ml and 10.71 g/ml, respectively. According to the assay results, there was a 99.96% concentration of

Cetirizine hydrochloride and a 99.88% concentration of Griseofulvin in the medication. For Cetirizine Hydrochloride, the recovery percentage ranges from 100.63% to 99.77%. The method was found to be accurate, with %RSD values for intra-day and inter-day for Griseofulvin and Cetirizine hydrochloride, respectively, being less than 2%. The developed method is, in conclusion, it is concluded that the method established is straightforward, exact, and appropriate for routine analysis. According to ICH rules, the created methods were validated and determined to be within bounds.

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