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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR FENTICONAZOLE NITRATE IN CAPSULE DOSAGE FORM

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ABSTRACT
A reversed-phase liquid chromatographic method has been developed and validated for estimation of Fenticonazole Nitrate in capsule Dosage Form. RP-HPLC method, Column used was 150 x 4.6mm C_{18}, Hypersil BDS with mobile phase containing 10mM phosphate buffer pH 4.5 : Acetonitrile (70%:30%v/v) . The flow rate (1.0 ml/min) and wavelength (255 nm). The retention time was found to Fenticonazole Nitrate was found to be 5.469 ± 0.01 min. Correlation co-efficient for Fenticonazole Nitrate was found to be 0.999. Assay result of marketed formulation was found to be in 99.7 % for Fenticonazole Nitrate. The proposed method was validated with respect to linearity, accuracy, precision and robustness. Percentage recovery for Fenticonazole Nitrate was found to be 100.1 – 100.3%. Analysis proves that the developed method was successfully applied for the analysis of pharmaceutical formulations and can be used for routine analysis of drugs in Quality Control laboratories.

KEYWORDS: Fenticonazole Nitrate, HPLC, Stability indicating method, AMV, ICH, USFDA, Chromatography.

INTRODUCTION
The IUPAC name of the Fenticonazole Nitrate is 1-[2-(2,4-dichlorophenyl)-2-{[4-(phenylsulfanyl)phenyl]methoxy}ethyl] 1*H*-imidazole. With molecular formula and molecular weight $C_{24}H_{20}Cl_2N_2OS,HNO_3$. and 518.4 g/mol respectively.

The molecular structure of the Fenticonazole Nitrate is given in Fig.A

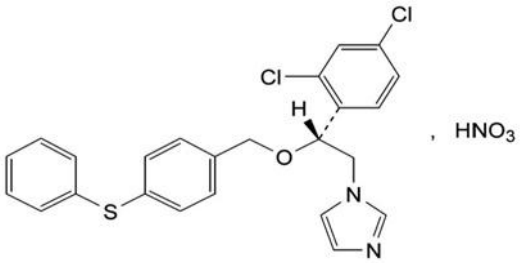

Figure A.

- Fenticonazole Nitrate is used as a antifungal.
- The main mechanism of action of Fenticonazole is based on the inhibition of the synthesis of aspartic protease, a virulence enzyme of fungus.

However no HPLC method has been reported till date for the estimation of Fenticonazole Nitrate using the stability indicating RP-HPLC method in capsule dosage form. The present paper describes the development and validation of stability indicating RP-HPLC method for Fenticonazole Nitrate in capsule dosage form. The proposed method are optimized and validated as per ICH guidelines.

MATERIALS AND METHODS
Materials
a) Instruments
- Analytical Weighing Balance
- Sonicator
- HPLC system
- HPLC software
- pH meter
- Micro balance
- UV Spectrophotometer
- Vacuum oven
- Vacuum Filtration Assembly

b) Glasswares
- Beaker
- Conical flask
- Measuring cylinder
- Pipette
- Volumetric flask

c) Chemicals
- Standard Fenticonazole Nitrate Gifted by Akhil healthcare, Vadodara.

- The commercial fixed dose Fenza 1 Capsule 600mg manufactured by Glenmark Pharmaceutical was procured from local market. All solvents (HPLC grade) were obtained from S.D. fine chemical.

d) Method

- Chromatographic method

METHODS

Working Standard preparation

- **Solution Preparation of Fenticonazole : (60.0 µg/ml)**

About 6 mg of Fenticonazole Nitrate API was weight and dissolve in 50 ml of methanol.

- **Sample Preparation for marketed formulation**

Transferred 1 soft gelatine capsule in to 100mL volumetric flask and added 20-25 mL of water, sonicated for 45 minutes and then it was shaked for 30 minutes by mechanical means, capsules were checked visually if they got dispersed and then volume was made up to mark with methanol and mixed well. The solution was filtered through 0.45 µ PVDF filter. Further 1mL of filtrate was transferred to 100mL of volumetric flask and volume was made up to mark with methanol and it was injected.

METHOD VALIDATION

Chromatographic conditions and System Suitability Parameters

Mode of chromatography: Reversed Phase Chromatography
Mode of Elution: Isocratic
Flow Rate: 1.0 ml/min
Oven: Oven Temperature: 25° ± 2°C
Detector: Type: UV detector
Wavelength: 255 nm
Column: 150 x 4.6mm C_{18}, Hypersil BDS
Sample Volume: 20 µl
Run time: 10 min
Mobile Phase: 10 mM phosphate buffer pH 4.5: Acetonitrile (70:30%v/v)

System Suitability Parameters.

Table 1: System Suitability Test Parameters for Fenticonazole Nitrate.

Sr. No.	System suitability parameter	Fenticonazole Nitrate
1	Retention time (min)	5.469
2	Resolution (R)	-
3	Theoretical plate number (N)	14236
4	Tailing factor (T)	1.0

Linearity and Range (n=3)

- The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analytes in sample within a given range.
- The range of analytical method is the interval between the upper and lower levels of analytes that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.
- The linearity was determined at five levels over the range of 1-5 µg/ml for Fenticonazole Nitrate and 30-90. Peak area of above linearity solution preparations were taken at each concentration three times. Mean Peak Area at each concentration was calculated and Graph of Mean Peak Area (y axis) versus Concentration (x-axis) was plotted.

Precision

Repeatability

Six replicate of 60 ug/ml concentration of Fenticonazole Nitrate were prepared and chromatographic were recorded at the optimized condition. SD and RSD were calculated.

Intraday Precision and Interday Precision

Variations of results within the same day (intra-day), variation of results between days (inter-day) were analyzed. Intra-day precision was determined by analyzing both standard solutions for three times in the same day. Interday precision was determined by analyzing the drugs daily for three days. %RSD was calculated.

Accuracy (% Recovery)

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy 5 tablet powder were weighed and analysis was carried out as per assay. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples. These solutions were subjected to re-analysis by the proposed method and Results are calculated.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the standard deviation of y-intercept of calibration curve (σ) and average of slope (S) of the calibration curve.

$LOD = 3.3 \times \sigma / s$

$LOQ = 10 \times \sigma / s$

Robustness

The robustness of the method was established by making deliberate minor variations in the following method parameter

a) Flow rate: ±0.2 ml/min

b) Change in the ratio of component in the mobile phase: ± 4%.

c) pH of mobile phase: ±4

VALIDATION PARAMETER

Linearity and Range

Linear correlation was obtained between peak area and concentration of Fenticonazole Nitrate in the range of

30-90 µg/ml. The linearity of the calibration curves was validated by the value of correlation coefficients of the regression (r).

Table 2: Linearity data for Fenticonazole Nitrate

% Linearity Level	Concentration (µg/ml)	Mean area	Correlation Coefficient
50	30.0	3483782	0.99999
75	45.0	5233707	
100	60.0	6981977	
125	75.0	8727217	
150	90.0	10518746	

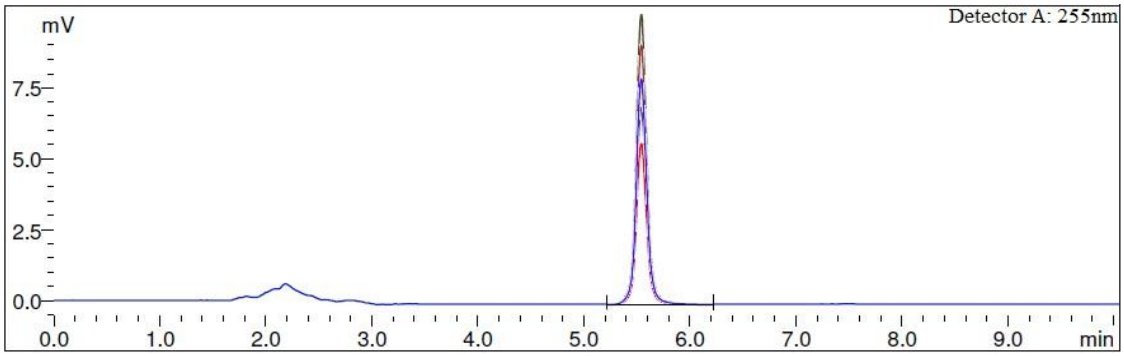

Figure 1: Overlay chromatogram of different concentration of Fenticonazole Nitrate.

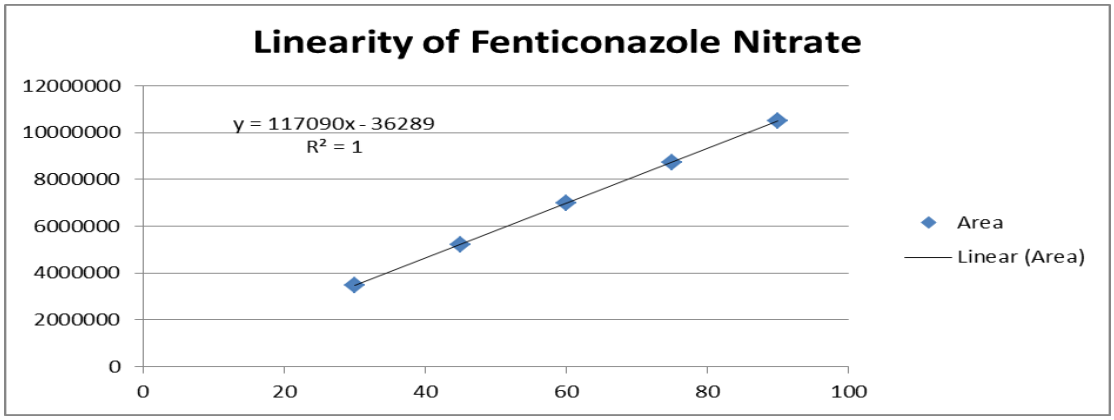

Figure 2: Calibration curve.

ACCURACY

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery for Fenticonazole Nitrate was found to be 100.1 – 100.3%.

Table 3: Recovery Data of Fenticonazole Nitrate.

Accuracy Level %	Set no.	Amount Added (mg)	Amount Recovery (mg)	% Recovery	Mean	% RSD
80	1	2400.00	2387.55	99.5	100.1	0.8
	2	2430.00	2455.07	101.0		
	3	2370.00	2366.62	99.9		
100	1	3030.00	3078.49	101.6	101	0.8
	2	3000.00	3037.81	101.3		
	3	2985.00	2987.07	100.1		
120	1	3615.00	3622.63	100.2	100.3	0.7
	2	3630.00	3616.50	99.6		
	3	3594.00	3630.14	101.0		

PRECISION

Repeatability (Method precision, n=6)

Table 4: Repeatability of Fenticonazole Nitrate.

Sr. no	Area	Mean	SD	%RSD
1	6958699	6957948	18138.8	0.3
2	6948569			
3	6968569			
4	6987415			
5	6948569			
6	6935869			

Repeatability

The data for repeatability of Fenticonazole Nitrate is shown in Table 4. The % RSD for Repeatability data was found to be 0.3%.

Intraday precision

The data for intraday precision for Fenticonazole Nitrate is shown in Table-5. The % RSD For intraday precision was found to be 0.233%.

Table 5: Intraday precision for Fenticonazole Nitrate(n=3)

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	30.0	3479320±8014.1	0.23
2	60.0	6960494±14465.3	0.21
3	90.0	10486379±27703.4	0.26
Mean			0.233%

Interday precision

The data for interday precision for Fenticonazole Nitrate is shown in Table-6. The % RSD For intraday precision was found to be 0.806%.

Table 6: Interday precision for Fenticonazole Nitrate (n=3).

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	30.0	3454793±24854.7	0.72
2	60.0	6915599±53173.2	0.77
3	90.0	10417217±96633.3	0.93
Mean			0.806

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The Limit of detection (LOD) and Limit of quantitation (LOQ) Fenticonazole Nitrate as mention below table 7.

Table 7: Results of LOD and LOQ.

Drug	Fenticonazole Nitrate
LOD	4.383
LOQ	13.28

ROBUSTNESS

Robustness

The method is found to be robust as the results were not significantly affected by slight variation in composition of mobile phase, Mobile phase pH and flow rate of the mobile phase.

Table 8: Change the flow rate

Standard repetitions (n=6)	0.9ml/min Fenticonazole Nitrate	1.1ml/min Fenticonazole Nitrate
Mean Area ±SD	7636355±36982	6320087±18350
% RSD	0.5	0.3

Table 9: Change the mobile phase composition.

Standard repetitions (n=6)	Buffer:Solvent 68:32	Buffer:Solvent 72:28
	Fenticonazole Nitrate	Fenticonazole Nitrate
Mean Area ±SD	6986936±29290	6988675±48204
% RSD	0.4	0.7

Table 10: Change the mobile phase pH.

Standard repetitions (n=6)	4.3	4.7
	Fenticonazole Nitrate	Fenticonazole Nitrate
Mean Area ±SD	6966647± 18992	6965019±16953
% RSD	0.3	0.2

System Suitability tests

Table 11: System Suitability Test Parameters for Fenticonazole Nitrate.

Sr. No.	System suitability Parameter	Fenticonazole Nitrate
1	Retention time (min)	5.469
2	Resolution (R)	-
3	Theoretical plate number (N)	14236
4	Tailing factor (T)	1.0

Assay preparation (Marketed formulation- Fenza)

Label claim: Fenticonazole Nitrate-600mg.

Sample stock solution

Transferred 1 soft gelatine capsule in to 100mL volumetric flask and added 20-25 mL of water, sonicated for 45 minutes and then it was shaked for 30 minutes by mechanical means, capsules were checked visually if they got dispersed and then volume was made up to mark with methanol and mixed well. The solution was filtered through 0.45 µ PVDF filter. Further 1mL of filtrate was transferred to 100mL of volumetric flask and volume was made up to mark with methanol and it was injected.

Working sample preparation

Weigh accurately 6mg of Fenticonazole API and transfer in 100mL of volumetric flask, add 70mL of diluent and sonicate it for about 15minutes to dissolve the API completely. Make the solution up to mark using diluent and mix well to achieve 60µg/mL concentration of Fenticonazole.

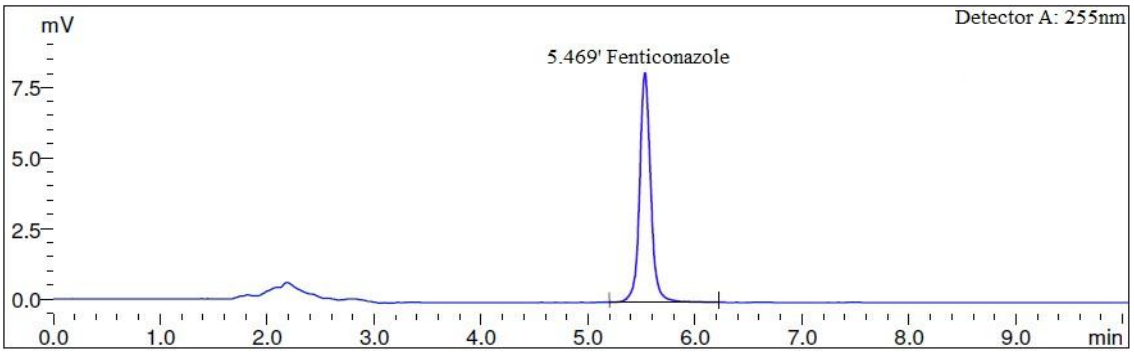

Figure 3: Injection of marketed formulation.

Peak Table

Table 12: Injection of marketed formulation.

Sr. No	Peak name	Retention time	Area	Tailing factor	Theoretical Plates	Resolution time
1	Fenticonazole Nitrate	5.469	6938088	1.0	14236	-

OBSERVATIONS

In formulation sample preparation, peak is found well separated with good peak shape.

% Assay Results from Formulation.
Table 1.

Sr. No.	Sample name	% Assay of Fenticonazole Nitrate
1	Fenza (Fenticonazole Nitrate 600mg)	99.7%

SUMMARY OF REGRESSION PARAMETERS
Table 14: Summary of Regression Parameters for Fenticonazole Nitrate.

Sr. No.	Parameters	Fenticonazole Nitrate	REMARK
1	Linearity (µg/ml)	30-90 µg/ml	Linear
2	%Recovery	100.1-103.3	Accurate
3	Precision (%RSD)	0.3%.	Precise (%RSD < 2)
	Repeatability (n=6)		
	Intra-day (n=3)	0.23%	
	Inter-day (n=3)	0.80%	
4	LOD (µg/ml)	4.383	Sensitive
5	LOQ (µg/ml)	13.28	Sensitive
6	Specificity	Specific	Specific (No interference)
7	Robustness	Robust	(No difference in result)

DISCUSSION

A simple, accurate and precise stability indicating RP-HPLC method for the Fenticonazole Nitrate in capsule Dosage form has been developed and validated. 10mM phosphate buffer pH 4.5: Acetonitrile (70%:30%v/v). Separation of drugs was carried out using mobile phase at 10 min. run time and 255 nm. The Rt value for Fenticonazole Nitrate were found to be 5.469 ± 0.01 min.

The drug response with respect to peak area was linear over the concentration range 30-90µg/ml Fenticonazole Nitrate. The percentage recovery of Fenticonazole Nitrate was found to be 100.1-100.3.

The %RSD values for intra-day precision study and inter-day study were ≤ 2.0%, confirming that the method was sufficiently precise. The limit of detection and limit of quantitation were found to be 4.38µg/ml and 13.28 µg/ml for Fenticonazole Nitrate.

The %RSD values of Robustness study were ≤ 2.0%, confirming that the proposed method was found to be robust enough to withstand such deliberate changes and allow routine analysis of the sample. Interference studies reveals that the common excipients and other additives usually present in the dosage form did not interfere in the proposed method.

So it is concluded that the developed method is specific. The system test parameters were also performed and were found to be within acceptable criteria. The method can be successfully employed for the development and validation of stability indicating RP-HPLC method for Fenticonazole Nitrate in capsule dosage form.

CONCLUSION

A simple, economic, specific and robust RP-HPLC method has been developed and validated for the Development and validation of stability indicating RP-HPLC method for Fenticonazole Nitrate in capsule dosage form. There was no interference from any excipients in the determination of drugs in capsules which indicates the method is specific. All method validation parameters lie within its acceptance criteria as per ICH Q2(R1) guideline so we can conclude that method is Specific, Linear, Accurate and Precise. Hence it can be successfully used for the routine analysis of Fenticonazole Nitrate in capsule dosage form.

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