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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF CHOLINE FENOFIBRATE IN PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC

E. Samyukta and Prathima Srinivas*

Sri Venkateshwara College of Pharmacy and Research Center, Madhapur, Hyderabad-81, Andhra Pradesh, India

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Correspondence to Author:

Dr. Prathima Srinivas

Sri Venkateshwara College of Pharmacy and Research Center, Madhapur, Hyderabad-8, Andhra Pradesh, India

E-mail: drpssvcp@gmail.com

ABSTRACT: A simple, precise, accurate and stability indicating RP-HPLC method was developed and validated for the determination of choline fenofibrate in delayed release tablet dosage form. Separation was achieved under optimized chromatographic conditions on an inertsil ODS column (150 x 4.6 mm, 5µ particle size). The mobile phase consisted of potassium dihydrogen phosphate buffer pH 2.5 and acetonitrile in the ratio 32:68, an isocratic elution at a flow rate of 1.5mL/minute at ambient temperature. The detection was carried out at 300nm using Shimadzu UV-visible detector HPLC system. The retention time of fenofibrate was found at 3.70 minutes and the calibration curve was linear in the concentration range of 18-42µg/mL (r^2=1). The limit of detection and the limit of quantification were found to be 2.40µg/mL and 7.28µg/mL respectively. The amount of fenofibrate present in dosage form was 100.9%. The proposed method was validated as per the ICH guidelines and during recovery studies the % recovery was found to be 98.37%. The method was found to be simple, linear, specific, rugged and suitable for the routine analysis of choline fenofibrate in the delayed release tablet dosage form.

INTRODUCTION: Choline fenofibrate is a choline salt of fenofibric acid, belonging to the class lipid modifying agents. Its chemical name is ethanaminium, 2-hydroxy-N, N, N-trimethyl, 2-{4-(4-chlorobenzoyl) phenoxy] -2-methylpropanoate (1:1) with the following structural formula in **Figure 1**:

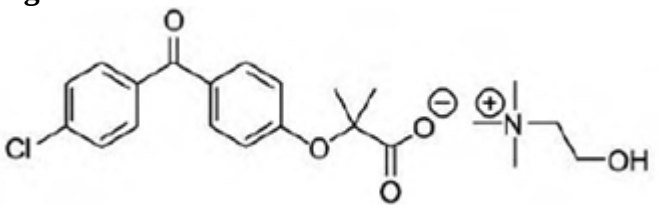

FIGURE 1: STRUCTURAL FORMULA OF CHOLINE FENOFIBRATE

QUICK RESPONSE CODE

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The empirical formula is $C_{22}H_{28}CINO_5$ and the molecular weight is 421.91. Choline fenofibrate is freely soluble in water.

The melting point is approximately 210°C. Choline fenofibrate is a white to yellow crystalline powder, which is stable under ordinary conditions.

EXPERIMENTAL:

Chemicals and reagents: The reference sample and API was a kind gift from MSN Labs**,** Hyderabad, India.

HPLC grade water, methanol, acetonitrile were purchased from E. Merck Co., Mumbai, India, and Potassium dihydrogen ortho phosphate, dipotassium hydrogen, ortho phosphate AR grade were purchased from S D Fine Chem Limited, Mumbai, India.

Apparatus and HPLC conditions: Shimadzu high-pressure liquid chromatography instrument supplied with Inertsil ODS C_{18} column (150x4.6mm; 5µ) with UV detector was used in the study.

1. **Preparation of Buffer solution:** 3.4gm of potassium dihydrogen ortho phosphate was dissolved in 1000mL MilliQ water, pH of this solution was adjusted to 2.5±0.05 with ortho phosphoric acid solution and was mixed well. This solution was then filtered through 0.45µ membrane filters.

2. **Diluents:** Diluent A: Water; Diluent B: Methanol: Water 80:20v/v

3. **Mobile Phase:** Mobile phase was prepared by mixing pH 2.5 buffer solutions and acetonitrile in the ratio 32:68v/v.

Sample Preparation:

1. **Preparation of standard stock solution:** Accurately weighed 100mg of choline Fenofibrate working standard was transferred into a 100mL volumetric flask, to which 50mL of diluent B was added and sonicated to dissolve the material completely, then the volume was made up to 100mL using diluent B. 10mL of this solution was diluted to 100mL using diluent A (stock II).

2. **Preparation of sample solution:** Twenty tablets were finely crushed in a mortar pestle and 100mg equivalent weight of the powder was weighed into a 100mL volumetric flask. To this 50mL of diluent B was added and sonicated to dissolve the material completely. Then the volume was made upto 100mL using diluent B (stock I). 10mL of this solution was diluted to 100mL using diluent A and mixed well.

3. **Calibration:** Five different concentrations (18, 24, 30, 36 and 42µg/mL) of Choline fenofibrate solutions were prepared for linearity studies. The responses were measured as peak areas and plotted against concentration.

4. **Estimation of Choline fenofibrate from tablet formulation:** 10µL standard solution and the sample solution were injected separately into the chromatographic system and the area for the Choline fenofibrate peak was measured. The %Assay was calculated by using the following formula**.**

Amount of Choline Fenofibrate present in the portion of tablet powder (% label claim) =

$$\frac{A2}{A1} \times \frac{W1}{D2} \times \frac{D1}{W2} \times \frac{TW}{LC} \times P$$

Where; A1= average area of standard preparation, A2= average area sample preparation, W1= weight of standard (mg), W2= weight of sample (mg), D1= dilution of standard solution, D2= dilution of sample solution, TW= average weight of tablets, LC=label claim, P= % purity of standard

5. **System Suitability:**

1) Tailing factor for the peak due to Choline Fenofibrate in Standard solution should not be greater than 2.0.
2) Theoretical plates for the Choline Fenofibrate peak in Standard solution should not less than 2500.

Standard chromatogram: The Standard chromatogram is represented in **Figure 2.**

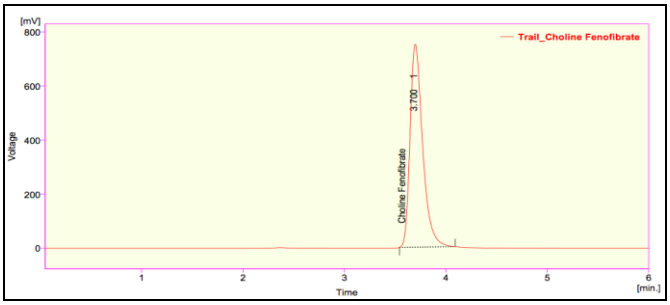

FIGURE 2: STANDARD CHROMATOGRAM OF CHOLINE FENOFIBRATE

Validation Parameters [3, 4, 5]:

1. **Specificity:** The specificity of the test method was demonstrated by studying the interference from diluent and placebo. Specificity of the developed method was determined by injecting 3 replicates of working standard solution (10µg/mL) and 3 replicates of working sample solution (10µg/mL) separately.

2. **Linearity:** From stock solution 18µg/mL, 24µg/mL, 30µg/mL, 36µg/mL and 42µg/mL solutions were prepared and injected into the chromatographic system. A graph was plotted for peak area and concentration (on x-axis concentration and on y-axis peak area) illustrated in **figure 3**. The correlation coefficient value is mentioned in **table 1**. The linearity chromatograms are represented in **figures 4-8**.

TABLE 1: LINEARITY READINGS

Conc. (µg/mL)	Area
18	812.57
24	1083.42
30	1354.28
36	1625.14
42	1896
Correlation coefficient (r^2)	1.00

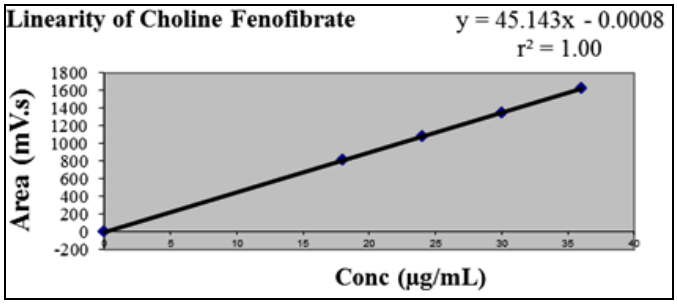

FIGURE 3: CALIBRATION CURVE OF CHOLINE FENOFIBRATE

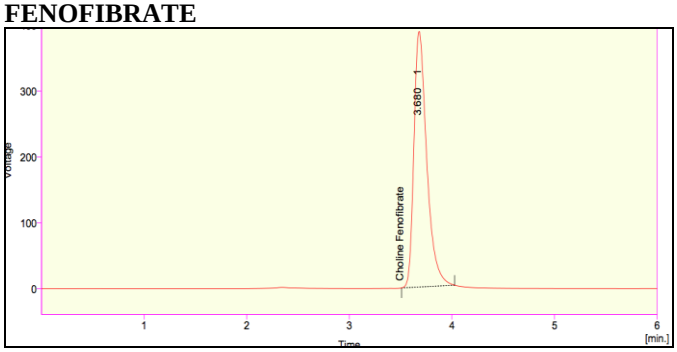

FIGURE 4: LINEARITY SAMPLE 01- 18µg/mL

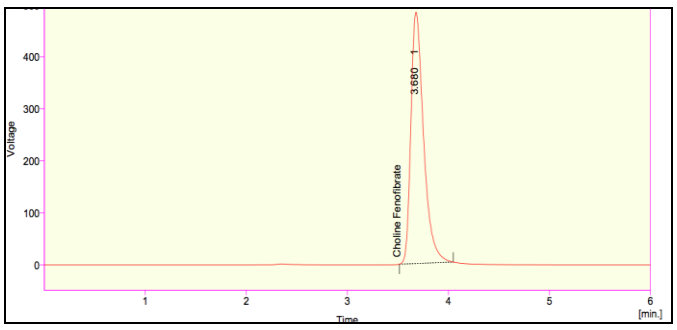

FIGURE 5: LINEARITY SAMPLE 02 - 24µg/mL

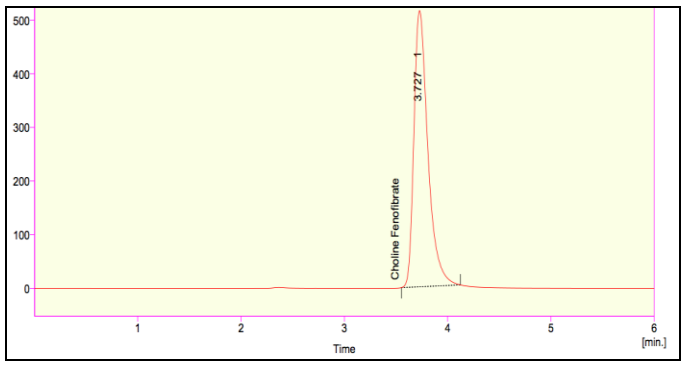

FIGURE 6: LINEARITY SAMPLE 03- 30µg/mL

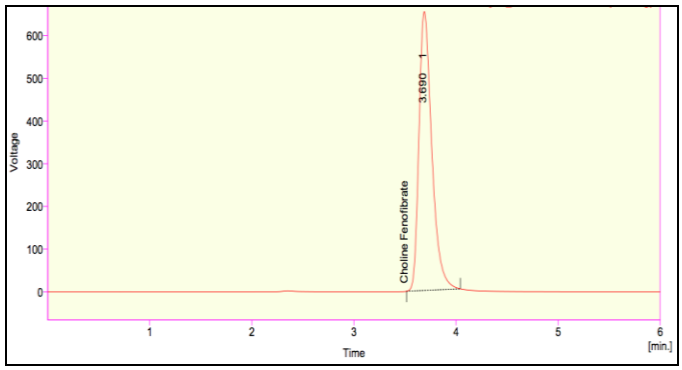

FIGURE 7: LINEARITY SAMPLE 04- 36µg/mL

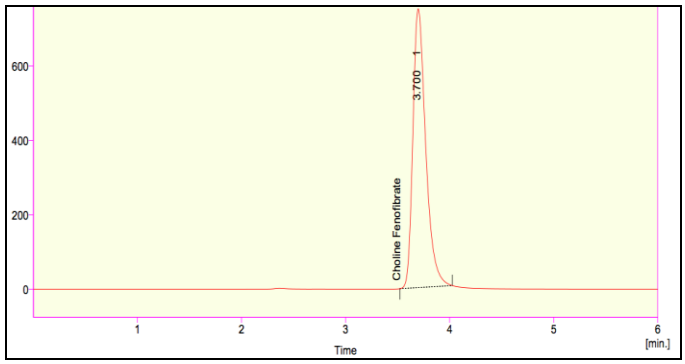

FIGURE 8: LINEARITY SAMPLE 05- 40µg/mL

3. **Accuracy:** Accuracy -80%, Accuracy - 100% and Accuracy -120% solutions prepared using the standard stock solution and injected separately into the chromatographic system. The amount found and amount added for Choline fenofibrate and the individual recovery and mean recovery values were Calculated and tabulated in **Table 2**.

4. **Precision:**

   a. **System Precision:** System precision was evaluated by injecting standard solution six times and the %RSD was calculated and tabulated in **Table 3**.

TABLE 2: ACCURACY READINGS

Level	Spiked level (mg)	Amount Recovered (mg)	% Recovery	Average Area	% RSD
1	80% [80+20]	98.96	98.96	4828	0.17
2	100% [100+20]	117.72	98.10	5688	0.21
3	120% [120+20]	137.26	98.06	6414	0.82

TABLE 3: SYSTEM PRECISION READINGS

Injection No	Standard Area
1	4771
2	4864
3	4845
4	4825
5	4804
6	4822
Average	4822
SD	32.17
% RSD	0.67

b. **Repeatability:** Repeatability of the method was demonstrated by preparing six different sample preparations as per test method representing a single batch. Assay of these samples was determined and the precision of the method was evaluated by computing the %RSD of the assay results are represented in **table 4**.

TABLE 4: REPEATABILITY READINGS

Injection No.	Standard Area
1	4910.10
2	4865.32
3	4883.83
4	4856.69
5	4856.40
6	4771.76
Average	4857.35
SD	46.63
% RSD	0.96

5. **Intermediate Precision/ Ruggedness:** To evaluate the ruggedness of the method, different analysts performed precision on different days. The values of the relative standard deviation of five replicate injections of the standard solutions were calculated and represented in **table 5 and 6**.

TABLE 5: ANALYST 1 READINGS

Injection No	Area
1	4771.764
2	4864.089
3	4845.742
4	4825.584
5	4804.118
6	4822.145
Average	4822.240
%RSD	**0.66**

TABLE 6: ANALYST 2 READINGS

Injection No	Area
1	4910.105
2	4865.312
3	4883.831
4	4856.694
5	4856.404
6	4771.764
A Average	4857.352
% RSD	**0.96**

6. **Limit of Detection:** The limit of detection was calculated using the formula:

LOD= 3.3* S.D/Slope

The slope and standard deviation (SD) values were calculated using the linearity graph and the limit of detection was found to be 2.40µg/mL.

7. **Limit of Quantification:** The limit of quantification was calculated using the formula:

LOQ = 10* S.D/Slope

The slope and standard deviation (SD) values were calculated using the linearity graph and the limit of quantification was found to be 7.28µg/mL.

8. **Robustness:** As part of the Robustness, deliberate changes were made in the Flow rate and wavelength to evaluate the impact on the method.

a) The flow rate of 1.3 mL/min and 1.7 mL/min were considered. Standard solution 10 µ/mL

was prepared and analyzed using the varied flow rates along with method flow rate.

b) The wavelength was varied from 298nm and 302nm. Standard solution 10 µ/mL was prepared and analyzed using varied wavelengths along with method wavelength.

When flow rate and wavelength were varied it was found that the method was not affected significantly. Hence it indicates that the method is robust even by change in the flow rate (±0.2%) and wavelength (±2nm of λ_{max}). The results are represented in **table 7**.

TABLE 7: ROBUSTNESS READINGS

Condition	Theoretical Plates (NLT 3000)	Asymmetry (NMT 2.0)
Wavelength 298nm	4399	1.679
Wavelength 302nm	4439	1.786
Flow rate 1.3mL	4367	1.78
Flow rate 1.7mL	4048	1.56

9. **Stability of solution and the mobile phase:** The solution stability of Choline fenofibrate was determined by leaving test solution and standard solutions in tightly capped volumetric flasks at room temperature for up to 48 hrs and measured the drug content at every 24 hrs against freshly prepared standard solution. The stability of the mobile phase was also determined by freshly prepared solutions of Choline fenofibrate at 24 hrs and for 48 hrs interval. The mobile phase was not changed during the study. The variability in the estimation of drug content was within ± 10% during solution stability and mobile phase stability. The results from solution stability and mobile phase stability experiments confirmed that sample solution; standard solution and mobile phase were stable up to 48 hrs. The results obtained are represented in **Table 8**.

TABLE 8: SOLUTION STABILITY READINGS

Time (hours)	Bench top stability	Refrigerator stability	Mobile phase stability
	% Assay of Choline Fenofibrate	% Assay of Choline Fenofibrate	% Assay of Choline Fenofibrate
Initial	102	102	102
After 24 hours	102	102	102
After 48 hours	102	102	102

10. **Forced degradation studies:** Stress studies were performed using Choline fenofibrate 135 mg tablet to provide an indication of the stability-indicating property and specificity of proposed method. The stress conditions employed for degradation study included acid hydrolysis (1 N HCl at 60°C for 30 mins), base hydrolysis (1N NaOH at 60°C for 30 mins), oxidation (10% H_2O_2 at room temperature for 24hrs), thermal (105°C for 30 mins), and photolytic degradation (drug product exposed to UV radiation for 2 hrs).

11. **Stability studies:**

a. **Accelerated stability studies:** These were performed on both API and tablet dosage form for 6 months under two conditions: 25°C / 60% RH and 40°C/75% RH. The results obtained at the end of the period are tabulated in **table 9**.

TABLE 9: ACCELERATED STABILITY STUDIES READINGS

Condition	API/Tablet	Rt	Area	Theoretical plates	Asymmetry	%assay
CONTROL	API	3.66	3495	6144	1.50	101
CONTROL	TABLET	3.62	3576	6011	1.60	101
25° C / 60% RH	API	3.63	3495	6428	1.50	99.25
25° C / 60% RH	TABLET	3.62	3570	6404	1.56	99.49
40° C / 75% RH	API	3.62	3523	6404	1.50	98.20
40° C / 75% RH	TABLET	3.62	3546	6392	1.56	98.82

RESULTS AND DISCUSSION:

System suitability results:

1) Tailing factor obtained from the standard injection is 1.60

2) Theoretical Plates obtained from the standard injection is 4266.

Assay Results: % Assay of Choline fenofibrate delayed release tablets = 100.9

DISCUSSION:

1. The diluent and excipients did not interfere with the drug peaks and thus the method was specific. The HPLC chromatograms recorded for the diluent and drug matrix (mixture of the drug and excipients) showed no interfering peaks within retention time ranges.

2. The method was found to be linear in the range of 18µg/mL, 24µg/mL, 30µg/mL, 36µg/mL and 42µg/mL concentration with r^2=1.

3. The recovery results were in the range of 98-102%. The study proves that the method was accurate.

% Recovery at 80% level= 98.96, % RSD=0.17

% Recovery at 100% level= 98.10, % RSD=0.21

% Recovery at 120% level= 98.06, % RSD=0.82

4. The % RSD for six replicate standard injections of Choline Fenofibrate during system precision was 0.67.

5. The % RSD for six replicate standard injections of Choline Fenofibrate during repeatability was 0.96.

6. The %RSD of analyst 1 was 0.56 and analyst 2 was 0.69 during intermediate precision studies.

7. By changing flow rate and wavelength and the method was found pass system suitability parameters.

8. The LOQ was found to be 2.40µg/mL and LOD was 7.28µg/mL.

9. % Assay values are found between 98.0 and 102.0 and the results obtained meet the system suitability requirements, which indicate that the analyte solutions and mobile phase are stable up to 48 hours.

10. No degradation was observed under any stress conditions and No interferences due to any degradation products were seen in the main peak.

11. The dosage form was found to be stable during accelerated stability studies with acceptable assay % between 98-102.

CONCLUSIONS: A simple and efficient stability indicating RP-HPLC method was developed and validated for quantitative analysis of Choline fenofibrate in delayed release tablet dosage form. The method was found to be precise, accurate, linear, robust and rugged during validation. Satisfactory results were obtained from the validation of the method. Stability of the analyte solutions and mobile phase were found to be stable upto 48hrs and no significant change in %assay was observed during accelerated stability studies.

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