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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF CHOLINE FENOFIBRATE IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM

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Choline fenofibrate, RP-HPLC, Capsules, Method validation

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ABSTRACT: A simple and precise RP-HPLC method was developed and validated for the determination of Choline fenofibrate in pharmaceutical dosage forms. Chromatography was carried out on Agilent make Zorbax C_{18} column (4.6 mm x 15 cm), 5 µ particle size using a mobile phase of phosphate buffer (pH 6.8) : acetonitrile (70 : 30 % v/v) at a flow rate of 0.8 ml/min. The analyte was monitored using UV detector at 298 nm. The retention time was found to be 3.207 min for Choline fenofibrate. The proposed method was found to be having linearity in the concentration range of 5-35 µg/ml with correlation coefficient 0.999. The mean recoveries obtained for Choline fenofibrate were in the range of 99.4-100.65 %. The developed method has been statistically validated according to ICH guidelines and found to be simple, precise and accurate with the prescribed values. Thus the proposed method was successfully applied for the estimation of Choline fenofibrate in routine quality control analysis in bulk and its formulations.

INTRODUCTION: Choline fenofibrate is a choline salt of fenofibric acid. It is chemically (**figure 1**) known as [1-({2-[4-(4-Chlorobenzoyl) phenoxy]-2- methylpropanoyl} oxy)-2-hydroxy ethyl] trimethylazanium and its empirical formula is $C_{22}H_{28}ClNO_5$ and molecular weight is 421.91. It is an Anti lipedimic agent and belong to a group of fibrates, used to lower low density lipoproteins (LDL) and increases high density lipoproteins (HDL).

It is an active ingredient in the cholesterol medication generally known as fenofibric acid, it is used for the management of high LDL and cholesterol help reduce triglycerides and increase the quantity of HDL cholesterol in the blood [1].

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This medication is also used to reduce the level of fatty acids in the blood. The fenofibric acid contains a carboxylic moiety instead of an ester moiety which is an important role in formation of rare acid to ketone hydrogen bond type packing interaction. It is sold under brand names Trilipix and Fibricor.

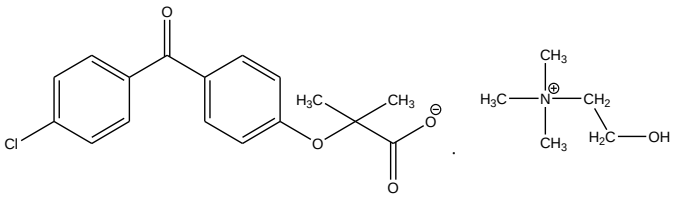

FIG. 1: STRUCTURE OF CHOLINE FENOFIBRATE

Literature survey reveals that the fenofibrate in both single and simultaneous with other drugs can be estimated by HPLC in biological fluids [2-3], RP-HPLC in pharmaceutical dosage forms [4-8], UPLC [9] and spectrophotometric method [10] but no RP-HPLC method for the estimation of Choline fenofibrate in pharmaceutical dosage forms have not been reported so far.

The objective of this study is to develop a simple, fast, economical, selective, accurate, precise and sensitive RP-HPLC-UV method for the determination of Choline fenofibrate in bulk and its pharmaceutical dosage forms suitable for routine quality control analysis.

MATERIALS AND METHODS:

Chemicals and solutions: HPLC grade methanol and acetonitrile (MERCK) were used for the analysis, potassium dihydrogen ortho phosphate (AR grade SD Fine-chem. Ltd, Mumbai) were used. All solutions were filtered through 0.45 µm nylon membrane filter using vacuum pump.

Instrumentation: Quantitative HPLC was performed on Agilent technologies1200 series, PDA detector module equipped with auto injector with Ezchrome elite software. A reverse phase Agilent Zorbax Eclipse XDB C_{18} (150 x 4.6mm, particle size 5µm) analytical column was used. Weighing was done on Schimadzu balance (Model AX 200).

Chromatographic conditions: Preliminary studies were conducted and trails are made for the method development. Separation and analysis was carried out on Agilent Zorbax C_{18} column (4.6 x 15 cm), 5 µ particle size. The optimized mobile phase consisting of methanol and phosphate buffer (pH adjusted to 6.8 with triethylamine) in the ratio of 70:30 % v/v, filtered through 0.45 µm nylon membrane filter using vacuum pump. Flow rate was maintained at 0.8 ml/min and run time for 10 min, prior to sample injection, column was saturated with mobile phase for 40 min and injection volume was 10 µl injected by auto sampler. The detection response was measured at 298 nm and maintained at ambient temperature.

Preparation of standard stock solution: Accurately weighed and transferred 10 mg of Choline fenofibrate working standard into a 100 ml clean dry volumetric flask, added 70 ml methanol to dissolve completely and made the volume up to the mark with the methanol to obtain concentration of 100 µg/ml.

Preparation of sample stock solution: 20 capsules of choline fenofibrate (Average weight = 270.72 mg, Label claim = 135 mg) were accurately weighed and powdered using a motor and pestle. 20.05 mg of powder was taken which is equivalent to 10 mg of Choline fenofibrate was accurately weighed & transferred into a 100 ml volumetric flask. To this 70 ml methanol was added and sonicated for 15 min and then made up the volume with methanol to obtain a final concentration of 100 µg/ml.

Method validation:

1. **System suitability:** Standard solution was injected six times into system and chromatograms were recorded, % RSD (relative standard deviation) of retention time & peak area, theoretical plates and tailing factor were calculated.

2. **Accuracy:** Accuracy was determined in terms of % recovery. Sample solutions were prepared at three different concentration levels 50 %, 100 % and 150 %. Predetermined amount of standard was added to these solutions by spiking standard drug solution to the sample. % recovery was calculated by assaying these solutions.

System precision and method precision: The system and method precision of the proposed method is ascertained by injecting 6 replicates of test and standard sample, % RSD were calculated.

1. **Specificity:** Standard solution, sample solution, blank solution and placebo solution were injected simultaneously into the system and chromatograms were recorded.

2. **Linearity:** From the above standard stock solution, aliquots of 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml, 30 µg/ml and 35 µg/ml were prepared and injected simultaneously into the system and chromatograms were recorded. Graph was plotted between concentration V_s peak area, R^2 was calculated.

Robustness and Ruggedness: Robustness was carried out by changing small variations in method parameters like mobile phase composition (± 5 % organic phase), flow rate (± 0.2 ml/min), wave length (± 2 nm) were considered. Ruggedness wad done by studying changes with variation of analyst

to analyst, column to column and provides an indication of its reliability.

RESULTS AND DISCUSSION: Choline fenofibrate is not official in any pharmacopoeia and no RP-HPLC methods were reported for the estimation of this drug in pharmaceutical dosage forms. The availability of commercial formulations containing this drug and RP-HPLC methods are limited. Hence, we planned to develop a validated RP-HPLC method for the estimation of choline fenofibrate in pharmaceutical dosage form.

From this study, it was found that a simple, precise, accurate, sensitive and efficient RP-HPLC method has been developed and validated for the estimation of choline fenofibrate in pharmaceutical dosage form. Separation was done by using mobile phase composed of phosphate buffer (pH 6.8) and methanol in the ratio (70: 30 % v/v).

Chromatographic separation were carried out on Agilent Zorbax C_{18} column (4.6 mm x 15 cm) 5 µ particle size at a flow rate 0.8 ml/min using UV detection at 298 nm. The retention time was found to be 3.2 min.

Linearity was evaluated in the concentration range of 5-35 µg/ml. The calibration curve was described by the equation Y = 78607x + 25899 with correlation coefficient 0.999 as shown in **figure 2**. System suitability parameters as shown in **table 1**.

The % RSD in precision, accuracy and robustness studies were found to be less than 2.0 %, indicating that the method is precise, **accurate and robust. Accuracy data as shown in table** 2. The assay results and validation summary obtained from the marketed formulations are given in **table 3**.

TABLE 1: SYSTEM SUITABILITY RESULTS

S. No.	Name	Retention time (min)	Area (mV.s)	Efficiency (th.pl)	Asymmetry
1	Choline fenofibrate	3.30	1708538	2257	1.08

TABLE 2: ACCURACY RESULTS

Sample	Accuracy	Peak area	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Over all mean % recovery
Choline Fenofibrate	50 %	814236	10	10.02	100.2 %	MEAN = 99.73
	50 %	807256	10	9.94	99.4 %	S.D = 0.416
	50 %	809138	10	9.96	99.6 %	% RSD = 0.417
	100 %	1608538	20	20.13	100.65 %	MEAN = 101.38
	100 %	1628025	20	20.38	101.9 %	S.D = 0.65
	100 %	1623424	20	20.30	101.6 %	% RSD = 0.641
	150 %	2385234	30	30.01	100.03 %	MEAN = 100.73
	150%	2408113	30	30.30	101 %	S.D = 0.611 %
	150 %	2412110	30	30.35	101.16 %	RSD = 0.606

System precision and method precision: The % RSD values were found to be 1.279 and 1.878.

Linearity: R^2 values was found to be 0.999 and regression equation Y = 78607x + 25899.

Specificity: The chromatograms of standard and sample were identical to each other as shown in **figure 3 and 4**. The blank and placebo injections were also identical without any interference from the excipients.

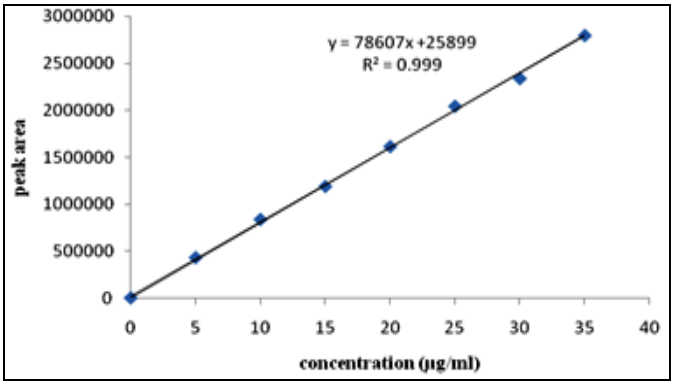

FIGURE 2: LINEARITY GRAPH OF CHOLINE FENOFIBRATE

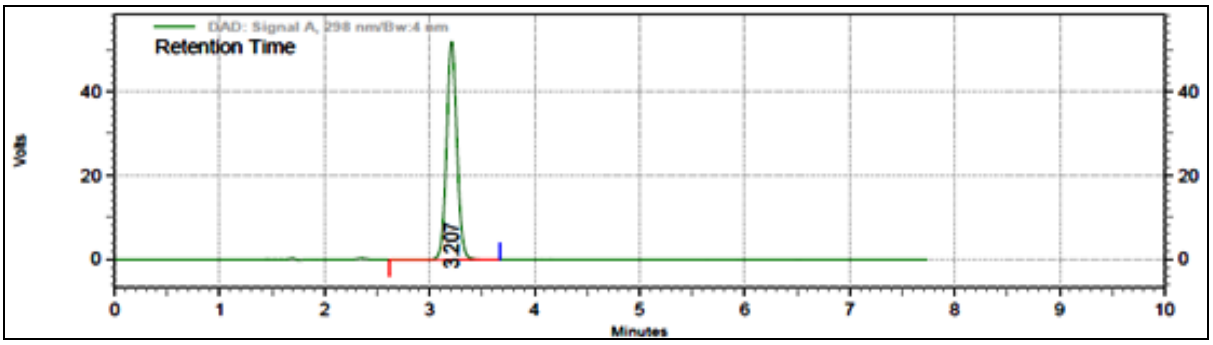

FIGURE 3: CHROMATOGRAM OF STANDARD SOLUTION

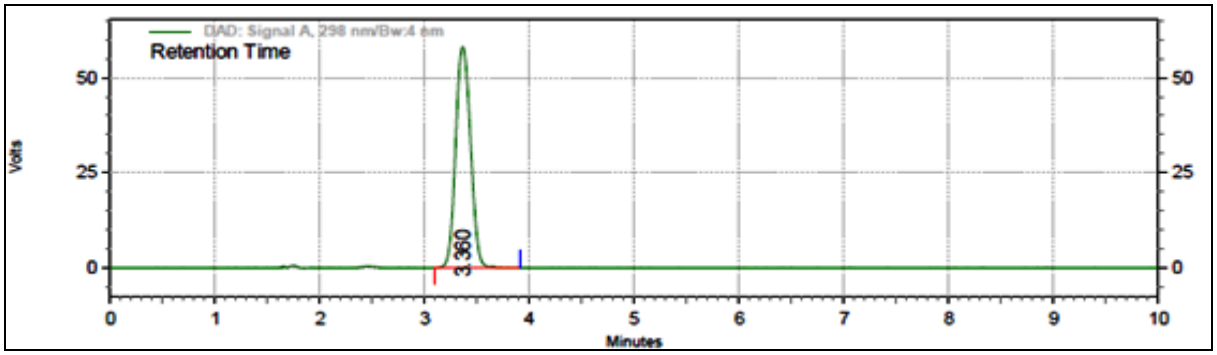

FIGURE 4: CHROMATOGRAM OF SAMPLE SOLUTION

TABLE 3: RESULTS OF SUMMARY OF VALIDATION PARAMETERS

S. No.	Parameter	Results			
1.	System suitability	The retention time for choline fenofibrate peak is 3.20.			
2.	Accuracy	Mean % recovery is 99.4 - 101.9 %.			
3.	System precision	% RSD = 1.279.			
4.	Method precision	% RSD = 1.878.			
5.	Linearity	The correlation coefficient value is 0.999.			
6.	Robustness	**Change in mobile phase variation**			
		Mobile phase	**Efficiency**	**Retention time (min)**	**Asymmetry**
		Low 65 : 35	2329	3.57	1.30
		Actual 70:30	4043	3.32	1.13
		High 75 : 25	2601	3.14	1.35
		Change in flow rate			
		Flow rate (ml/min)	**Efficiency**	**Retention time (min)**	**Asymmetry**
		0.6	2010	3.17	1.22
		0.8	2304	3.22	1.30
		1	2218	3.51	1.43
		Change in detector wavelength			
		Wavelength	**Efficiency**	**Retention time (min)**	**Asymmetry**
		296	2358	3.24	1.26
		298	2456	3.26	1.35
		300	2371	3.22	1.13

CONCLUSION: From this study, it is concluded that the proposed RP-HPLC method was found to be simple, accurate, precise, rapid and useful for routine analysis of choline fenofibrate in bulk & its pharmaceutical dosage form. The statistical parameters and recovery studies were carried out and reported. The obtained results were satisfactory as per ICH guidelines.

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