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Development and Validation of UPLC Method for the Determination of Related Substances in Fenoprofen Calcium

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ABSTRACT

A new, rapid, specific and sensitive Ultra Performance Liquid Chromatographic (UPLC) method with gradient elution was developed and validated for the determination of related substances in Fenoprofen calcium. The successful separation of Fenoprofen calcium and its impurities was achieved using BEH C18 column (Size: 100 × 2.1 mm; 1.7 µm particle size) column maintained at 30°C with mobile phase consisting of Water : Acetic acid (980:20) as Mobile phase-A (MP-A) and Acetonitrile : Acetic acid (980:20) as Mobile phase-B (MP-B) in a gradient programme. The mobile phase flow rate was 0.3 ml/min and the detection wavelength was 270 nm. The retention time of Fenoprofen calcium and its impurities in the present method was comparatively less than the reported HPLC methods, offering less time consuming, minimum usage of chemical reagents and fast analytical method. As part of the method validation, specificity, limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy, precision, robustness and ruggedness were determined.

Keywords: Fenoprofen calcium; Related substances; Impurities; Development; Validation; Ultra performance liquid chromatographic (UPLC) method

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INTRODUCTION

Fenoprofen calcium is a nonsteroidal, anti-inflammatory drug. Chemically, Fenoprofen calcium is a calcium salt of 2-(3- phenoxyphenyl)propanoic acid. Fenoprofen calcium is available as stable dihydrate form. It has a molecular formula of $C_{30}H_{26}CaO_6 \cdot 2H_2O$ and molecular weight of 558.65. The pKa of Fenoprofen calcium is 4.5 at 25°C. It is a white crystalline powder. At 25°C, it dissolves to a 15 mg/mL solution in alcohol (95%). It is slightly soluble in water and insoluble in benzene. Fenoprofen calcium is used in the relief of mild to moderate pain in adults and for the relief of signs and symptoms of rheumatoid arthritis and osteoarthritis. Fenoprofen calcium is commercially available in the form of capsules and tablets in the dose range from 200 mg to 600 mg [1-7]. Fenoprofen calcium is official in United States Pharmacopoeia [8]. Literature survey revealed HPLC method to measure plasma and urine Fenoprofen levels in equine biofluids [9], isocratic RP HPLC method for model determination of Fenoprofen in PHEA-fenoprofen conjugates [10], HPLC method for the analysis of Fenoprofen calcium capsules and its related substances [11] and RP-HPLC method for simultaneous determination of Fenoprofen Calcium and its related process impurities [12].

There is no UPLC method reported for the estimation of related substances in Fenoprofen calcium. Hence, the aim of the present work was to develop and validate a new UPLC method for determination of Fenoprofen calcium impurities. In this work, we show how the HPLC method for Fenoprofen calcium has been transferred to UPLC. A

comparison was made between HPLC and UPLC efficiency on the basis of resolution and sensitivity. The developed method was validated in accordance with International Conference on Harmonization (ICH) guidelines [13-16].

EXPERIMENTAL SECTION

Standards and Reagents

Fenoprofen calcium and its impurities viz. Impurity-A, Impurity-B, Impurity-C, Impurity-D, Impurity-E and Impurity-F were obtained from Suven Life sciences Ltd., Hyderabad, India. In addition, analytical reagent grade acetonitrile and acetic acid was purchased from RANKEM chemicals. HPLC grade water was used from Milli-Q water purification system. All other chemicals and solvents used were of analytical grade. The chemical names of the impurities of Fenoprofen calcium were given in Table 1 and the chemical structures of Fenoprofen calcium and its impurities were given in Figure 1.

Table 1: Chemical names of fenoprofen calcium impurities

S. No.	Impurity Name	Chemical Name
1	Impurity-A (Process Impurity)	2-(3'-Phenoxyphenyl) acetic acid
2	Impurity-B (Process Impurity)	2-(3'-Phenoxyphenyl)-2-methyl propionic acid
3	Impurity-C (Intermediate)	m-Phenoxybenzyl alcohol
4	Impurity-D (Intermediate)	m-Phenoxybenzyl chloride
5	Impurity-E (Intermediate)	m-Phenoxybenzyl cyanide
6	Impurity-F (Intermediate)	2-(3'-Phenoxyphenyl) propionitrile

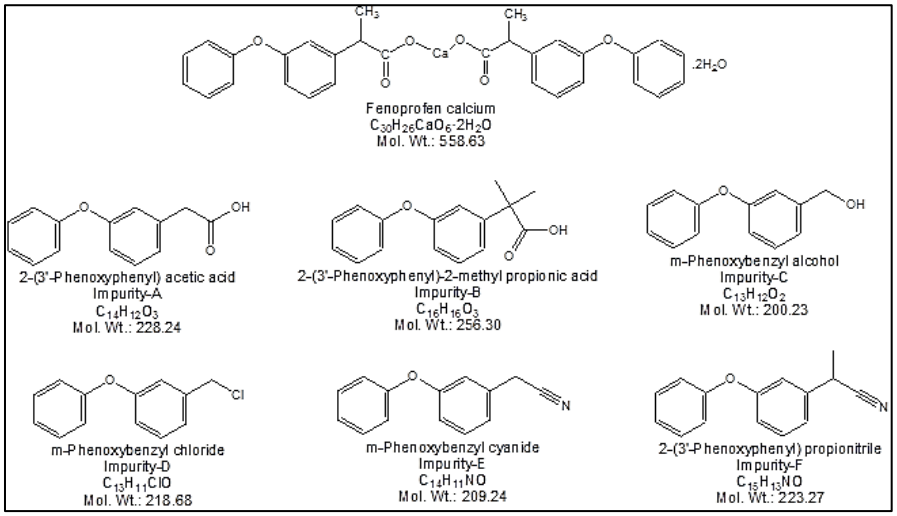

Figure 1: Chemical structures of fenoprofen calcium and its impurities

Instrumentation and Software

High performance liquid chromatography:

The HPLC system used for initial chromatographic development was a Waters Alliance separation module with a 2487 UV detector (Milford, MA). YMC pack C8 column (250 × 4.6 mm, 5 µm) was used for separation. Mobile phase consisting of a mixture of A: Water and Acetic acid with a ratio of 98:2 and B: Acetonitrile and Acetic acid with a ratio of 98:2. The timed gradient program: T (min)/%B: 0/30, 3/30, 35/50, 40/50, 50/90, 55/90, 55.10/30 and 60/30 with the flow rate of 1.5 mL/min was employed. The injection volume was 20 µL while detector was set at UV 270 nm. The column was maintained at 30°C.

Ultra-performance liquid chromatography:

The chromatographic analysis was performed using a Waters Ultra performance Liquid chromatography system equipped with a PDA detector, pump, Auto sampler, Column compartment, Degasser and Data handling system. Chromatographic separation was achieved using BEH C18 column (Size: 100 × 2.1 mm; 1.7 µm particle size) using a gradient program at a flow rate of 0.3 ml/min. The column oven temperature was set at 30°C temperature. The

injection volume was 2 µl with wavelength detection at UV 270 nm. The total run time of analysis was 20 minutes. Water: Acetonitrile mixture in the ratio of 50:50 was used as a diluent.
Chromatographic separation was achieved through gradient program. Mobile phase-A (MP-A) consists of Water:Acetic acid (980:20) and Mobile phase-B (MP-B) consists of Acetonitrile : Acetic acid (980:20) previously filtered through 0.45 µm cellulose membrane filter. The gradient program was given in Table 2.

Table 2: Gradient program

Time (min)	Flow (mL/min)	%MP-A	%MP-B
0	0.3	70	30
9	0.3	50	50
12	0.3	50	50
14	0.3	10	90
16	0.3	10	90
17	0.3	70	30
20	0.3	70	30

The reported HPLC method by Dasari Purnachand et al. [12] for determination of impurity profile in Fenoprofen calcium describes the usage of C8 column (Size: 250 × 4.6 mm; 5 µm particle size) using a gradient program at a flow rate of 1.5 mL/min. and an injection volume of 20 µL with wavelength detection at UV 270 nm. However, this method reports a run time of 60 minutes. Ultra performance liquid chromatography (UPLC) is relatively novel technique which can be used to decrease the analysis time and solvent consumption. UPLC systems can withstand high system back pressure and comprising special analytical columns with sub 2 µm particles. UPLC system allows shortening analysis time up to nine times compared to conventional HPLC system [17].

Preparation of Standard Solutions

Stock solutions (A):

Individual stock solutions were prepared by dissolving accurately weighed 25 mg of Fenoprofen Calcium, 50 mg of each Impurity-A, B, C, D, E and F into a volumetric flask and adjusted to 50 mL with diluent i.e., Water : ACN (50:50) (1000 µg/mL).

Stock solutions (B):

Pipette out 10 mL of Stock Solution-A into a 50 mL volumetric flask dilute the volume with diluent and mix.

Sample solution:

Accurately weigh and transfer about 100 mg of Fenoprofen Calcium test sample into a 10 mL volumetric flask. Dissolve and dilute to volume with diluent.

RESULTS AND DISCUSSION

HPLC Method Development and Transfer to UPLC

The main target of the chromatographic method was to achieve separation and quantification of impurities of Fenoprofen calcium. Initially, the gradient HPLC conditions were optimized for Fenoprofen Calcium in drug substance, which was then transferred to UPLC. Gradient system is always preferred over isocratic system in order to achieve improved peak shape and resolution. With the isocratic system, sometimes peak is eluted late so the gradient system was used to reduce run time. Hence, it was decided to use gradient HPLC mode. The response of Fenoprofen Calcium was found to be adequate at UV 270 nm. The HPLC chromatographic separation was achieved on a YMC pack C8 column (250 × 4.6 mm, 5 µm) maintained at 30°C temperature.
The basic chromatographic conditions such as stationary phase, solvents, and UV detection employed in HPLC were taken into account while developing the new UPLC method. The detection wavelength, column temperature, buffer and solvent and flow rate used in HPLC were kept constant. The stationary phase C18 was chosen in order to have similar chemistry as that used in the HPLC. Primarily a BEH C18 column (100 × 2.1 mm, 1.7 µm) was used, but the pressure found was very high (20000 psi) and not acceptable. In the next trials decreased the flow rate to 0.3 mL/min from 0.919 mL/min. The observed pressure is 6900 psi which is acceptable. The injection volume in UPLC was scaled to 2 µL from 20 µL as used in HPLC, whereas the mobile phase containing a mixture of A: water and acetic acid and B: Acetonitirle and Acecetic acid remained the same on a timed gradient program T (min)/%B: 0/30, 9/50, 12/50, 14/90, 16/90, 17/20 and 20/30 with the flow rate of 0.3 mL/min was used. The results obtained from different trials carried out during the optimization of chromatographic conditions were given in Table 3.

Table 3: UPLC method development experiments

Trial No	Column anf Dimensions	Experimental Conditions	Conclusion
1	BEH C18, 100 × 2.1 mm, 1.7 µm	Gradient elution programmed with 7.75 minutes at 0.92 mL/min	Observed column pressure is 12000 psi which is close to higher limit of 15000 psi
2	BEH C18, 100 × 2.1 mm, 1.7 µm	Gradient elution programmed with 24 minutes at 0.3 mL/min	Pressure is found to be satisfactory (about 7000 psi). To reduce the run time further optimization is required.
3	BEH C18, 100 × 2.1 mm, 1.7 µm	Gradient elution programmed with 20 minutes at 0.3 mL/min	All the Impurities are separated

Final Optimized Method

Chromatographic separation on BEH C18 column (Size: 100 × 2.1 mm; 1.7 µm particle size) column maintained at 30°C with mobile phase consisting of Water: Acetic acid (980:20) as Mobile phase-A (MP-A) and Acetonitrile: Acetic acid (980:20) as Mobile phase-B (MP-B) in a gradient programme. Gradient program is given in Table 2.

Results of Forced Degradation Experiments

Forced degradation of Fenoprofen calcium was carried out, to confirm that during stability study or throughout the shelf life, any degradation product if found will not interfere with the impurity peaks. In addition, the forced degradation study would help to identify the type of degradation pathway (whether oxidative, alkali hydrolysis, acid hydrolysis, photolytic etc.) for each of the degradant. Forced degradation was performed by degrading the sample with 1 N HCl, 1 N NaOH, 10% hydrogen peroxide and Neutral, Thermal and Photolytic degradation. All the samples are exposed for 6 hours. From the forced degradation studies, slight degradation was observed in Oxidative degradation. Degradation was not observed in acid, base, neutral, thermal and photolytic degradation conditions. The results were given in Table 4.

Table 4: Results forced degradation studies

Type of degradant	%Area						
	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	Imp-F	Any other impurity
As such sample	0.06	0.01	0.01	ND	ND	ND	0.02
Acid degradation: (With 1 N HCL heat at 80°C up to 6 hours)	0.06	0.01	0.01	ND	0.01	ND	0.02
Base degradation: (With 1 N NaOH heat at 80°C up to 6 hours)	0.06	0.01	0.01	ND	ND	ND	0
Oxidative degradation: (With 10% H_2O_2 heat at 80°C up to 6 hours)	0.06	0.01	0.12	ND	ND	ND	0.37
Neutral degradation (at 80°C for 6 hours)	0.06	0.01	0.01	ND	ND	ND	0.02
Thermal degradation (at 80°C for 6 hours)	0.06	0.01	0.01	ND	ND	ND	0.02
Photolytic degradation	0.06	0.01	0.01	ND	ND	ND	0.02

Method Validation

Specificity / Selectivity:

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. The specificity of the method was performed by injecting diluent, individual impurity standard solutions, impurities spiked with sample solution. The chromatograms were verified for interferences from other impurities or the sample matrix. The developed analytical method was found to be specific since no interfering peak was observed at the retention time of each impurity and no interference between each other. The chromatogram obtained for Fenoprofen calcium along with its impurities was shown in Figure 2. The Retention Time (RT) and Relative Response Factor (RRF) values for Fenoprofen calcium and its impurities were given in Table 5.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD and LOQ of the impurities were estimated by Residual standard deviation method (serial dilution). Standard solutions of 5%, 10%, 20%, 30%, 40% and 50% were prepared and injected. Then the SLOPE and STEYX were calculated by plotting the graph between concentration on (x-axis) and response on (y-axis). The values of LOD and LOQ were calculated from SLOPE and STYEX from the graph. After establishing the LOD and LOQ concentrations, solutions at LOD and LOQ concentrations were prepared and injected. LOD solution was injected in duplicate and peaks were observed visually at LOD concentration. LOQ Solution was injected in to six replicates and calculated the precision at LOQ level and well within acceptance criteria for each impurity. The LOD and LOQ of Fenoprofen calcium and its impurities were given in Table 6.

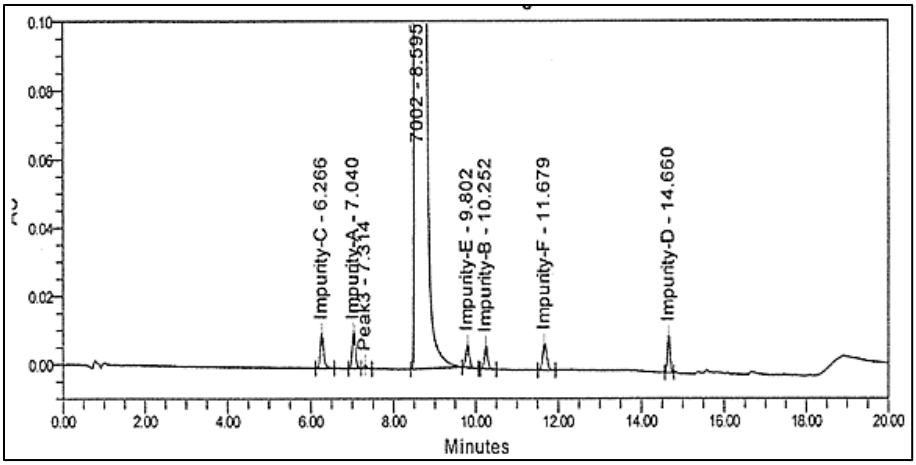

Figure 2: Chromatogram containing fenoprofen calcium and its impurities

Table 5: Retention time and relative response factor values of fenoprofen calcium and its impurities

S. No	Name of the compound	Retention Time in minutes (RT)	Relative Response Factor (RRF)
1	Fenoprofen calcium	8.62	1
2	Impurity-A	7.09	0.9
3	Impurity -B	10.28	0.81
4	Impurity -C	6.32	1.32
5	Impurity -D	14.67	1.08
6	Impurity -E	9.83	1.07
7	Impurity -F	11.7	1.07

Table 6: Results of LOD, LOQ and range

Name of the compound	Limit of detection (%)	Limit of Quantification (%)	Range (%)
Impurity-A	0.002	0.005	LOQ to 150%
Impurity-B	0.001	0.004	LOQ to 150%
Impurity-C	0.001	0.002	LOQ to 150%
Impurity-D	0.001	0.004	LOQ to 150%
Impurity-E	0.001	0.002	LOQ to 150%
Impurity-F	0.001	0.004	LOQ to 150%
Fenoprofen calcium	0.002	0.006	LOQ to 150%

Accuracy (% Recovery):

Accuracy of the method was evaluated by calculating the % recovery of impurities at three different concentrations of LOQ, 100% and 150% of specification level. Each level has been analyzed in triplicate and reported. The recovery of all the substances was found to be in between the predefined acceptance criteria of 80 to 120%. The obtained % recovery values for the impurities LOQ, 100% and 150% concentration level were given in Table 7. Hence it was concluded that the method was found to be accurate.

Table 7: Accuracy data

Level	Preparation	Impurity-A	Impurity-B	Impurity-C	Impurity-D	Impurity-E	Impurity-F
LOQ	1	114.1	106.2	90.7	108.5	85.2	95.1
	2	118.3	104.6	94.8	103.8	86.7	99.1
	3	113.5	108.1	92.6	105.7	86.9	95.2
100%	1	98.1	98.4	99.7	99.1	96.1	98.6
	2	98.5	98.6	98.6	98.8	96.3	98.9
	3	98.6	98.8	98.5	98.2	96.7	98.8
150%	1	97.6	98	102.3	100.5	96.2	98
	2	97	97.7	101.8	99.9	95.7	97.7
	3	97.3	97.7	102	100.2	95.9	98.1
Average		103.7	100.9	97.9	101.6	92.9	97.7

Linearity and range:

The linearity of the method was established by injecting Fenoprofen calcium and the impurities at 6 different concentrations ranging from LOQ to 150% (LOQ, 10, 20, 50, 75, 100, 120, 150%) of specification level.

The linearity graphs for Fenoprofen calcium and its impurities were given in Figure 3. The correlation coefficient values of all the impurities were more than 0.999. Hence, it was concluded that the method was linear.

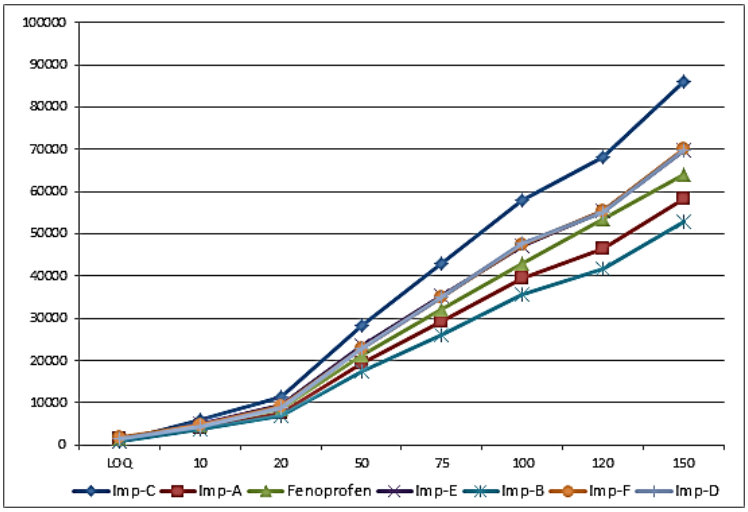

Figure 3: Linearity graphs of fenoprofen and its impurities

System precision:

The system precision was evaluated by analyzing six replicate injections of standard solution containing Fenoprofen calcium, Impurity-B and Impurity-E mixture. The %RSD for the retention time and area response of Fenoprofen calcium, Impurity-B and Impurity-E was determined to assess the system precision. The %RSD for retention time and area response Fenoprofen calcium, Impurity-B and Impurity-E was given in Table 8. Hence the system was precise.

Table 8: System precision data

Injection Number	Retention time			Area response		
	Fenoprofen	Impurity-B	Impurity-E	Fenoprofen	Impurity-B	Impurity-E
1	9.28	10.82	10.37	27557	31139	42679
2	9.27	10.83	10.37	27589	31104	42721
3	9.31	10.87	10.41	27806	31384	43098
4	9.3	10.86	10.41	27745	31305	42942
5	9.3	10.86	10.41	27988	31482	43068
6	9.33	10.89	10.44	27978	31576	43160
Average	9.3	10.86	10.4	27777	31332	42945
% RSD	0.22	0.21	0.24	0.66	0.6	0.47

Method precision:

The method precision was evaluated by analyzing six replicate preparations of spiked sample and the area response was calculated. The % RSD of area response of impurities was given in Table 9. Hence the method was precise.

Table 9: Method precision data

Preparation	Impurity-A	Impurity-B	Impurity-C	Impurity-D	Impurity-E	Impurity-F
1	52900	39490	60540	63077	39034	43520
2	52033	38840	59593	62059	38410	42821
3	52146	38956	59776	62352	38534	42879
4	52318	39093	59842	62592	38659	43031
5	52023	38829	59484	62093	38411	42713
6	51934	38764	59447	61984	38316	42732
Average	52226	38995	59780	62360	38561	42949
% RSD	0.68	0.69	0.68	0.67	0.68	0.7

Ruggedness:
The system suitability was performed on different instruments on different day by different analyst at different labs and the retention times were compared. All the impurities were well separated on two different UPLC systems. Resolution between Impurity-B and Impurity-E on Instrument-1 and Instrument-2 were 2.81 and 2.98 respectively. Tailing factor for Fenoprofen Calcium on Instrument-1and Instrument-2 were 1.07 and 1.00 respectively.

Robustness:
The robustness of the method was studied by deliberately changing the flow rates to 0.29 ml/min and 0.31 ml/min (optimized flow rate was 0.3 ml/min), changing the column oven temperature to 28°C and 32°C (optimized temperature was 30°C) and changing of mobile phase composition by increasing and decreasing the acetic acid composition in the mobile phase. All the impurities were well separated in each parameter. The robustness data was given in Table 10. Hence it was concluded that the method was robust even after deliberate changes in the flow rate, column oven temperature and mobile phase composition.

Table 10: Robustness data

Impurity	Retention Time					
	Change in flow rate		Change in column temperature		Change in mobile phase composition	
	At 0.29 mL/min	At 0.31 mL/min	At 28°C	At 32°C	With increase in Acetic acid composition	With decrease in acetic acid composition
Impurity-A	7.42	7.08	7.35	7.13	7.41	7.36
Impurity-B	10.73	10.35	10.64	10.42	10.69	10.66
Impurity-C	6.61	6.28	6.53	6.34	6.6	6.55
Impurity-D	14.98	14.71	14.9	14.77	14.94	14.93
Impurity-E	10.27	9.86	10.19	9.91	10.26	10.17
Impurity-F	12.26	11.79	12.17	11.84	12.23	12.16
Fenoprofen	9.04	8.68	8.95	8.74	9.01	8.96

CONCLUSION

The proposed method was a new UPLC method for the determination of related substances in Fenoprofen calcium. The method was fully validated according to the ICH guidelines and presented good specificity, linearity, accuracy, precision and robustness. And it was also found to be simple, sensitive, selective, and stability indicating. The Limit of Detection and Limit of Quantification values were established by using Slope and STYEX method. The proposed method can be successfully applied for the determination of Fenoprofen related substances for routine testing in quality control.

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