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DETERMINATION OF 2, 4 DIFLUORONITROBENZENE AS A GENOTOXIC IMPURITY IN FLURBIPROFEN DRUG SUBSTANCE BY USING UPLC

Vinayak Vele*, Amol Kumbhar Vishal Telvekar, Shivaji Kadam, Rupesh Kelaskar and Mohan Chandavarkar

R & D Synthetic API Analytical Development Laboratory, FDC Ltd., 142-148, S.V. Road, Jogeshwari (W), Mumbai-400102, Maharashtra, India.

***Corresponding Author: Vinayak Vele**
R & D Synthetic API Analytical Development Laboratory, FDC Ltd., 142-148, S.V. Road, Jogeshwari (W), Mumbai-400102, Maharashtra, India.

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ABSTRACT

A simple and accurate method was developed for the determination of 2, 4 Difluoronitrobenzene in Flurbiprofen. Chromatographic separation between 2, 4 Difluoronitrobenzene and Flurbiprofen was achieved using a C18 column using a mobile phase containing buffer and acetonitrile in gradient mode. The resolution between the between 2, 4 Difluoronitrobenzene and Flurbiprofen was found to be more than 5.0. The limit of detection (LOD) and limit of quantification (LOQ) of the 2, 4 Difluoronitrobenzene was 0.11 and 0.22 µg mL-1 respectively, for 3 µL injection volume. The percentage recoveries of the 2, 4 Difluoronitrobenzene ranged from 90.36% to 92.84% in the samples of Flurbiprofen. The developed method was validated as per International Conference on Harmonization guidelines in terms of specificity, limit of detection, limit of quantification, precision, linearity, accuracy and ruggedness.

KEYWORDS: Development, Validation, Flurbiprofen, 2, 4 Difluoronitrobenzene, Genotoxic, UPLC.

Flurbiprofen[1,2] (trade name Ansaid), [1,1'-biphenyl]-4-acetic acid, 2-fluoro-alphamethyl-, (±) (Figure 1), is member of the phenylalkanoic acid derivative family of nonsteroidal anti-inflammatory drugs (NSAIDs). It is primarily indicated as a pre-operative anti-miotic (in an ophthalmic solution) as well as orally for arthritis or dental pain.[2,3]

2, 4 Difluoronitrobenzene was used as one of key starting material in the manufacturing process. Flurbiprofen. So the study is proposed and conducted for the method development and further validation of method for determination of 2, 4 Difluoronitrobenzene in Flurbiprofen drug substance. As per the IARC monograph of 2, 4 Difluoronitrobenzene, it is listed in 2A group (Probably carcinogenic to humans) and also it is genotoxic as per the final evaluation.[4,5] The maximum daily dose and treatment of duration of Flurbiprofen is 300 mg/day and for a month respectively considering the therapeutic uses. Thus as per ICH M7 (R1) guideline based on the maximum daily dose and treatment period of around 12 months, the 2, 4 Difluoronitrobenzene is need to control and evaluate at 20.0 µg/g level.[6,7] The limit for the 2, 4 Difluoronitrobenzene obtained is 66.67 µg/mL. In this study the evaluation performed at stringent limit i.e. 25.0 µg/mL. The results obtained after completion of validation are all within the set acceptance criteria.

MATERIALS AND METHODS

Samples of Flurbiprofen were obtained from R & D synthetic Department of FDC Ltd, Mumbai, India. HPLC-grade Acetonitrile, Acetic acid was procured from Fischer Scientific, USA. 2, 4 Difluoronitrobenzene with certified purity procured from Sigma-Aldrich.

Chromatographic condition

UPLC system used was Waters (H-class series, US) system equipped with auto sampler, quaternary pump, degasser, and a UV Detector. The output signal was monitored and processed using Chromeleon 7.2 software. The chromatographic column used was BEH, C18 Acquity (100 mm X 2.1mm, 1.7 µm), (Waters, USA). The mobile phase used was water & acetic acid (923:77 v/v) as buffer, was used as a mobile phase A and acetonitrile was used as a mobile phase B. The gradient program time (minutes) % mobile phase B (T%B) was set as 0/40, 2/40, 14/90, 15/90, 18/40 and 20/40 respectively. The flow rate of the mobile phase was 0.3 mL/min. The column temperature was maintained at 25°C and the eluent was monitored at a wavelength of 254 nm. The injection volume used was 3.0 µL. The diluent used was Water: Acetonitrile (30:70 v/v).

Preparation of Standard and Sample Solutions

The stock solution of the 2, 4 Difluoronitrobenzene and Flurbiprofen were prepared individually by dissolving an

appropriate amount of the substances in diluent. For quantitation of 2, 4 Difluoronitrobenzene in Flurbiprofen a solution of 0.75 µg/mL concentration was used. The target analyte concentration was fixed as 30.0 mg mL-1.

Method Development

A solution of Flurbiprofen and 2, 4 Difluoronitrobenzene (30mg/mL & 0.75µg/mL) prepared in diluents for method establishment. To develop a rugged and suitable UPLC method for the separation, different stationary phases and mobile phases were employed. Preliminary column screening involved different types of C18, C8, Cyano, Amino and Phenyl columns were employed. On Waters BEH C18 (100 mm X 2.1mm, 1.7 µm) column provided selectivity between the Flurbiprofen and the 2, 4 Difluoronitrobenzene peak using a mobile phase consisting of buffer-methanol (30:70, v/v), but the resolution between Flurbiprofen, known impurities and 2, 4 Difluoronitrobenzene were closed. We continued to select the best mobile phases that would give optimum resolution and selectivity for the 2, 4 Difluoronitrobenzene and Flurbiprofen. Good separation was achieved on C18 column and buffer-acetonitrile (60:40 v/v) as the mobile phase in gradient mode.

METHOD VALIDATION

Precision

The precision of an analytical procedure expresses the closeness of agreement among a series of measurements obtained from multiple samplings of the same homogenous sample under prescribed conditions. The system and method precision for the 2, 4 Difluoronitrobenzene were checked at its specification level i.e. 0.75µg/ml with respect to analyte concentration, 30.0 mg mL-1). The percentage RSD of method repeatability and system repeatability for the 2, 4 Difluoronitrobenzene were found to be 0.47% and 0.96%, respectively, which confirms good precision of the method.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample. The linearity of the method for the 2, 4 Difluoronitrobenzene was checked at six concentration levels, i.e. from limit of quantitation (LOQ) (30%) to 150% of the 2, 4 Difluoronitrobenzene specification level (0.75µg/ml), which is with respect to of Flurbiprofen analyte concentration. The coefficient of regression of the calibration curve was found to be 0.9998, thus confirming the excellent correlation between the peak area and concentration of the 2, 4 Difluoronitrobenzene.

Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and limit of quantification were achieved by injecting a series of dilutions of 2, 4 Difluoronitrobenzene.[8] The precision of the developed method for 2, 4 Difluoronitrobenzene at LOD and LOQ was checked by analyzing six test solutions prepared at the LOD and LOQ level and calculating the percentage relative standard deviation of area. The limit of detection and quantification for 2, 4 Difluoronitrobenzene was found to be 0.11 µg mL-1 and 0.22 µg mL-1 respectively for the 3.0 µL of injection volume.

Ruggedness and Robustness

The ruggedness[8] of a method was defined as degree of reproducibility of results obtained by analysis of the same sample under a variety of normal test conditions such as different laboratories, different analysts, different instruments and different days. The standard addition and recovery experiments carried out for the 2, 4 Difluoronitrobenzene in Flurbiprofen samples at the same concentration levels tested. The data obtained from both the experiment was well in agreement with each other, thus proving the method ruggedness. The robustness[8] of an analytical procedure is measured by its capability to remain unaffected through small, but deliberate, variations in method parameters and provide an indication of its reliability during normal usage. In the varied chromatographic conditions like flow rate, mobile phase ratio and column temperature, the resolution between the peaks of 2, 4 Difluoronitrobenzene and Flurbiprofen was found to be >5.0 illustrating the robustness of the method.

Recovery (Accuracy) of 2, 4 Difluoronitrobenzene

The standard addition and recovery experiments were conducted for the 2, 4 Difluoronitrobenzene in bulk samples of Flurbiprofen in triplicate at LOQ (30%) (0.22 µg/mL), 50% (0.38 µg/mL) 100% (0.75 µg/mL) and 150% (1.13 µg/mL) with respect to test concentration. The percentage recovery ranged from 93.2% to 115.7% (Table 2).

RESULTS AND DISCUSSION

Due to the better chromatographic results obtained on the C18 column, further method optimization and quantification of the 2, 4 Difluoronitrobenzene were carried out on this column. Based on the data obtained from method development and optimization activities, the Inert sustain C18 (100 mm X 2.1mm, 1.7 µm) column with the mobile phase of buffer-acetonitrile in gradient was selected for the final method. The flow rate of the experimental method was 0.3 mL/min with an injection volume of 3.0µL. The column temperature was 25°C, and the detection wavelength was 254 nm. Under these conditions, 2, 4 Difluoronitrobenzene and Flurbiprofen were separated well and the peak of the 2, 4 Difluoronitrobenzene eluted before the peak of Flurbiprofen. In the optimized method, the typical retention times of the Flurbiprofen and 2, 4 Difluoronitrobenzene were approximately 2.42 and 5.35 min, respectively. Baseline separation of Flurbiprofen and 2, 4 Difluoronitrobenzene was obtained with a total run time of 20 min. The system suitability results were given in Table 1.

Table 1: System suitability criteria.

Component	Retention time (min)	Relative retention time (min)	Resolution	Tailing factor
2, 4 Difluoronitrobenzene	2.42	0.45		1.1
Flurbiprofen	5.35	1.00	8.34	---

The structure of 2, 4 Difluoronitrobenzene and Flurbiprofen are displayed in Fig. 1. The typical chromatogram of the Flurbiprofen spiked with 2, 4 Difluoronitrobenzene displayed in Fig. 2.

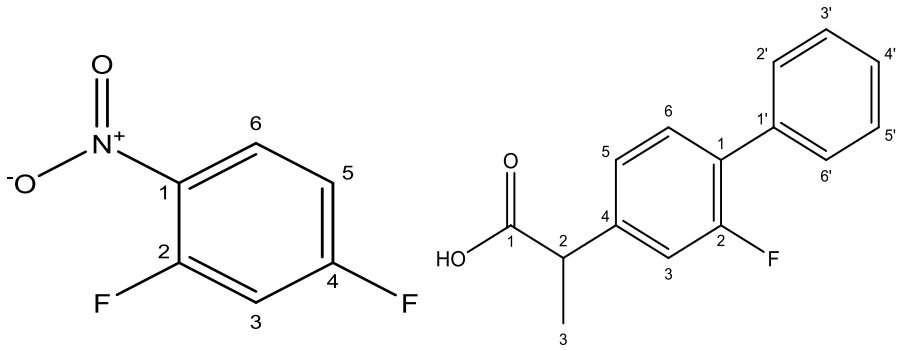

Fig. 1: Structure of 2, 4 Difluoronitrobenzene and Flurbiprofen.

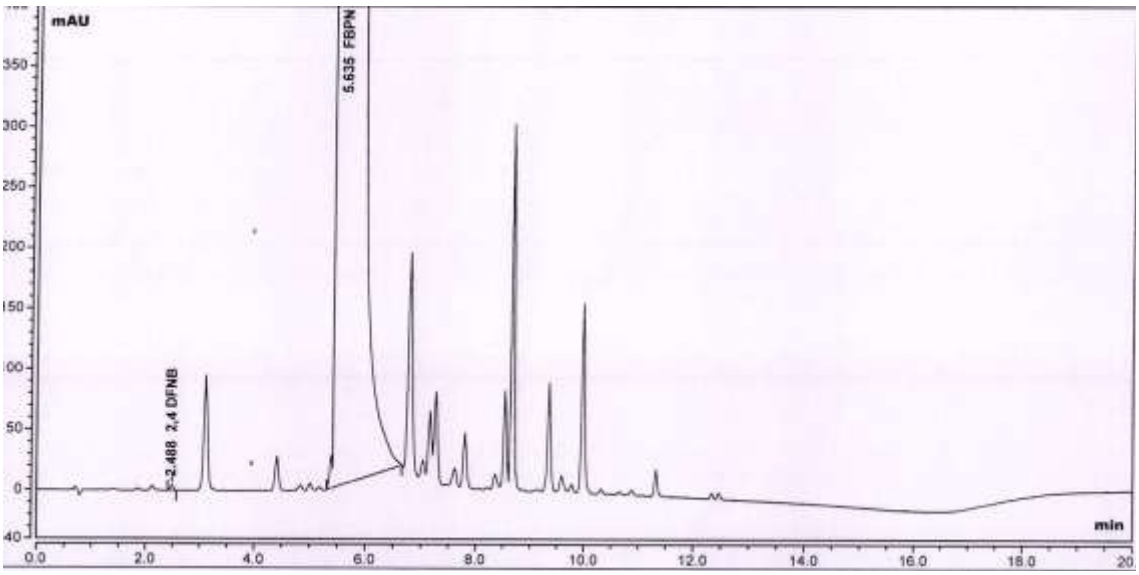

Fig. 2: A typical HPLC chromatogram of Flurbiprofen spiked with 2, 4 Difluoronitrobenzene at specification level.

Table 2: Summary of method validation data.

Parameter	µg mL^{-1}	r	% Mean recovery	% RSD
LOD	0.11	-	-	3.05
LOQ Linearity	0.22	-	-	1.79
(LOQ to 150%) Accuracy	-	0.9998	-	-
LOQ % spiking	-	-	91.79	2.43
50% spiking	-	-	90.36	0.88
100 % spiking 150 % spiking	- -	- -	91.97 92.84	0.62 0.21
Precision				
System precision	-	-	-	0.96

Method precision	-	-	-	0.47
Intermediate pre (Ruggedness)	-	-	-	0.29

CONCLUSION

A simple, rapid and accurate Ultra Performance Liquid Chromatography (UPLC) method in ordered to separate 2, 4 Difluoronitrobenzene and Flurbiprofen. Method validation was carried out using a C18 column due to the better chromatographic results achieved on the column. The validated method was demonstrated to be specificity, accurate, precise, selective, sensitive, rugged and robust. The developed and validated method can be implemented for the determination and quantitative of 2, 4 Difluoronitrobenzene in Flurbiprofen bulk drug.

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Conflict of Interests

The authors declared no conflict of interest.

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