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DETERMINATION OF DIETHYL (4-AMINO-3-FLUOROPHENYL) (METHYL) PROPANEDIOATE AS A GENOTOXIC IMPURITY IN FLURBIPROFEN DRUG SUBSTANCE BY USING HPLC

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ABSTRACT
A simple and accurate method was developed for the determination of Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate in Flurbiprofen. Chromatographic separation between Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate and Flurbiprofen was achieved using a C18 column using a mobile phase containing buffer and acetonitrile in gradient mode. The resolution between the between Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate and Flurbiprofen was found to be more than 10.0. The limit of detection (LOD) and limit of quantification (LOQ) of the Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate was 0.05 and 0.10 µg mL-1 respectively, for 50 µL injection volume. The percentage recoveries of the Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate ranged from 96.79% to 112.43% 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, Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate, Genotoxic, HPLC

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]

Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate was one of the process intermediate formed in the manufacturing process of Flurbiprofen. So the study is proposed and conducted for the method development and further validation of method for determination of Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate in Flurbiprofen drug substance. As per the IARC monograph of Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate, 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 Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate is need to control and evaluate at 20.0 µg/g level.[6,7] The limit for the Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate 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 and Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate were obtained from R & D synthetic Department of FDC Ltd, Mumbai, India. HPLC-grade Acetonitrile, Acetic acid was procured from Fischer Scientific, USA.

Chromatographic condition:
HPLC system used was Agilent (1200-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 Kromasil, C18 (150 mm X 4.6mm, 3.5 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, 15/40, 14/90, 18/90, 22/90, 25/40 and 30/40 respectively. The flow rate of the mobile phase was 1.0 mL/min. The column and autosampler temperature was maintained at 25°C and 10°C respectively. The eluent was monitored at a wavelength of 254 nm. The injection volume used was 50.0 µL. The diluent used was Water: Methanol (80:20 v/v).

Preparation of Standard and Sample Solutions

The stock solution of the Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate and Flurbiprofen were prepared individually by dissolving an appropriate amount of the substances in diluent. For quantitation of Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate in Flurbiprofen a solution of 0.50 µg/mL concentration was used. The target analyte concentration was fixed as 20.0 mg mL-1.

Method Development

A solution of Flurbiprofen and Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate (20mg/mL & 0.50µg/mL) prepared in diluents for method establishment. To develop a rugged and suitable HPLC 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 Kromasil C18 (150 mm X 4.6mm, 3.5 µm) column provided selectivity between Flurbiprofen and Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate peak using a mobile phase consisting of buffer-acetonitrile (25:75, v/v), but the resolution between Flurbiprofen, known impurities and Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate were closed. We continued to select the best mobile phases that would give optimum resolution and selectivity for the Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate 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 Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate were checked at its specification level i.e. 0.5µg/ml with respect to analyte concentration, 20.0 mg mL-1). The percentage RSD of method repeatability and system repeatability for the Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate were found to be 2.97% and 1.69%, 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 Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate was checked at six concentration levels, i.e. from limit of quantitation (LOQ) (20%) to 150% of the Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate specification level (0.50µg/ml), which is with respect to of Flurbiprofen analyte concentration. The coefficient of regression of the calibration curve was found to be 0.9999, thus confirming the excellent correlation between the peak area and concentration of the Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate.

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 Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate.[8] The precision of the developed method for Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate 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 Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate was found to be 0.05 µg mL-1 and 0.10 µg mL-1 respectively for the 50.0 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 Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate 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 Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate and Flurbiprofen was found to be >10.0 illustrating the robustness of the method.

Recovery (Accuracy) of Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate

The standard addition and recovery experiments were conducted for the Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate in bulk samples of Flurbiprofen in triplicate at LOQ (20%) (0.10 µg/mL), 50% (0.25 µg/mL) 100% (0.50 µg/mL) and 150% (0.75 µg/mL) with respect to test concentration. The percentage recovery ranged from 96.79% to 112.43% (Table 2).

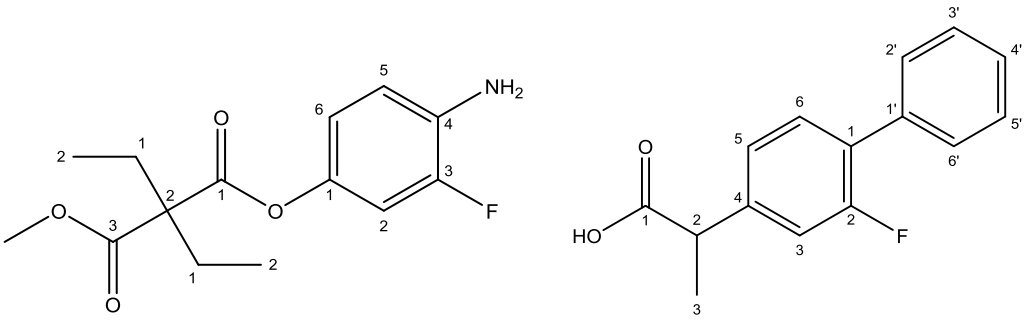
RESULTS AND DISCUSSION

Due to the better chromatographic results obtained on the C18 column, further method optimization and quantification of the Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate were carried out on this column. Based on the data obtained from method development and optimization activities, the Kromasil C18 (150 mm X 4.6mm, 3.5 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 1.0 mL/min with an injection volume of 50.0µL. The column temperature was 25°C, and the detection wavelength was 254 nm. Under these conditions, Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate and Flurbiprofen were separated well and the peak of the Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate eluted before the peak of Flurbiprofen. In the optimized method, the typical retention times of the Flurbiprofen and Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate were approximately 11.29 and 18.59 min, respectively. Baseline separation of Flurbiprofen and Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate was obtained with a total run time of 30 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
Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate	11.29	0.58		1.1
Flurbiprofen	18.59	1.00	13.24	---

The structure of Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate and Flurbiprofen are displayed in Fig. 1. The typical chromatogram of the Flurbiprofen spiked with Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate displayed in Fig. 2.



Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate Flurbiprofen

Fig. 1: Structure of Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate and Flurbiprofen.

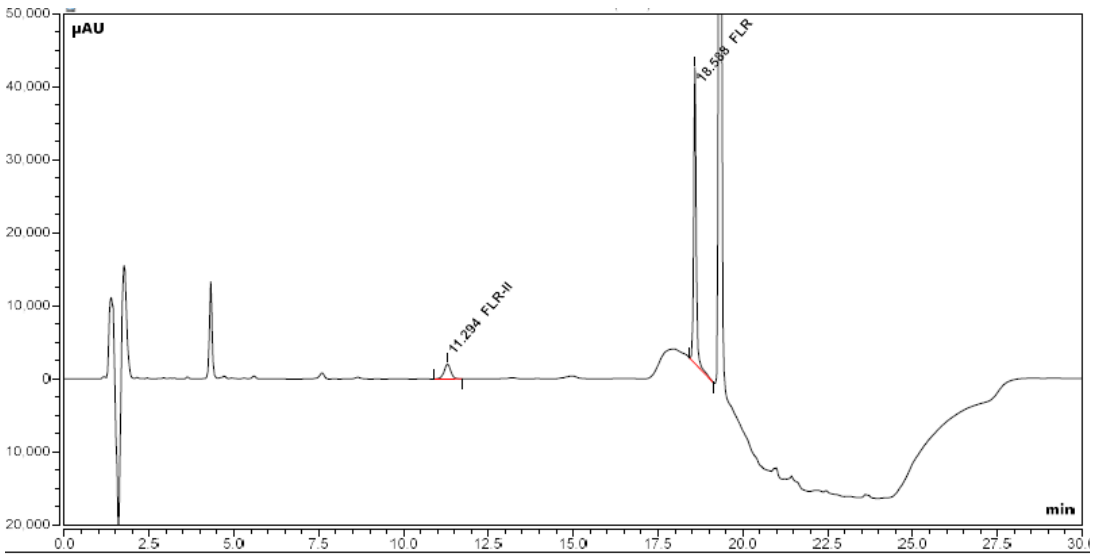


Fig. 2: A typical HPLC chromatogram of Flurbiprofen spiked with Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate at specification level.

Table 2: Summary of method validation data.

Parameter recovery	µg mL^{-1}	r	% Mean	% RSD
LOD	0.05	-	-	8.41
LOQ	0.10	-	-	7.74
Linearity (LOQ to 150%)	-	0.9999	-	-
Accuracy				
LOQ % spiking	-	-	112.43	5.90
50% spiking	-	-	104.91	2.88
100 % spiking	-	-	96.79	2.20
150 % spiking	-	-	96.81	3.64
Precision				
System precision	-	-	-	1.69
Method precision	-	-	-	2.97
Intermediate pre (Ruggedness)	-	-	-	2.32

CONCLUSION

A simple, rapid and accurate High Performance Liquid Chromatography (HPLC) method in ordered to separate Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate 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 Diethyl (4-amino-3-Fluorophenyl) (methyl) propanedioate in Flurbiprofen bulk drug.

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Conflict of Interests

The authors declared no conflict of interest.

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