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Short communication

Isolation, synthesis and characterization of impurities in Celecoxib a cox-2 inhibitor

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Abstract

During the impurity profile of Celecoxib, four polar impurities (impurity I, II, III and IV) and one non-polar impurity (impurity V) with respect to Celecoxib were detected by HPLC. LC-MS has been employed in this impurity profile study. The three polar impurities (I, II and III) were found to be process related while impurities (IV and V) turned out to be isomers. The impurities III, IV and V were isolated with the help of preparative HPLC. The structure of impurities III, IV (*ortho*-isomer) and V (*regio*-isomer) were confirmed as [5-(4-methylphenyl)-3-trifluoromethyl-1H-pyrazole], 4-[5-(2′-methyl phenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide, and 4-[4-(4′-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]-benzenesulfonamide, respectively. The structures of impurities I, II, III and IV were confirmed by synthesis and structural characterization using spectral data. However, the impurity V was not synthesized.

Keywords: Celecoxib; Impurity; Spectroscopy; Identification; Liquid chromatography–mass spectrometry; Preparative HPLC; Characterization; Synthesis

1. Introduction

Celecoxib is cox-2 class of anti-inflammatory compound with little or no gastric side effects.

The HPLC analysis data of Celecoxib were reported in the literature [1]. The metabolites of Celecoxib have been characterized by LC-MS-MS and were reported in the literature [2]. The present study describes the identification of the five impurities present in crude Celecoxib drug. Impurities I and II were characterized by LC-MS data and further confirmed by synthesis. Impurities III, IV and V were isolated by a simple reversed phase preparative HPLC and were characterized using spectral data.

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2. Experimental

2.1. Chemicals

Ammonium acetate (AR grade, SD fine chemicals, India) and acetonitrile (HPLC grade, Merck, India) were purchased. High pure Milli-Q water was used with the help of Millipore Milli-Q plus purification system. Crude Celecoxib drug sample was analysed and taken up for this study.

2.2. High performance liquid chromatography (analytical)

Waters alliance system was used for the entire analysis. A Hichrom C18 (250 mm × 4.6 mm) 5 μm column was used for the separations. The sample detection was monitored at a wavelength of 252 nm. A mixture of 0.01 M KH_2PO_4 in water and acetonitrile in the ratio of 45:55 was used as the mobile phase at a flow rate of 1.0 ml/min.

2.3. High performance liquid chromatography (preparative)

A Shimadzu preparative HPLC which was equipped with an LC-8A pump, SCL-8A system controller, SPD-6AV UV-Vis detector, FCV-100B fraction collector and a Rheodyne injector model 7725i with a 2.0 ml loop was used with FC100E basic program carried fraction collection. The data was collected and processed using Shimadzu C-R7A Chromatopak. A 250 mm × 10 mm i.d. column packed with 8 μm Hyperprep-C18 (Shandon, Life Sciences International (Europe) Ltd.) was employed for separation. The mobile phase consisted of acetonitrile and water in the ratio of 50:50 (v/v). The flow rate was kept at 2.0 ml/min. Detection was carried out at 252 nm. The fractions of impurity III, IV and V were collected from several injections and pooled. These fractions were concentrated separately by using Buchi Rotavapor model R124.

2.4. Mass spectrometry

LC-MS consisted of Hewlett-Packard 5989A mass spectrometer with a HP-1050 pump. The interface used was HP59980B particle beam interface. Shimadzu SPD-M10Avp DAD has been used for chromatography work.

2.5. NMR spectroscopy

NMR spectra were recorded on Varian Gemini 200 instrument (200 MHz for ^1H, and 50 MHz for ^{13}C) ^1H and ^{13}C chemical shifts are reported on the δ-scale in ppm, relative to TMS (δ 0.00) and DMSO (δ 39.5) as internal standards, respectively.

2.6. Sample preparation

The Celecoxib sample was prepared at a concentration of 1 mg/ml in mobile phase for the analytical HPLC and 10 mg/ml for the preparative HPLC analysis.

2.7. LC–MS conditions

The mobile phase used was 0.01 M CH_3COONH_4, and acetonitrile in the ratio of 40:60. The sample was monitored at 254 nm. Flow was kept at 0.4 ml/min. A Zorbax C18 (250 mm × 4.6 mm) column was used. The particle beam interface temperature was maintained at 70 °C with helium as the nebuliser gas at 60–70 psi. The injected volume was 20 μl. The temperatures of the source manifold and quadrapole temperatures were maintained at 250 and 100 °C, respectively.

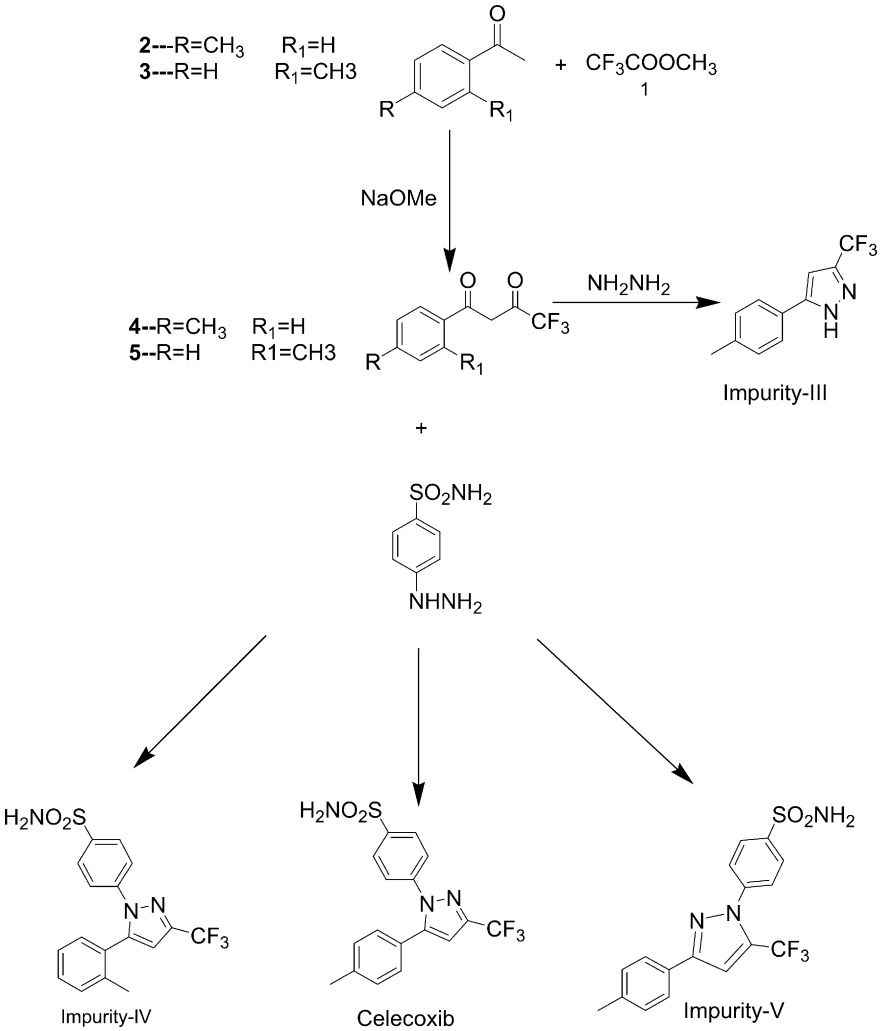
2.8. Synthesis of Impurities

2.8.1. Impurity I [4-methyl acetophenone]

This is one of the starting materials. This impurity's presence was confirmed by co-injection with Celecoxib MLs in the HPLC.

2.8.2. Impurity II [methyl-4-methyl benzoate]

A solution of 4-methylbenzoic acid (5 g, 36 mmol) in 5% methanolic sulfuric acid (50 cc) was refluxed for 4 h. The solvent was evaporated and the residue was dissolved in ethylacetate (50 cc). It was washed with saturated sodium bicarbonate solution and evaporated to get the title compound, a brown colour liquid.



Scheme 1. For synthesis of possible impurities.

2.8.3. Impurity III [5-(4-methylphenyl)-3-trifluoromethyl-1H-pyrazole]

A solution of hydrazine hydrate (5 g, 100 mmol) in methanol (75 cc) was slowly added to a solution of 1-(4-methyl phenyl)-4,4,4-trifluorobutan-1,3-dione (Scheme 1) (23 g, 100 mmol) in methanol (25 cc). It was refluxed for 14 h. The solvent was evaporated completely and the residue was diluted with water (50 cc). It was extracted into ethylacetate (3 × 75 cc). The combined extracts were dried over anhydrous sodium sulphate and evaporated to one-third of the original volume. After precipitating with hexane (250 cc), the title compound was filtered and dried at 60–70 °C and subjected to spectral analysis.

^{1}H NMR (DMSO-d_6) δ 2.3(s, 3H). 7.2–7.6 (d, 4H, ArH), 6.8 (s, 1H), 14.0 (s, 1H, NH). Mass: $m/z = 226$.

2.8.4. Impurity IV 4-[5-(2′-methyl phenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide

Dry hydrochloride gas was passed in to a solution of 4-hydrazine benzene sulphonamide (Scheme 1) (4 g, 21 mmol) in methanol (75 cc) till pH reads 2.0. A solution of 1-(2-methylphenyl)-4,4,4-trifluorobutan-1, 3-dione (Scheme 1) (5 g, 21 mmol) in methanol

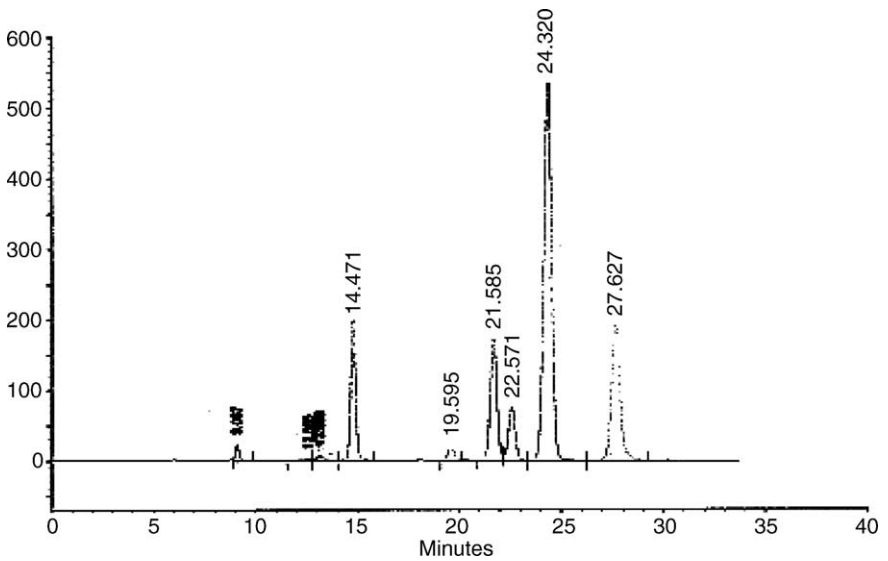


Fig. 1. Celecoxib LC–MS data. Column: Zorbax C18, 250 mm × 4.6 mm, mobile phase: 0.01 M CH_3COONH_4 in water: acetonitrile (40:60), flow rate = 0.4 ml/min, wavelength at UV 254 nm.

(15 cc) was slowly added to the reaction mixture. It was refluxed for 16 h. The solvent was evaporated completely. The residue was diluted with water (35 cc). It was extracted into ethylacetate (3 × 50 cc). The combined organic layers were dried over sodium sulphate and evaporated to one-third of the original volume. It was precipitated with hexane (150 cc). The title compound was filtered and dried at 60–70 °C.

For NMR data see Table 2. Mass: m/z = 381, 300, 184, 116, 91.

2.9. Spectral data of impurity V 4-[4-(4′-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]-benzenesulfonamide

Mass: (EI; relative intensity, %) 91 (15), 116 (10), 206 (9), 232 (10), 281 (15), 300 (25), M^+ = 381 (100). For NMR data see Table 2.

3. Results and discussion

The HPLC analysis of crude Celecoxib drug in which the targeted impurities were present greater than 2.0% was taken up for a detailed study. The LC–MS analysis of Celecoxib is shown in Fig. 1, and results are given in Table 1. The impurity I was confirmed by co-injecting with Celecoxib in HPLC analysis.

The structure of impurity II was characterized as methyl-4-methyl benzoate based on the molecular ion information and fragmentation pattern obtained by

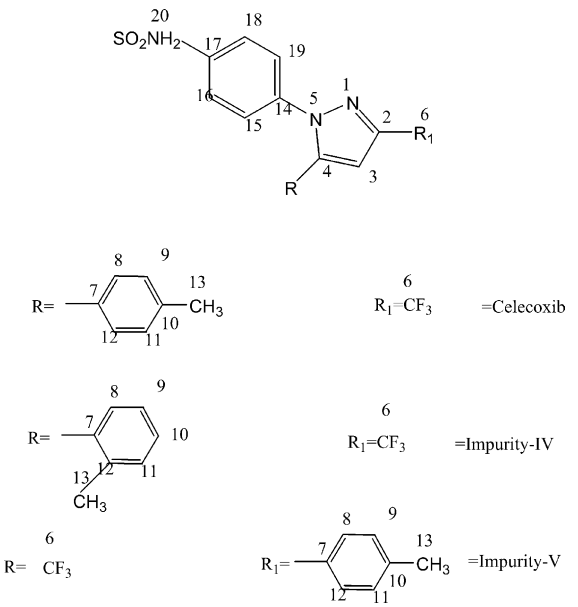
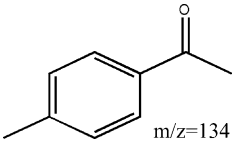
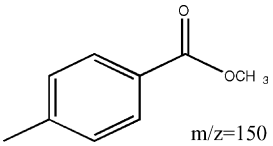
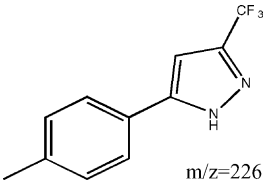
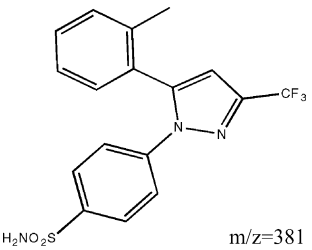
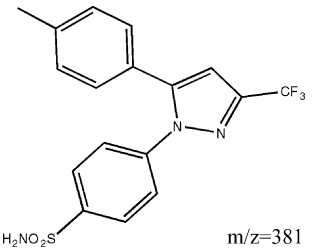
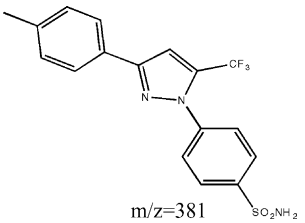


Fig. 2. Structural formula for numbering.

Table 1

IMP No.	RT (LC–MS)	Structure	Confirmed by	Area %*
I	14.7	 m/z=134	Synthesis, spectral analysis & HPLC co-injection	
II	19.6	 m/z=150	Synthesis, spectral analysis & HPLC co-injection	0.05
III	21.7	 m/z=226	Preparative HPLC, synthesis, spectral analysis & HPLC co-injection	0.15
IV	22.5	 m/z=381	Preparative HPLC, synthesis, spectral analysis & HPLC co-injection	0.1
Celecoxib	24.3	 m/z=381	Synthesis, spectral analysis & HPLC co-injection	API
V	27.6	 m/z=381	Preparative HPLC, spectral analysis & HPLC co-injection	0.38

* This result was obtained in a purified batch sample.

LC–MS data. The impurity III was isolated by preparative HPLC and characterized as 5-(4-methylphenyl)-3-trifuloromethyl pyrazole by LC–MS data. These impurities were further confirmed by the synthesis.

The mass spectral data of the impurity IV and V displayed the molecular ion at $m/z = 381$ as that of Celecoxib indicating that they are isomers. To investigate the structures of impurity IV and V, they were

Table 2
^{1}H, ^{13}C and DEPT NMR assignments of Celecoxib, impurity IV and impurity V

Position	Celecoxib[a]				DEPT	Impurity IV[a]				DEPT	Impurity V[a]				DEPT
	^{1}H	δ (ppm)	J (Hz)[b]	^{13}C		^{1}H	δ (ppm)	J (Hz)[b]	^{13}C		^{1}H	δ (ppm)	J (Hz)[1]	^{13}C	
2	–	–	–	142.8 (q, 38)[c]	–	–	–	–	142.4 (q, 38)[c]	–	–	–	–	132.7 (q, 39)[c]	–
3	1H	7.2	s	106.1	CH	1H	7.1–7.6	S	107.3	CH	1H	7.8	s	107.5	CH
4	–	–	–	141.2	–	–	–	–	141.2	–	–	–	–	140.9	–
6	–	–	–	121.3 (q, 269)[c]	–	–	–	–	121.4 (q, 267)[c]	–	–	–	–	119.6 (q, 268)[c]	–
7	–	–	–	125.4	–	–	–	–	128.2	–	–	–	–	128.2	–
8	H	7.2	–	128.8	CH	H	7.1–7.6	–	130.0	CH	H	7.8 –8.1	–	125.6	CH
9	H	7.2	–	129.4	CH	H	7.1–7.6	–	130.7	CH	H	7.3	d,7.9	129.5	CH
10	–	–	–	139.2	–	H	7.1–7.6	–	130.7	CH	–	–	–	138.5	–
11	H	7.2	–	129.4	CH	H	7.1–7.6	–	126.3	CH	H	7.3	d,7.9	129.5	CH
12	H	7.2	–	128.8	CH	–	–	–	136.9	–	H	7.8 –8.1	–	125.6	CH
13	3H	2.3	s	20.8	CH_3	3H	2.0	–	19.5	CH_3	3H	2.4	s	20.8	CH_3
14	–	–	–	145.3	–	–	–	–	143.9	–	–	–	–	151.7	–
15 and 9	2H	7.8	d, 8.6	126.9	CH	2H	7.8	d, 8.3	126.9	CH	2H	7.8 –8.1	–	126.1	CH
16 and 18	2H	7.5	d, 8.6	126.0	CH	2H	7.1–7.6	–	124.8	CH	2H	8.1	d, 8.6	127.0	CH
17	–	–	–	144.1	–	–	–	–	144.4	–	–	–	–	144.8	–
20	NH_2[d]	7.5	s	–	–	NH_2[d]	7.1–7.6	–	–	–	NH_2[d]	7.6	s	–	–

[a] Refer structural formula for numbering (Fig. 2).
[b] This column gives the multiplicity and the ^{1}H–^{1}H coupling constant; s-singlet, d-doublet.
[c] C–F couplings in Hz and multiplicity q-quartet.
[d] Exchangeable proton.

isolated by preparative HPLC. The spectral data of impurity IV and V were collected. The data was compared with those of Celecoxib (Table 2).

3.1. Structure elucidation of impurity III

The mass spectrum of impurity III displayed the molecular ion at $m/z = 226$ and was confirmed by the protonated ion in chemical ionization mode. The presence of two doublets integrated for four protons in the aromatic region (7.2–7.6 ppm) lend support to the presence of *p*-disubstituted phenyl ring. In ^{13}C NMR one quaternary carbon was observed as a quartet due to the carbon fluorine coupling. This confirmed the presence of CF_3 group in the structure. In IR spectrum the secondary amine N–H stretching was observed at 3350 cm^{-1}. On the basis of these observations the structure was confirmed as 5-(4-methylphenyl)-3-trifluoromethyl-1H-pyrazole ($C_{11}H_9F_3N_2$).

3.2. Structure elucidation of impurity IV

The impurity IV molecular ion was observed at $m/z = 381$ as Celecoxib in mass spectrum. The aromatic proton splitting pattern in ^1H NMR of impurity IV was different when compared with Celecoxib. This was confirmed as an *ortho*-isomer with respect to the methyl group on the phenyl ring. The presence of the methyl group in the *ortho* position disturbed the aromatic region splitting pattern. The methyl group substitution was confirmed with 1D-'nOe' experiments. This corresponds to the molecular formula $C_{17}H_{14}F_3N_3O_2S$.

3.3. Structure elucidation of impurity V

Impurity V was also detected at $m/z = 381$ the same as that of Celecoxib. This was predicted as one of the possible isomers. This was confirmed as a *regio*-isomer with respect to phenyl sulphonamide ring substitution on nitrogens. To conclude this structure unequivocally the following points lends support to the *regio*-isomer.

Though the number of signals of Celecoxib and the impurity V are same, the chemical shifts are different, which are shown in Table 2.

3.4. Formation of impurities

Impurity I (4-methyl acetophenone) was found to be a starting material for Celecoxib.

Impurity II (methyl-4-methyl benzoate) was found as an impurity in the starting material (4-methyl acetophenone).

Impurity III [5-(4-methylphenyl)-3-trifluoromethyl-1H-pyrazole] is formed during the final condensation with the possible parallel reaction of left over hydrazine hydrate present in 4-hydrazino benzene sulphonamide (intermediate) and 1-(4-methylphenyl)-4,4,4-trifluoromethylbutan-1,3-dione (Scheme 1).

The impurity IV is formed during the final condensation of 4-hydrazino benzene sulphonamide and 1-(2-methylphenyl)-4,4,4-trifluoromethylbutan-1,3-dione (which in turn formed as a result of condensation of 2-methyl acetophenone and with CF_3COOCH_3) (Scheme 1).

The impurity V is formed during the final condensation of 4-hydrazino benzene sulphonamide and 1-(4-methyl phenyl)-4,4,4-trifluromethyl butan-1,3-dione (Scheme 1).

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