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Short communication

Isolation and characterisation of process-related impurities in rofecoxib

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Abstract

Two unknown impurities in rofecoxib bulk drug at levels below 0.1% were detected by a simple isocratic reverse phase high performance liquid chromatography (HPLC). These impurities were isolated from crude sample of rofecoxib using reverse phase preparative HPLC. ^{1}H, ^{13}C and Mass spectroscopic investigations revealed the structures of the impurities as 4-[4-(methylsulphonyl)phenyl]-3-phenyl-5-hydroxyfuran-2-one (**I**) and 4-[4-(methylsulphonyl)phenyl]-3-phenyl-2,5-furandione (**II**), respectively. These structures were further confirmed by prepared synthetic standards of the impurities. The tentative mechanism for the formation of these impurities was discussed.

Keywords: Rofecoxib; Impurities; Spectroscopy; Identification and characterisation

1. Introduction

Rofecoxib, (4-[4-(methylsulphonyl)phenyl]-3-phenyl-5-hydroxyfuran-2-one, is a potent and selective COX-2 inhibitor that was shown to be very effective for the treatment of arthritis and acute pain [1,2]. High performance liquid chromatographic (HPLC) method was cited in the literature for the purity testing of rofecoxib bulk material [3]. The stringent purity requirement [4] that all the individual impurities, which are $\geq 0.1\%$, must be identified and characterised, this paper aims at the isolation and characterisation of two minor impurities (**I** and **II**) in rofecoxib, which are present at a level of $\leq 0.1\%$. To the best of our knowledge, impurity **II** was not known and impurity **I** was known as a metabolite [5,6] but not as an impurity of rofecoxib.

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2. Experimental

2.1. Samples

The investigated samples of rofecoxib bulk material (B.No. PC/2015/V/MB/RC2) and crude samples (B.Nos. PC/2015/V/34 2nd cr. and PC/2015/V/AC-33 Resd) were obtained from Process Research and Technology Development Department of Dr Reddy's Research Foundation, Hyderabad, India.

2.2. High performance liquid chromatography (analytical)

A Waters Model Alliance 2690 Separation module equipped with a Waters 996 photo diode array UV detector was used. A Hichrom RPB column with the dimensions of 250 × 4.6 mm i.d. (Hichrom, UK) was used for the separations. The column eluent was monitored at a wavelength of 225 nm and the data was recorded using Waters Millennium 32 software. A mixture of water and acetonitrile in the ratio of 50:50 (v/v) was used as mobile phase at a flow rate of 1.0 ml/min [3].

2.3. High performance liquid chromatography (preparative)

A Shimadzu preparative HPLC equipped with LC-8A pump, SCL-8A System controller, SPD-6AV UV–Vis detector, FCV-100B Fraction collector and Rheodyne Injector Model 7725i with 2.0 ml loop. The data was collected and processed using Perkin–Elmer PE-1022 Integrator. A 250 × 10 mm i.d. column packed with 10 μ Hyperprep-HS-C18 (Shandon, UK) was employed for separation. The mobile phase consisted of water–acetonitrile in a ratio of 65:35 (v/v). The flow rate was set at 3.0 ml/min. Detection was carried out at 225 nm.

2.4. Mass spectrometry

Mass spectra were run on HP5989 with ionisation electron beam energy of 70 eV. The sample was introduced into the source with the help of a particle beam interface connected to LC by by-passing the column. The source manifold and Quadrupole temperatures were maintained at 250 and 100 °C, respectively. The CI reagent gas used was isobutane.

2.5. NMR spectroscopy

NMR measurements were performed on a Varian Gemini 2000 model 200 MHz instrument (both for ^{1}H and ^{13}C) at 25 °C in $CDCl_3$ and DMSO-d_6.

The chemical shift values were reported on the δ scale in ppm, relative to TMS ($\delta = 0.00$) and DMSO-d_6 ($\delta = 39.5$ ppm) as internal standard, respectively. Assignments were further confirmed by running two-dimensional chemical shift correlation experiments.

2.6. FT-IR spectroscopy

IR spectra for rofecoxib, impurity **I** and **II** were recorded in the solid state as KBr dispersion using Perkin–Elmer 1650 FT-IR spectrophotometer.

2.7. Synthesis of impurities

Synthetic impurities [5] were obtained through arial oxidation of rofecoxib. The procedure cited in the literature was slightly modified by stirring a solution of rofecoxib in ethyl acetate in the presence of activated charcoal under 2 kg of air pressure at 90 °C for 72 h.

3. Results and discussions

3.1. Detection of impurities

Typical analytical LC chromatograms of rofecoxib, impurity **I** and **II** obtained by using the LC method discussed in Section 2.2 are shown in Fig. 1. The target impurities under study are marked as **I** and **II** with retention times of about 6 and 11 min, respectively, while rofecoxib eluted at about 7 min. These impurities were isolated by chromatographing the crude sample of rofecoxib on preparative LC.

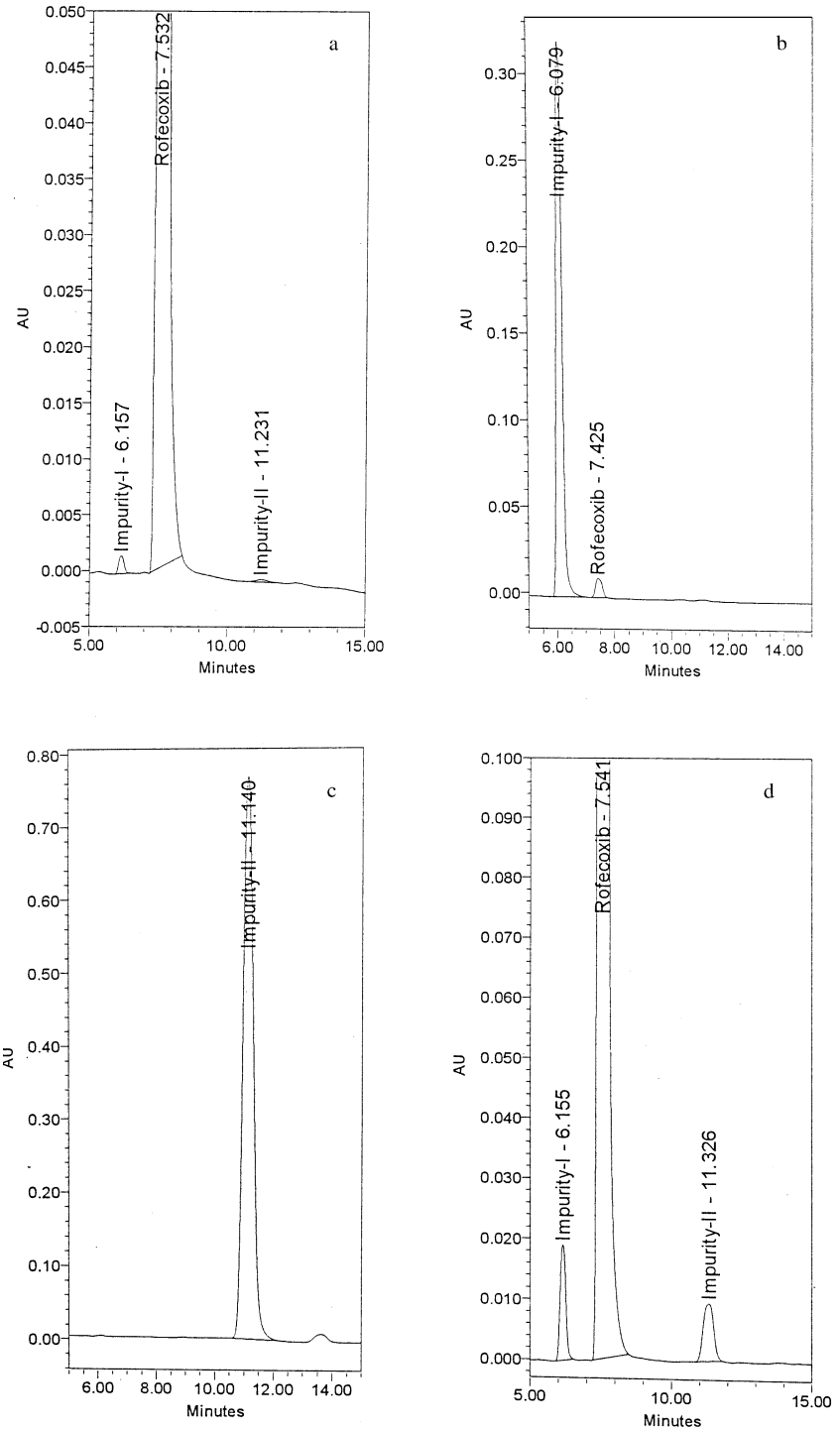


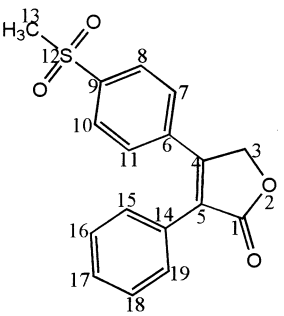
Fig. 1. LC chromatograms of rofecoxib (a), impurity **I** (b), impurity **II** (c) and spiked chromatogram of rofecoxib with impurities (d).

3.2. Isolation of the impurities by preparative HPLC

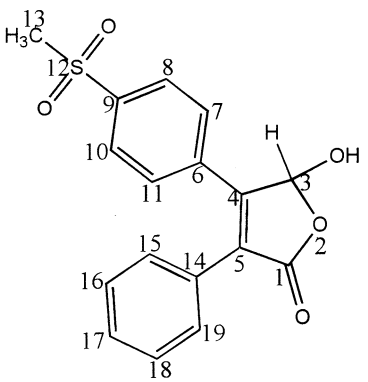
A simple reverse phase solvent system discussed in Section 2.3 was used for isolating these impurities. In this solvent system rofecoxib eluted at about 20–23 min whereas the impurities **I** and **II** eluted at about 9–12 and 30–35 min, respectively. Both the fractions of impurities isolated, were concentrated under high vacuum on a Buchii Rotavapor Model R124. Purity of these impurities was tested in analytical mode and found to be 93 and 91%, respectively for carrying out spectroscopy experiments.

3.3. Structure elucidation of impurities **I** and **II**

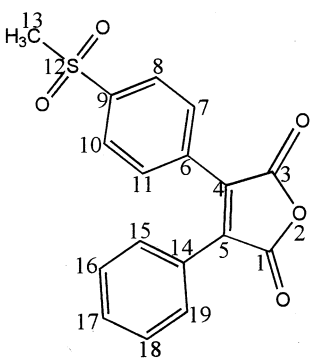
The spectral data of impurities **I** and **II** were compared with those of rofecoxib. The FT-IR spectrum of impurity **I** exhibited a characteristic absorption band between 3380 and 3400/cm indicating the presence of OH group which was absent in that of rofecoxib. The CI mass spectrum displayed a protonated molecular ion peak at m/z



Rofecoxib



Impurity I



Impurity II

Fig. 2. Chemical structures of rofecoxib, impurities **I** and **II**.

Table 1
NMR assignments of rofecoxib, impurities **I** and **II**

Position	Rofecoxib			Impurity **I**			Impurity **II**		
	^{1}H	ppm/*J*	^{13}C	^{1}H	ppm/*J*	^{13}C	^{1}H	ppm/*J*	^{13}C
1	–	–	172.5	–	–	170.6	–	–	164.6
3	2H	5.2/s	70.7	1H	6.5/s	97.1	–	–	164.7
4	–	–	155.8	–	–	152.9	–	–	140.0
5	–	–	126.9	–	–	135.8	–	–	136.9
6	–	–	141.8	–	–	130.8	–	–	132.6
7 and 11	2H	7.5/d (8.4)	128.6	2H	7.6/d (8.4)	128.9	2H	7.4/d (8.5)	130.4
8 and 10	2H	7.9/d (8.4)	127.4	2H	7.8/d (8.4)	127.6	2H	8.0/d (8.5)	127.3
9	–	–	135.6	–	–	141.2	–	–	142.0
13	3H	3.2/s	43.1	3H	3.1/s	44.1	3H	3.3/s	43.2
14	–	–	129.7	–	–	128.4	–	–	126.9
15 and 19	2H	7.3–7.4	128.9	2H	7.4–7.5	129.7	2H	7.4–7.5	129.5
16 and 18	2H	7.3–7.4	128.5	2H	7.4–7.5	129.2	2H	7.4–7.5	128.9
17	1H	7.4–7.5	128.7	1H	7.4–7.5	129.7	1H	7.4–7.5	131.0

Refer structural formula for numbering (Fig. 2). d, doublet; s, singlet; *J*, coupling constant.

331 which is 16 atomic mass units (amu) more than that of rofecoxib and the corresponding molecular formula of the impurity could be $C_{17}H_{14}O_5S$. This indicates the possible incorporation of oxygen in rofecoxib. The ^{1}H NMR spectrum showed a methylene group signal at δ 5.2 ppm in rofecoxib, which disappeared in the spectrum of impurity **I** and a signal at δ 6.5 ppm corresponding to the formation of methine group. The methylene signal at 70.7 ppm for rofecoxib in ^{13}C NMR spectrum disappeared and a methine signal appeared at 97.1 ppm in impurity **I**. The above data can be rationalised in terms of the incorporation of hydroxyl group. The structure of impurity **I** was characterised as 4-[4-(methylsulphonyl)phenyl]-3-phenyl-5-hydroxyfuran-2-one, is shown in Fig. 2.

The spectral data of impurity **II** was very similar with those of impurity **I** and rofecoxib. The FT-IR spectrum of impurity **II** exhibited two carbonyl absorption bands at 1760 and 1830/cm indicating the presence of two carbonyl groups in impurity **II**. The CI mass spectrum showed a protonated molecular ion peak at 329, which was two amu less than that of impurity **I** and the corresponding molecular formula of the impurity **II** could be $C_{17}H_{12}O_5S$. The mass spectral data indicated the loss of two mass units from impurity **I**. Neither the methylene group (δ 5.2 ppm) of rofecoxib nor methine group (δ 6.5 ppm) could be seen in the ^{1}H spectrum of impurity **II**. This observation along with an additional IR band at 1830/cm indicates an additional carbonyl group in impurity **II** which is due to the oxidation secondary OH in impurity **I**. The ^{13}C NMR spectrum displayed an additional quaternary carbon signal at δ 164.6 ppm corresponding to the additional carbonyl group in impurity **II**. The struc-

Table 2
FT-IR frequency assignments of rofecoxib, impurities **I** and **II**

Mode of vibration	Rofecoxib	Impurity **I**	Impurity **II**
OH stretching	–	3474	–
Aromatic C–H stretching	3017	3018	3019
Aliphatic C–H stretching	2928	2918	2922
Lactone C=O stretching	1747	1754	1829, 1765
S=O stretching	1308, 1149	1305, 1149	1308, 1162
C–O stretching	1283, 956	1274, 962	1271, 971

All frequencies are in per cm.

Table 3
Mass spectral data (possible fragments) of rofecoxib, impurities **I** and **II** in Electron Ionisation mode

Compound	Possible fragments (m/z)
Rofecoxib	314 (M^+), 257, 178, 165, 152, 131 and 103
Impurity **I**	330 (M^+), 302, 257, 193, 178, 165 and 151
Impurity **II**	328 (M^+), 300, 256, 249, 193, 176, 165 and 151

ture of impurity **II** was characterised as, 4-[4-(methylsulphonyl)phenyl]-3-phenyl-2,5-furandione, is shown in Fig. 2.

Chemical shift values for rofecoxib, impurities **I** and **II** obtained from NMR (^1H and ^{13}C) studies are listed in Table 1. Characteristic FT-IR absorption frequencies recorded for rofecoxib, impurities **I** and **II** are given in Table 2. Possible mass fragments obtained from mass spectral data for rofecoxib, impurities **I** and **II** are tabulated in Table 3.

3.4. Synthesis of impurities

The reaction mass obtained after the synthesis (discussed in Section 2.7) was subjected to LC using the method discussed in Section 2.2. Interestingly both the impurities were formed in the same reaction, which was observed in LC analysis. The percentages of impurities **I** and **II** were found to be 40 and 55%, respectively. It was also observed that unreacted rofecoxib was around 2%. The impurities mixture was subjected to column purification using 35% ethyl acetate in petroleum ether. Purity of individual fractions was also checked on LC method. Purities (column purified fractions) of impurities **I** and **II** were 93 and 97%, respectively. The spectral data of **I** and **II** were generated. The spectral data of impurities **I** and **II** matched well with those isolated by preparative HPLC.

This was further confirmed by co-eluting the impurities isolated by preparative HPLC and the synthesised impurities by using LC method [7] as shown in Fig. 1d.

The ^1H NMR data of impurity **I** matched well with the reported data [6].

3.5. Formation of impurities

Formation of these impurities can be rationalised in terms of consecutive air oxidation viz. rofecoxib $\Rightarrow$ **I** $\Rightarrow$ **II**.

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