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Identification, characterization and synthesis of impurities of zafirlukast☆

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ABSTRACT

Zafirlukast is a drug in the treatment of pulmonary disorders such as asthma. During the process development of zafirlukast, five unknown impurities were detected at levels of below 0.10% (ranging from 0.05 to 0.15%) in reverse phase gradient high performance liquid chromatography (HPLC) method. The molecular weights were determined by LC–MS analysis. These impurities were isolated from crude samples of zafirlukast using gradient reverse phase preparative HPLC and were subsequently synthesized. Based on the spectral data, the structures of these impurities were characterized as 3-methoxy-4-(5-methoxycarbonylamino-1-methyl-1*H*-indol-3-ylmethyl)-benzoic acid (Impurity 1), {3-[2-methoxy-4-(toluene-2-sulfonylaminocarbonyl)-benzyl]-1-methyl-1*H*-indol-5-yl}-carbamic acid methyl ester (Impurity 2), {3-[2-methoxy-4-(toluene-3-sulfonylaminocarbonyl)-benzyl]-1-methyl-1*H*-indol-5-yl}-acetic acid cyclopentyl ester (Impurity 3), {3-[2-methoxy-4-(toluene-4-sulfonylaminocarbonyl)-benzyl]-1-methyl-1*H*-indol-5-yl}-acetic acid cyclopentyl ester (Impurity 4), and 4-(5-cyclopentyloxy carbonylamino-1-methyl-1*H*-indol-3-yl methyl)-3-methoxy-benzoic acid methyl ester (Impurity 5). The separation of the impurities by reverse phase HPLC, the confirmation of their structures by IR, MS and NMR spectral data, the mechanism of their formation and their syntheses are discussed in detail.

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1. Introduction

Zafirlukast, 3-[2-methoxy-4-(toluene-2-sulfonylaminocarbonyl)-benzyl]-1-methyl-1*H*-indol-5-yl}-acetic acid cyclopentyl ester (see Fig. 1 and Scheme 1) is a drug used for the treatment of pulmonary disorders such as asthma. It acts by antagonizing one or more of the arachidonic acid metabolites such as leukotriene, which inhibits the activity of cytochrome CYP 3A4 and CYP 2C9. The CYP 3A4 isozyme is also responsible for the metabolism of many other drugs [1,2].

There are very few analytical techniques reported in the literature for the determination of zafirlukast. Mainly high performance liquid chromatography (HPLC) methods for the determination of zafirlukast in biological fluids have been presented. Ficarra et al. [3] reported a HPLC method with UV detection for routine control of zafirlukast in pharmaceutical formulations. Radhakrishna et al. [4] developed a stability indicating HPLC procedure for the determination of zafirlukast in bulk drug and its commercial formulations. Recently, a capillary electrophoretic method was developed and validated for the determination of zafirlukast [5].

To our knowledge, there is no report in the literature for the separation and identification of the impurities of zafirlukast. As per the general guidelines recommended by ICH [7] to qualify the drug substance, the amount of acceptable level for a known and unknown related compound (impurity) should be less than 0.15 and 0.10%, respectively. In order to meet the stringent regulatory requirements, the impurities present in the drug substance must be identified and characterized.

During the process development of zafirlukast (Scheme 1) [6], five impurities present in the laboratory batches in the range of 0.05–0.15% level were found by HPLC. In this article, we report isolation, spectral characterization/structure elucidation and synthesis of these impurities together with the pathway for their formation.

2. Experimental

2.1. Samples

The samples of zafirlukast were obtained from Dr. Reddy's Laboratories Ltd. Integrated Product Development, Innovation plaza,

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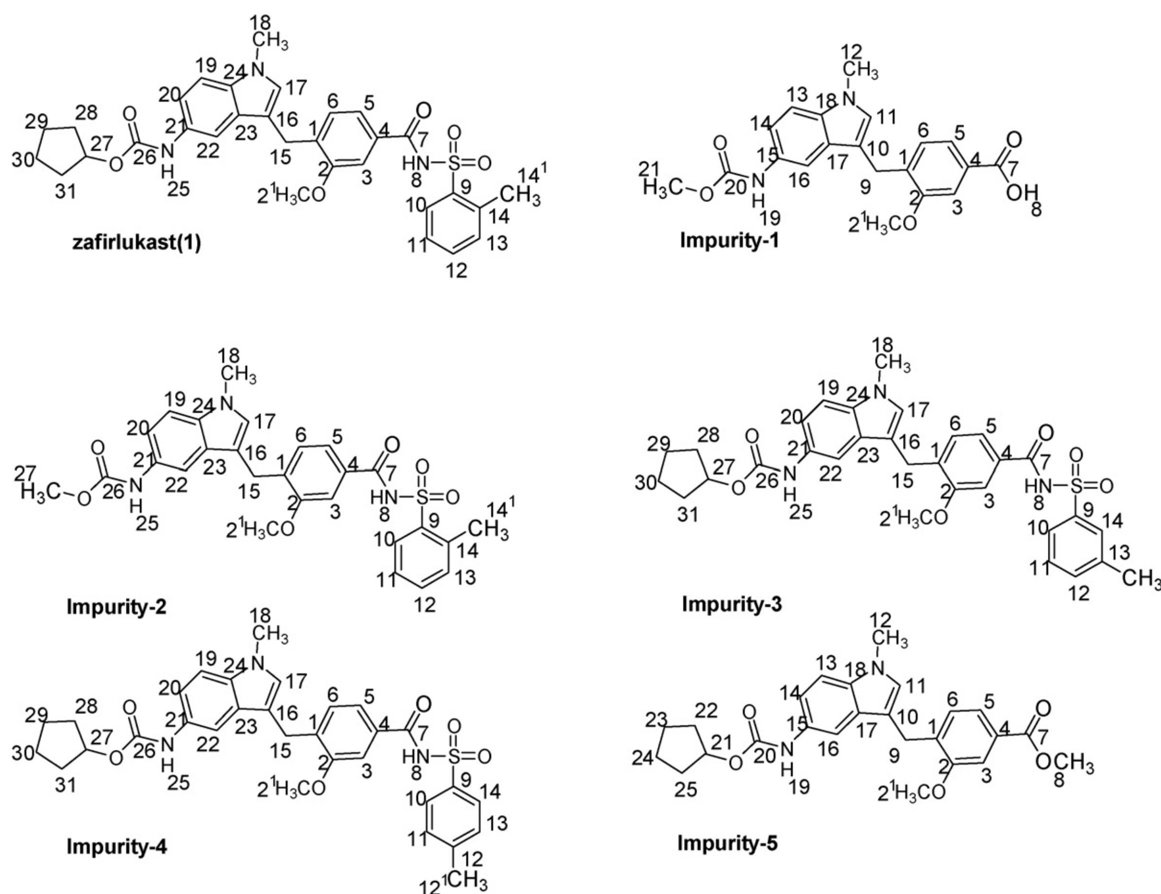


Fig. 1. Structures of zafirlukast and its impurities.

Bachupally, Hyderabad, India. The impurities were isolated from a crude sample (obtained from the mother liquor) by preparative HPLC. The reagents used for analysis included acetonitrile (HPLC grade), potassium dihydrogen orthophosphate (Merck) and 1-decanesulphonic acid sodium salt (S.D. fine chem.). The water used for preparing mobile phase was purified using a Millipore Milli-Q plus purification system.

2.2. High performance liquid chromatography (analytical)

A Waters Model Alliance 2690-separation module equipped with a Waters 996-photo diode array detector was used for the studies. The analysis was carried out on Zodiac 100, C18 columns, 250 mm × 4.6 mm i.d., 5 μm particle size with a mobile phase consisting of A: (degassed buffer) 7.27 g of KH_2PO_4 1.0 g of 1-decanesulphonic acid sodium salt in 1000 ml of milli Q water and pH adjusted to 4.0 with diluted phosphoric acid (1.0 g in 10.0 ml water) and methanol in the ratio of 85:15. B: acetonitrile, methanol and water of 850:100:50 (v/v). Sample was dissolved in diluent (acetonitrile:water, 8:2, v/v). The injection load was 20 μl. Gradient elution was used with UV detection at 220 nm at a flow rate of 0.8 ml/min (t(min)/A(v/v)/B(v/v) = 0/60/40, 5/60/40, 17/38/62, 33/38/62, 35/40/60, 45/26/74, 55/26/74, 60/14/86, 65/7/93, 75/60/40, 85/60/40). The column temperature was maintained at 27 °C. The data was recorded using Waters Millennium software.

2.3. Preparative liquid chromatography

The preparative HPLC system used was a Waters system equipped with W600 quaternary solvent delivery module and Delta prep 2487 dual wavelength UV detector. Data was processed through Waters Empower software. A Develosil ODS MG-10 column (250 mm × 20 mm, 10 μm particle size) was used for preparative work. The mobile phase employed was solution A as aqueous 0.01 M ammonium acetate and solution B as a mixture of acetonitrile and water 8:2 (v/v). The gradient program was as follows: t(min)/B (v/v): 01/20,05/20,20/55, 35/65, 50/75, 70/85, 85/95, 90/20, 95/20. The flow rate was kept at 20 ml/min and the column eluent was monitored at 220 nm.

2.4. Mass spectrometry

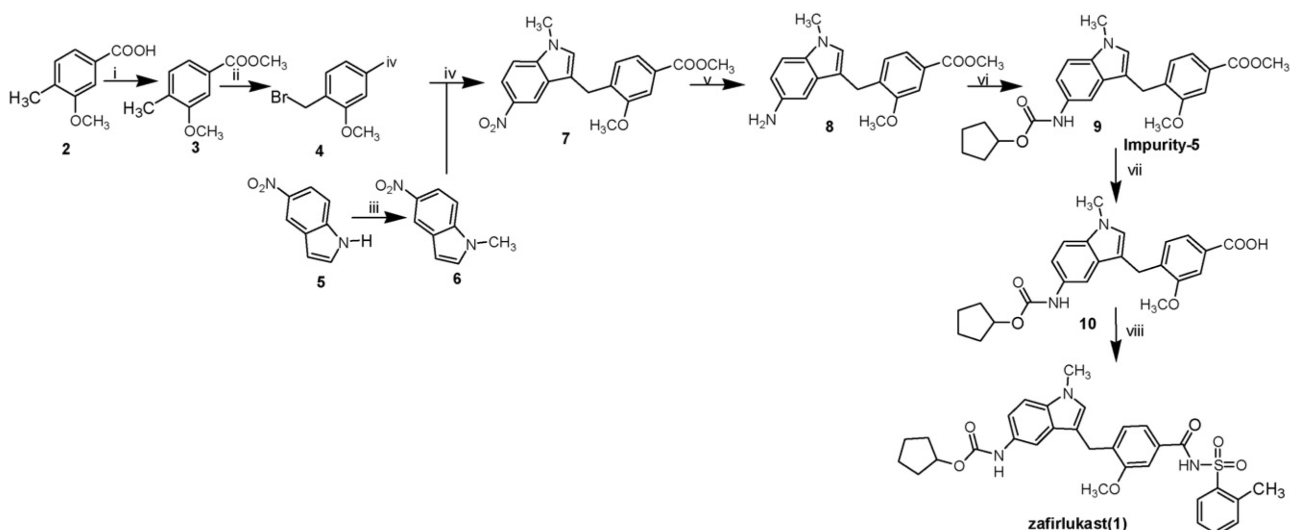
Mass spectra were recorded on an AB 4000Q Trap LC–MS/MS mass spectrometer. The samples were introduced into the system at the ion spray voltage of 4500 V and temperature was maintained at 400 °C. The GS_1 and GS_2 were optimized to 30 and 35 psi, respectively and declustering potential is 70 V. Detection of ions was performed in electrospray ionization, positive ion mode.

2.5. NMR spectroscopy

The ^{1}H NMR, ^{13}C NMR and DEPT spectra were recorded on Varian Mercury plus 400 MHz, Gemini 200 MHz, using DMSO-d_6, and a mixture of DMSO-d_6 and $CDCl_3$ as solvents and trimethylsilane (TMS) as the internal standard.

2.6. FT-IR spectroscopy

FT-IR spectra were recorded in the solid state using KBr dispersion on a PerkinElmer 1650 FT-IR spectrophotometer.



Scheme 1. Synthetic scheme of zafirlukast.

2.7. Sample preparation

The zafirlukast sample was prepared at concentration of 0.5 mg/ml in diluent for the analytical HPLC and 20 mg/ml for the preparative HPLC analysis.

2.8. LC–MS analysis

A LC–MS compatible method was developed for the analysis of zafirlukast and its impurities. A column (Zodiac100, C18, 250 mm × 4.6 mm i.d., 5 μm) with a mobile phase consisting of A: 0.01 M ammonium acetate pH adjusted to 4.0 with trifluoroacetic acid; B: mixture of acetonitrile methanol and water 85:10:5 (v/v), respectively, with gradient program of (t(min)/A(v/v)/B(v/v) = 0/60/40, 5/60/40, 17/38/42, 33/38/62, 35/40/60, 45/26/74, 55/26/74, 60/14/86, 65/7/93, 75/60/40, 85/60/40) with flow rate of 0.8 ml/min, sample concentration of 0.5 mg/ml and UV detection at 220 nm was used.

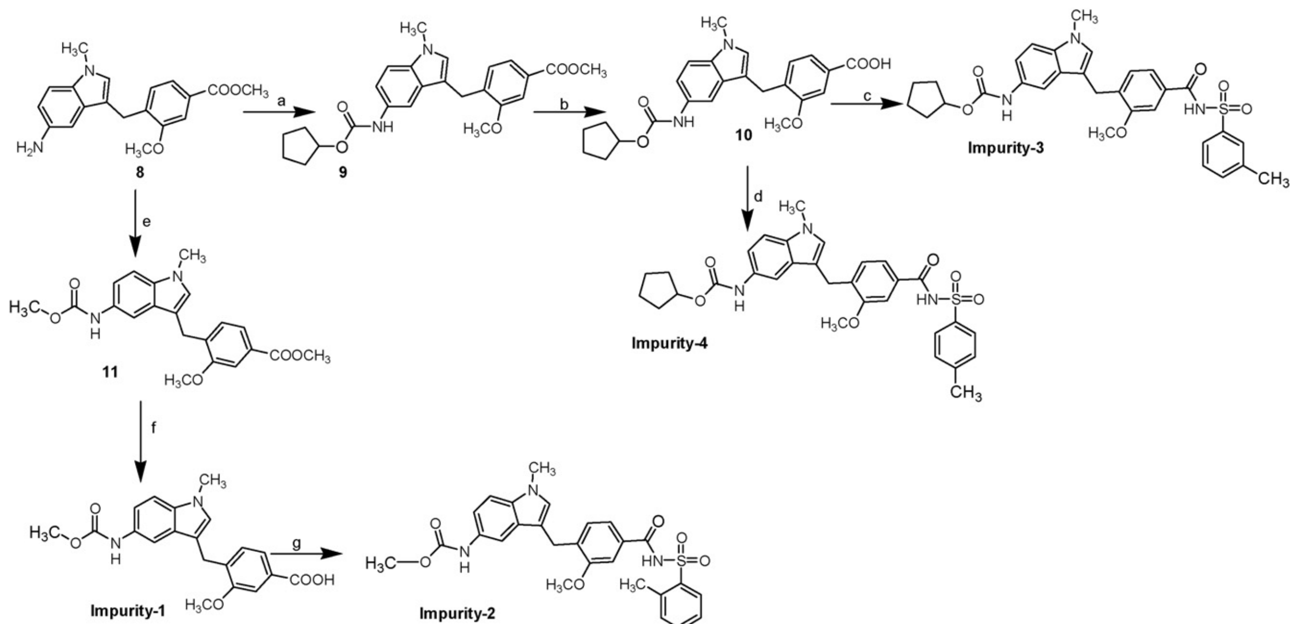
2.9. Melting point determination

Melting points were determined on a Polman digital melting point instrument.

2.10. Synthesis of zafirlukast

Zafirlukast was synthesized as shown in Scheme 1 [6].

Reagents, solvents and conditions: (i) $SOCl_2$ (1.125 equiv.), MeOH, 55–60 °C, 98.0%; (ii) 1,3-dibromo-5,5-dimethylhydantoin (0.725 equiv.), 2,2′-azobis-isobutyronitrile (0.008 equiv.), cyclohexane, 78–82 °C, 84.0%; (iii) dimethyl sulfate (1.17 equiv.), dimethyl formamide, water, 25–35 °C, 98.0%; (iv) cuprous oxide (3.0 equiv.), 1,4-dioxane, EtOAc, MeOH, 95–100 °C; (v) Ra–Ni, EtOAc, H_2, 5–6 kg/cm^2, 25–35 °C, 85.0%; (vi) cyclopentyl chloroformate (1.5 equiv.), *N*-methylmorpholine (1.20 equiv.), toluene, MeOH, 25–35 °C, 97.0%; (vii) $LiOH{\cdot}H_2O$ (1.5 equiv.), MeOH, 60–65 °C, 98.0%; (viii) 1,3-dicyclohexylcarbodiimide (1.14 equiv.), 4-(dimethylamino)pyridine



Scheme 2. Synthetic scheme of zafirlukast impurities.

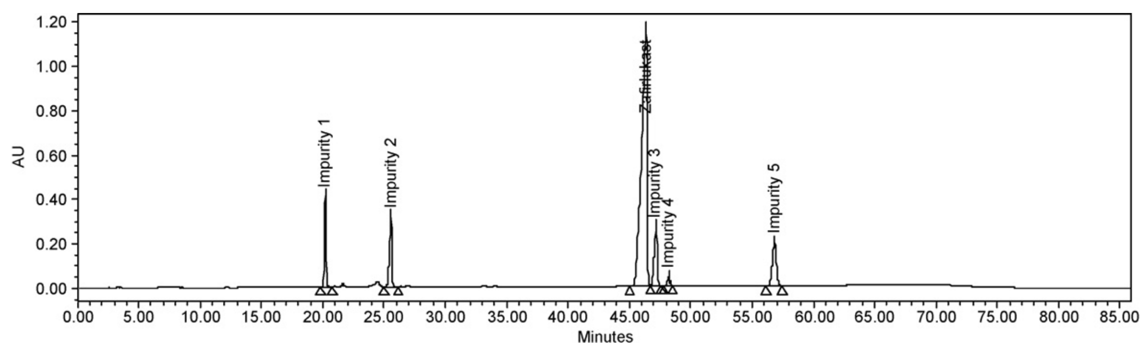


Fig. 2. HPLC of zafirlukast bulk drug spiked with impurities.

(1.2 equiv.), *o*-toluene sulfonamide (1.2 equiv.), CH_2Cl_2, acetonitrile, 25-35 °C, 86.0%.

2.11. Synthesis of zafirlukast impurities

Zafirlukast impurities were synthesized as shown in Scheme 2.
Reagents, solvents and conditions: (a) cyclopentyl chloroformate (1.5 equiv.), *N*-methylmorpholine (1.2 equiv.), toluene, MeOH, 25–35 °C, 97.0%; (b) LiOH·H_2O (1.5 equiv.), MeOH, 60–65 °C, 98.0%; (c) 1,3-dicyclohexylcarbodiimide (1.11 equiv.), 4-(dimethylamino) pyridine (1.0 equiv.), *m*-toluene sulfonamide (1.2 equiv.), CH_2Cl_2, acetonitrile; (d) (1.11 equiv.), 4-(dimethylamino) pyridine (1.0 equiv.), *p*-toluene sulfonamide (1.2 equiv.), CH_2Cl_2, acetonitrile; (e) methyl chloroformate (1.93 equiv.), *N*-methylmorpholine (1.2 equiv.), toluene, MeOH; (f) LiOH·H_2O (2.63 equiv.), MeOH; (g) 1,3-dicyclohexylcarbodiimide (0.967 equiv.), 4-(dimethylamino)pyridine (1.0 equiv.), *o*-toluene sulfonamide (1.05 equiv.), CH_2Cl_2, acetonitrile.

3. Results and discussion

3.1. Detection of impurities 1, 2, 3, 4 and 5

A typical analytical HPLC chromatogram of a production batch of zafirlukast bulk drug spiked with impurities is shown in Fig. 2. The impurities (marked as Impurities 1–5) were isolated from the crude sample of zafirlukast by preparative HPLC method and also synthesized subsequently to obtain sufficient quantities for further studies. The prepared and isolated impurities were co-injected with zafirlukast to confirm the identity of the impurities based on retention matching. All impurities were well resolved from zafirlukast and each other. Relative retention times of the impurities with respect to zafirlukast, melting ranges and structures are shown in Table 1 and Fig. 1, respectively. With the exception of Impurity 5 (a synthesis intermediate) the impurities studied in this paper have not yet been reported earlier. The spectral data for the synthesized and isolated impurities from preparative HPLC were found to be identical. Comparative ^{1}H NMR and ^{13}C NMR assignments for zafirlukast and its impurities are shown in Tables 2 and 3.

3.2. Isolation of impurities by preparative HPLC

A gradient system discussed in Section 2.3 was used for the isolation of these impurities. The fractions were concentrated and extracted with chloroform. The isolated solids obtained from the concentrated fractions were used to generate spectral data.

3.3. Structure elucidation

3.3.1. Impurity 1

The ESI mass spectrum exhibited a molecular ion at *m/z*, 369 [$(MH)^+$] in positive ion mode, indicating molecular weight of 368, which was lesser by 207amu than that of zafirlukast. The ^{1}H NMR spectrum (DMSO-d_6 + $CDCl_3$) showed an additional signal at δ 3.80 ppm indicating the presence of another methoxy group in the impurity. The ^{13}C NMR signal at δ 51.3 ppm supports the presence of extra methyl group. The absence of signals corresponding to the *o*-toluene sulfonamide substituted phenyl ring, and also of cyclopentyl ring protons when compared with zafirlukast ^{1}H NMR spectrum is remarkable. D_2O exchange studies showed two exchangeable protons at δ 9.35 ppm, corresponding to –NH and at δ 8.20 ppm corresponding to –OH of carboxylic acid. These spectral data indicate the absence of cyclopentyl ester and sulfonamide moieties. Furthermore, the IR spectrum did not show asymmetric, symmetric stretching frequency corresponding to sulfone group at 1340, and 1162 cm^{-1} as observed in zafirlukast. Based on the above spectral data, the molecular formula of this impurity was confirmed as $C_{20}H_{20}N_2O_5$ and the corresponding structure was characterized as 3-methoxy-4-(5-methoxycarbonylamino-1-methyl-1*H*-indol-3-ylmethyl)-benzoic acid.

3.3.2. Impurity 2

The ESI mass spectrum exhibited a molecular ion at *m/z*, 523 [$(MH)^+$] in positive ion mode, indicating molecular weight of 522, which was lesser by 53amu than that of zafirlukast. The ^{1}H NMR spectrum (DMSO-d_6) showed an additional signal at δ 3.80 ppm indicating the presence of another methoxy group in the impurity, and the absence of corresponding cyclopentyl ring protons when compared with zafirlukast ^{1}H NMR spectrum. Based on the above

Table 1
Relative retention time (RRT), melting range, FT-IR, mass spectral data of zafirlukast and its impurities.

S.No.	RRT	Compound	IR (KBr) cm^{-1}	MS m/z (Positive)	Melting range (°C)
1	0.43	Impurity 1	3240 (s), 2951 (br), 1707 (s, –C=O), 1279 (s, –OCH_3)	369	128–132
2	0.56	Impurity 2	1679 (s, –C=O), 1333 (s), 3343 (s)	522	118–122
3	1.0	Zafirlukast	3331(N–H,s),2960Ali(–C–H,s), 1690(–C=O,s),1455Ali(–C–H,b), 1340, 1162 (SO_2 Asy,Sym,s)	576	142–145
4	1.03	Impurity 3	1677 (–C=O, s), 1341(s), 3331(s), 1341,1157 (SO_2, Asy, Sym, s)	576	204–210
5	1.05	Impurity 4	1679 (–C=O, s), 1342 (s), 3326 (s), 1343,1166 –(SO_2, Asy, Sym, s)	576	248–252
6	1.21	Impurity 5	3247(–NH, s), 1692 (–C=O, s,), 1719 (–C=O, s), 1232 (–OCH_3,s)	437	128–132

Table 2
Comparative ^{1}H NMR assignments for zafirlukast and its impurities.

Position[a]	Zafirlukast, δ ppm multiplicity	Impurity 1, δ ppm multiplicity	Impurity 2, δ ppm multiplicity	Impurity 3, δ ppm multiplicity	Impurity 4, δ ppm multiplicity	Impurity 5, δ ppm multiplicity
1	–	–	–	–	–	–
2	–	–	–	–	–	–
2[1]	3.82 (s,3H)	3.82 (s,3H)	3.82 (s,3H)	3.82 (s,3H)	3.82 (s,3H)	3.82 (s,3H)
3	6.75 (s,1H)	6.75 (s,1H)	6.75 (s,1H)	6.75 (s,1H)	6.75 (s,1H)	6.75 (s,1H)
4	–	–	–	–	–	–
5	7.18 (d,1H)	7.18 (d,1H)	7.18 (d,1H)	7.18 (d,1H)	7.18 (d,1H)	7.18 (d,1H)
6	7.10 (d,1H)	7.10 (d,1H)	7.10 (d,1H)	7.10 (d,1H)	7.10 (d,1H)	7.10 (d,1H)
7	–	–	–	–	–	–
8	6.54 (s,NH)	8.20 (s,OH)	6.54 (s,NH)	6.54 (s,NH)	6.54 (s,NH)	3.70 (s,3H)
9	–	4.0 (s,2H)	–	–	–	4.0 (s,2H)
10	7.27 (d,1H)	–	7.27 (d,1H)	7.27 (d,1H)	7.27 (d,1H)	–
11	7.49 (t,1H)	6.74 (s,1H)	7.49 (t,1H)	7.49 (t,1H)	7.49 (t,1H)	6.74 (s,1H)
12	7.38 (t,1H)	3.68 (s,3H)	7.38 (t,1H)	7.38 (t,1H)	–	3.68 (s,3H)
12[1]	–	–	–	–	2.68(s,3H)	7.16 (d,1H)
13	8.24 (d,1H)	7.16 (d,1H)	8.24 (d,1H)	–	7.6 (d,1H)	7.06 (d,1H)
13[1]	–	–	–	2.68(s,3H)	–	–
14	–	7.06 (d,1H)	4.0 (s,1H)	7.58 (d,1H)	7.6 (d,1H)	4.0(s,2H)
14[1]	2.68 (s,3H)	–	2.68 (s,3H)	–	–	–
15	4.0 (s,2H)	–	4.0 (s,2H)	4.0 (s,2H)	4.0 (s,2H)	6.74(s,1H)
16	–	7.30 (s,1H)	–	–	–	7.30 (s,1H)
17	6.74 (s,1H)	–	6.75 (d,1H)	6.74 (s,1H)	6.74(s,1H)	–
18	3.68 (s,3H)	–	3.68 (d,3H)	3.68 (s,3H)	3.68(s,3H)	–
19	7.16 (d,1H)	9.35 (br,NH)	7.16 (d,1H)	7.16 (d,1H)	7.16(d,1H)	9.35 (br,NH)
20	7.06 (d,1H)	–	7.30 (s,1H)	7.06 (d,1H)	7.06(d,1H)	–
21	–	3.80 (s,3H)	–	–	–	5.18 (m,1H)
22	7.30 (s,1H)	–	7.30 (s,1H)	7.30 (s,1H)	7.30(s,1H)	1.85 (br,m,2H)
23	–	–	–	–	–	1.71 (br,m,2H)
24	–	–	–	–	–	1.58 (br,m,2H)
25	9.35 (br,NH)	–	9.35 (br,NH)	9.35 (br,NH)	9.35 (br,NH)	1.85 (br,m,2H)
26	–	–	–	–	–	–
27	5.18 (m,1H)	–	–	5.18 (m,1H)	5.18 (m,1H)	–
28	1.85 (br,m,2H)	–	–	1.85 (br,m,2H)	1.85 (br,m,2H)	–
29	1.71 (br,m,2H)	–	–	1.71 (br,m,2H)	1.71 (br,m,2H)	–
30	1.58 (br,m,2H)	–	–	1.58 (br,m,2H)	1.58 (br,m,2H)	–
31	1.85 (br,m,2H)	–	–	1.85 (br,m,2H)	1.85 (br,m,2H)	–

s = singlet; d = doublet; t = triplet; m = multiplet; br = broad singlet. a = For numbering of zafirlukast and the impurities see Fig. 1.

spectral data, the molecular formula of this impurity was confirmed as $C_{27}H_{27}N_3O_6S$ and the corresponding structure was characterized as {3-[2-methoxy-4-(toluene-2-sulfonylaminocarbonyl)-benzyl]-1-methyl-1*H*-indol-5-yl}-carbamic acid methyl ester.

3.3.3. Impurity 3

The ESI mass spectrum exhibited a molecular ion at *m/z*, 576 [(MH)$^+$] in positive ion mode, indicating molecular weight of 575, which is identical to that of zafirlukast. Its UV spectrum was also similar to the zafirlukast spectrum. The singlet at δ 7.78 ppm in the aromatic region of ^{1}H NMR spectrum (DMSO-d_6) indicated the presence of an m-substituted methyl group in the phenyl ring attached to the sulfonamide moiety. Based on the above spectral data, the molecular formula of this impurity was confirmed as $C_{31}H_{33}N_3O_6S$ and the corresponding structure was characterized as {3-[2-methoxy-4-(toluene-3-sulfonylaminocarbonyl)-benzyl]-1-methyl-1*H*-indol-5-yl}-acetic acid cyclopentyl ester (regioisomer of zafirlukast).

3.3.4. Impurity 4

The ESI mass spectrum exhibited a molecular ion at *m/z*, 576 [(MH)$^+$] in positive ion mode, indicating molecular weight of 575, which is also identical to that of zafirlukast. The UV spectra are also similar. The ^{1}H NMR spectrum (DMSO-d_6) clearly shows the presence of the methyl group substituted at the para position to the sulfonamide group. The *p*-disubstituted pattern of corresponding protons shows two doublets at δ 7.27 ppm and δ 7.6 ppm. Based on the above spectral data, the molecular formula of this impurity was confirmed as $C_{31}H_{33}N_3O_6S$ and the corresponding structure was characterized as {3-[2-methoxy-4-(toluene-4-sulfonylaminocarbonyl)-benzyl]-1-methyl-1*H*-indol-5-yl}-acetic acid cyclopentyl ester (another regioisomer of zafirlukast).

3.3.5. Impurity 5

The ESI mass spectrum exhibited a molecular ion at *m/z*, 437 [(MH)$^+$] in positive ion mode, indicating molecular weight of 436, which was lesser by 139 amu than that of zafirlukast. The ^{1}H NMR spectrum (DMSO-d_6) showed additional signal at δ 3.90 ppm and ^{13}C NMR signal at δ 52.0 ppm indicating the presence of a methyl group attached to an electron withdrawing group and it is indicating the presence of another methoxy group in the impurity, when compared with zafirlukast. The signals corresponding to *o*-toluene sulfonamide and substituted phenyl ring were absent in the ^{13}C, ^{1}H NMR spectra. Further the IR spectrum contained stretching frequency corresponding to the ester carbonyl group at 1719 cm^{-1} as observed in zafirlukast. Based on the above spectral data, the molecular formula of this impurity was confirmed as $C_{25}H_{28}N_2O_5$ and the corresponding structure was characterized as 4-(5-cyclopentyloxy carbonylamino-1-methyl-1*H*-indol-3-yl methyl)-3-methoxy-benzoic acid methyl ester.

3.4. Formation of impurities

The presence of methanol under basic conditions resulted in trans-esterification of cyclopentyl group with the methyl group to form Impurity 1. Impurity 2 is formed from Impurity 1 by condensation with *o*-toluene sulfonamide. The presence of *m*-toluene sulfonamide and *p*-toluene sulfonamide impurities in *o*-toluene sulfonamide contributes to the formation of Impurity 3 and 4,

Table 3
Comparative ^{13}C chemical shifts in zafirlukast and its impurities.

Position[a]	Zafirlukast, ^{13}C (δ ppm)/DEPT	Impurity 1, ^{13}C (δ ppm)/DEPT	Impurity 2, ^{13}C (δ ppm)/DEPT	Impurity 3, ^{13}C (δ ppm)/DEPT	Impurity 4, ^{13}C (δ ppm)/DEPT	Impurity 5, ^{13}C (δ ppm)/DEPT
1	136.0/–	134.5/–	135.2/–	135.2/–	136.5/–	135.2/–
2	157.1/–	156.5/–	156.6/–	156.5/–	156.5/–	156.7/–
2^1	55.3/CH_3	55.4/CH_3	55.7/CH_3	55.1/CH_3	55.6/CH_3	55.5/CH_3
3	127.7/CH	128.4/CH	128.4/CH	128.4/CH	128.4/CH	128.5/CH
4	129.5/–	130.7/–	130.05/–	130.0/–	130.1/–	130.9/–
5	119.9/CH	121.5/CH	120.6/CH	120.6/CH	120.0/CH	121.3/CH
6	115.2/CH	114.4/CH	114.5/CH	114.4/CH	114.0/CH	114.4/CH
7	154.4/–	154.2/–	154.3/–	153.6/–	153.6/–	153.6/–
8	–	–	–	–	–	52.0/CH_3
9	137.4/–	24.6/CH_2	137.5/–	139.4/–	144.0/–	24.6/CH_2
10	132.2/CH	111.1/–	132.3/CH	127.6/CH	129.2/CH	110.9/–
11	133.6/CH	129.7/CH	133.4/CH	124.7/CH	127.6/CH	129.4/CH
12	126.1/CH	32.2/CH_3	126.1/CH	128.9/CH	127.1/–	32.3/CH_3
12	–	–	–	–	21.0/–CH_3	–
13	131.1/CH	109.4/CH	130.4/CH	138.7/–	127.6/CH	101/CH
13^1	–	–	–	20.8/CH_3	–	110.4/–
14	136.7/–	110.6/CH	136.8/CH_3	134.2/CH	129.2/– CH	108.0/CH
14^1	20.1/CH_3	–	19.6/CH_3	–	–	–
15	24.9/CH_2	134.6/–	24.6/CH_3	24.6/CH_2	25.3/CH_2	135.2/–
16	111.8/–	108.4/CH	110.9/–	110.8/–	110.8/–	109.4/CH
17	128.0/CH	127.2/–	129.3/CH	129.3/CH	129.2/CH	127.2/–
18	32.4/CH_3	133.3/–	32.3/CH_3	33.4/CH_3	33.3/CH_3	133.2/–
19	109.1/CH	133.3/–	109.5/CH	109.4/CH	109.4/CH	–
20	129.7/CH	167.2/–	110.0/CH	110.0/CH	109.9/CH	166.1/–
21	134.0/–	51.3/CH_3	133.0/–	133.2/–	135.1/–	76.2/CH
22	109.4/CH	–	108.4/CH	108.2/CH	108.1/CH	32.3/CH_2
23	127.7/–	–	127.1/–	133.2/–	130.9/–	23.2/CH_2
24	132.2/–	–	130.8/–	127.1/–	133.1/–	23.2/CH_2
25	–	–	–	–	–	32.3/CH_2
26	164.8/–	–	164.8/–	164.8/–	164.8/–	–
27	77.6/CH	–	51.3/CH_3	76.1/CH	76.0/CH_3	–
28	32.5/CH_2	–	–	32.1/CH_2	32.3/CH_2	–
29	23.4/CH_2	–	–	23.2/CH_2	23.2/CH_2	–
30	23.4/CH_2	–	–	23.2/CH_2	23.2/CH_2	–
31	32.5/CH_2	–	–	32.1/CH_2	32.5/CH_2	–

a = For numbering of zafirlukast and the impurities see Fig. 1.

respectively. One of the intermediates used in the synthesis of zafirlukast is Impurity 5.

4. Conclusions

In conclusion, we have isolated and characterized five process related impurities including two regioisomers present in zafirlukast bulk drug. Structural elucidation of these compounds is based on their LC–MS, IR and NMR spectral data followed by their independent synthesis. Thus, we have met the regulatory requirement of characterizing all those impurities whose levels are close to 0.10%. Furthermore, by providing the impurity standards, we have facilitated the development of a robust validated analytical method.

This study is an example to characterize our general strategy that lead to the rapid identification of impurities. At first we propose a structure for the low level unknown impurities based on LC–MS or GC–MS, supplemented by high resolution MS (HRMS) and MS/MS for the determination of the molecular weight and fragmentation patterns. On the basis of the on-line mass spectroscopic and UV spectral data, the HPLC retention time and knowledge of the process chemistry, one or more possible structure(s) may be assigned for the impurities. This is followed by the isolation of the impurities by using preparative HPLC chromatography to obtain enough material for further MS and NMR studies. The identified impurities are then synthesized to obtain sufficient quantities for final confirmation of their structures and for being used in toxicological studies and as impurity standards in further quantitative analytical tests.

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