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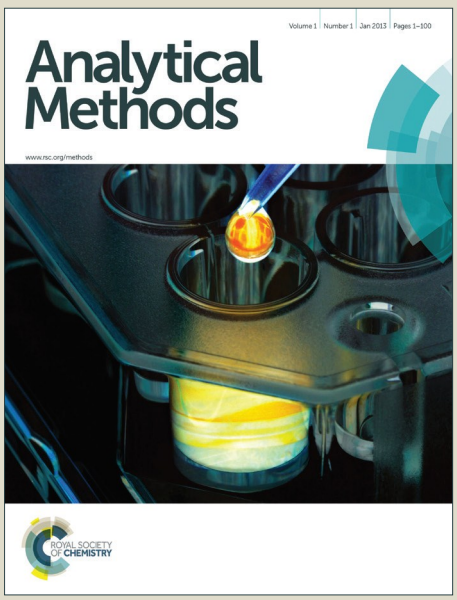

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Isolation and Identification of a potential unknown impurity in montelukast drug substance resulting from photolytic degradation

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Hemant Madhusudan Gandhi,*[a] Nageswara Rao Gollapalli,[b] Jaydeep Kumar D. Lilakar, Kirti Kumar Jain and Sandeep Mohanty[a]

During related substance analysis of montelukast bulk drug, a potential unknown impurity was detected in routine reverse phase impurity profiles by high performance liquid chromatography (HPLC). This impurity was identfied by LC-MS and characterized by (^{1}H NMR, ^{13}C NMR, gDQCOSY, gHSQC, LC/MS/MS, elemental analysis and FTIR) after isolation from montelukast drug substance exposed to sunlight. Based on sperctral data, the impurity was unambiguously named as (E)-2-(2-(3-(3-(2-(7-chloroquinolin-2-yl) vinyl)phenyl)-3-((2-methylenebutyl)thio) propyl)phenyl)propan-2-ol. To the best of our knowledge, this impurity has not been reported elsewhere. Structural elucidation of the impurity by spectral data is discussed in detail.

1. Introduction

Montelukast is a leukotriene receptor antagonist (LTRA)[1] used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. Montelukast is a CysLT1 antagonist;[2] it blocks the action of leukotriene D4 (and secondary ligands LTC4 and LTE4) on the cysteinyl leukotriene receptor CysLT1 in the lungs and bronchial tubes by binding to it. This reduces the bronchoconstriction otherwise caused by the leukotriene and results in less inflammation. Chemically montelukast sodium is (sodium (R,E)-2-(1-(((1-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-(2-(2-hydroxypropan-2-yl)phenyl)propyl)thio)methyl)cyclopropyl)acetate.[3] The empirical formula of montelukast is $C_{35}H_{35}ClNNaO_3S$ and molecular weight is 607.19. It belongs to quinoline series developed by Merck & Co. The molecular structure of montelukast is shown in Fig.1

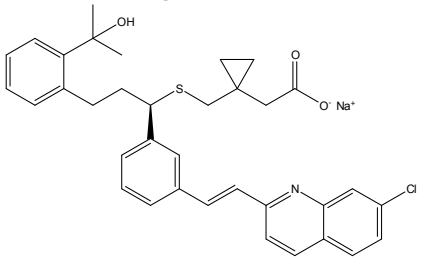

Fig. 1 Structure of Montelukast Sodium

During the related substance analysis of montelukast base, one non-polar impurity at RRT~1.53 was observed in the range of 0.10–0.30% level along with the main product peak in the HPLC analysis. As per International conference on Harmonization (ICH) guideline (ICH Q3A, R2) for a new drug substance having maximum daily dose ≤ 2 g/day, the reporting and identification thresholds for a unknown related compound (impurity) are 0.05% and 0.10%, respectively.[4] In order to meet the stringent regulatory requirements, a comprehensive study was undertaken to identify, synthesize and characterize potential unknown impurity of montelukast. The present manuscript deals with the identification, isolation and characterization of potential unknown impurity of montelukast by preparative HPLC followed by structure elucidation by LC/MS/MS, 1H, 13C and 2D NMR spectroscopy. This impurity was observed during the analysis of montelukast base [4] in process development[5] of montelukast sodium (Fig. 2).

Montelukast sodium is a light sensitive drug substance subjected to photolytic degradation. Chromatographic methods were reported using RP-HPLC[7] and UPLC methods for quantitative determination of montelukast and related substances. In some references Fluorescence[8] detector and mass spectrometry[11] has used for quantitation of montelukast in urine and plasma. Impurity profiling and metabolic study of montelukast was reported using mass spectrometric techniques.[12] Montelukast Sodium is a US pharmacopeia listed drug substance where six impurities are reported.[13] The impurity identified in present work is different from reported impurity.

[a]Dr Reddy's Laboratories Limited, CTO Unit-III, Plot No 116, I.D.A. Bollaram, Medak District, Telangana- 502325, India. E-Mail: hemantgandhi@drreddsys.com, Sandeepmohanty@drreddys.com
[b]Andhra University, Department of Inorganic & Analytical Chemistry, Visakhapatnam-530003, India. E-Mail: gollapallinr@yahoo.com.

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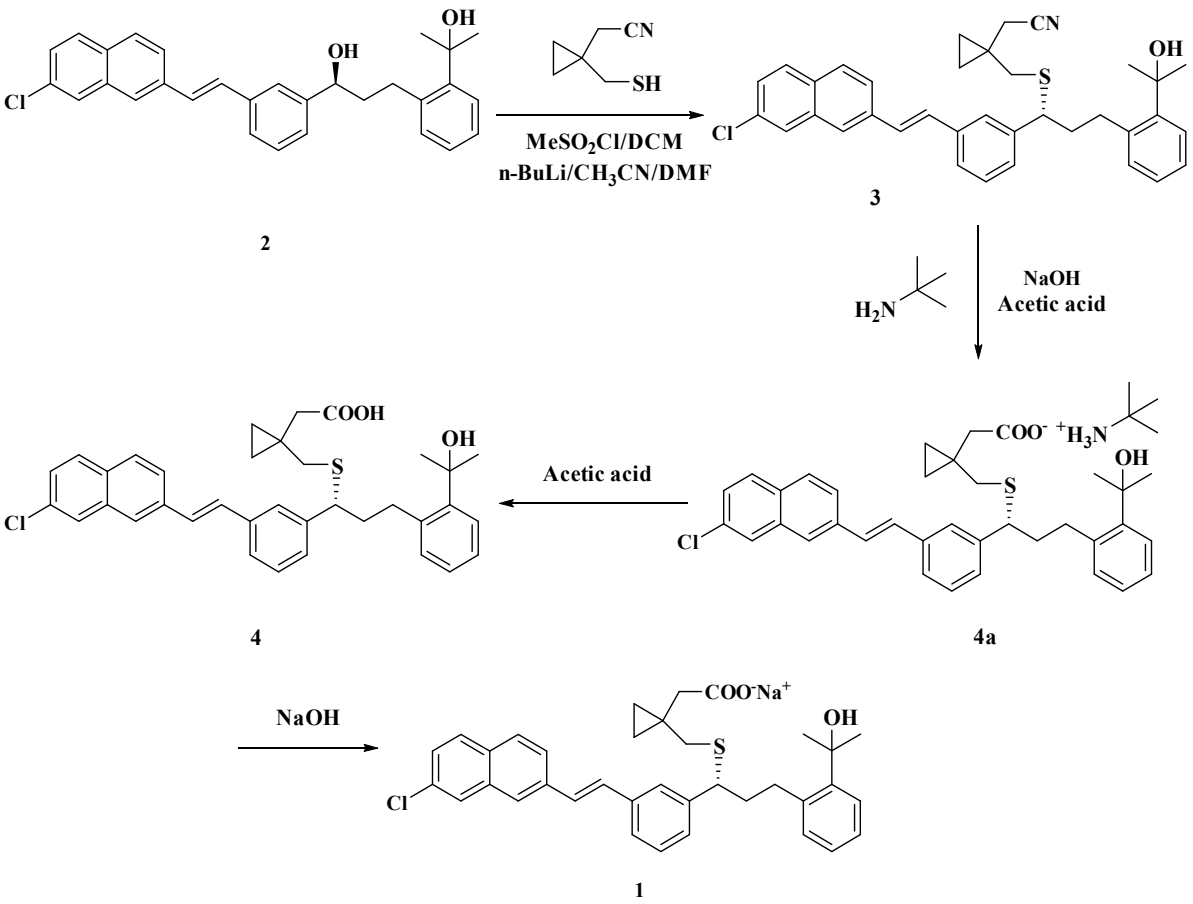

Fig. 2 Synthetic scheme for montelukast Sodium

2. Experimental

2.1. Materials and reagents

The investigated samples of montelukast and crude samples were in house synthesized [Dr. Reddy's Laboratories]. Analytical reagent acetonitrile and methanol were obtained from SD Fine Chemicals Limited, Mumbai, India. LC-MS grade acetonitrile and trifluroacetic acid used for LC-MS analysis was obtained from Biosolve BV, Valkenswaard, Netherland. Deuterated Chloroform-d and dimethylsulfoxide (DMSO-d6) were purchased from Aldrich Chemicals Co., USA. IR spectroscopy grade potassium bromide was procured from Merck (India) limited. Water used for the preparation of mobile phase was purified using Millipore Milli-Q plus (Milford, MA, USA).

2.2 High-performance liquid chromatography

A Waters Alliance 2690 separation module equipped with 2998-photodiode-array (PDA) detector and Empower pro data handling system [Waters Corporation, Miliford, MA, USA] was used. The analysis was carried out on a stainless steel column 150 mm long, 4.6 mm internal diameter filled with phenyl groups chemically bonded to porous silica partials of 3.5 µm diameter [Zorbax SB Phenyl column (make: Agilent technologies)] maintained at 30°C. Mobile phase A was aqueous solution of 0.015% trifluroacetic acid, prepared by dissolving 1.5 ml of trifluroacetic acid in 1000 ml water. Mobile phase B was 0.015% trifluroacetic acid in mixture of acetonitrile and water (95:5). Diluent was prepared by mixing

900 ml methanol with 100 ml water. The flow rate was kept as 1.5 ml min-1, injection volume was 20 µl, chromatographic data acquisition time was 35 min and UV detection was carried out at 230 nm. The pump was in gradient mode and time program was as follows: Time (min)/%B (v/v) 0.01/40, 3/40, 15/51, 20/60, 25/70, 30/70, 32/40, 35/40.

2.3 Preparative Liquid chromatography

Agilent 1200 series preparative liquid chromatograph equipped with G1315D PDA detector, Rheodyne 2260A series injector with 1.8 ml loop and G3146B Fraction collector [Agilent technologies, Santa Clara, CA, 95051 USA] was used. Phenomenex Luna C_{18} (2) 250 mm long, 21.2 mm i.d., Preparative HPLC column packed with 10 µm particle size was employed for isolation of impurity. Mobile phase consists of 0.01% trifluroacetic acid solution in mixture of acetonitrile and water (80:20). Flow rate was set as 15 ml min^{-1} and UV detection was carried out at 230 nm.

2.4 LC/MS/MS

LC/MS/MS analysis was carried out using AB SCIEX Triple TOF 4600 (Time of Flight) mass spectrometer (AB SCIEX, USA) coupled with Agilent 1290 series RRLC system. Analyst ® TF 1.6 software was used for data acquisition and data processing. Ion spray voltage for DuoSpray™ ion source in ESI mode was maintained at 4500V and temperature was set at 450°C. The auxiliary gas and curtain gas used was high purity nitrogen. Zero air was used as nebulizer gas. The resolution of AB Sciex 4600 Time of Flight mass spectrometer for ALILTLVS, a synthetic peptide [m/z 829.5398] was more than 25000. The High resolution mass spectra [HRMS] were acquired from m/z 50-1000 in accumulation time of 1000 ms. All chromatographic conditions used for HRMS analysis were same as mentioned under High-performance liquid chromatography.

2.5. NMR spectroscopy

^{1}H NMR, ^{13}C NMR and 2D NMR experiments were performed on Varian Mercury plus 400 MHz FT-NMR spectrometer [Agilent technologies, Palo Alto, California, USA] using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal standard.

2.6. FT-IR spectroscopy

The FT- IR spectra were recorded as KBr pellet on a Perkin-Elmer instrument model-spectrum one.

3. Results and Discussion

3.1. Detection and identification of impurity

Montelukast sample was accurately weighed and diluted to the required concentration (0.5 mg ml^{-1}) and injected into HPLC using chromatographic conditions mentioned above. One unknown peak was identified in the chromatogram at a relative retention time of about 1.53 with respect to the montelukast peak along with pharmacopoeial impurities. The same sample was subjected to LC-MS analysis to identify mass of the impurity. A typical representative HPLC chromatogram of montelukast is shown in Fig. 3. Unknown impurity at RRT-1.53 elutes at about 22.817.

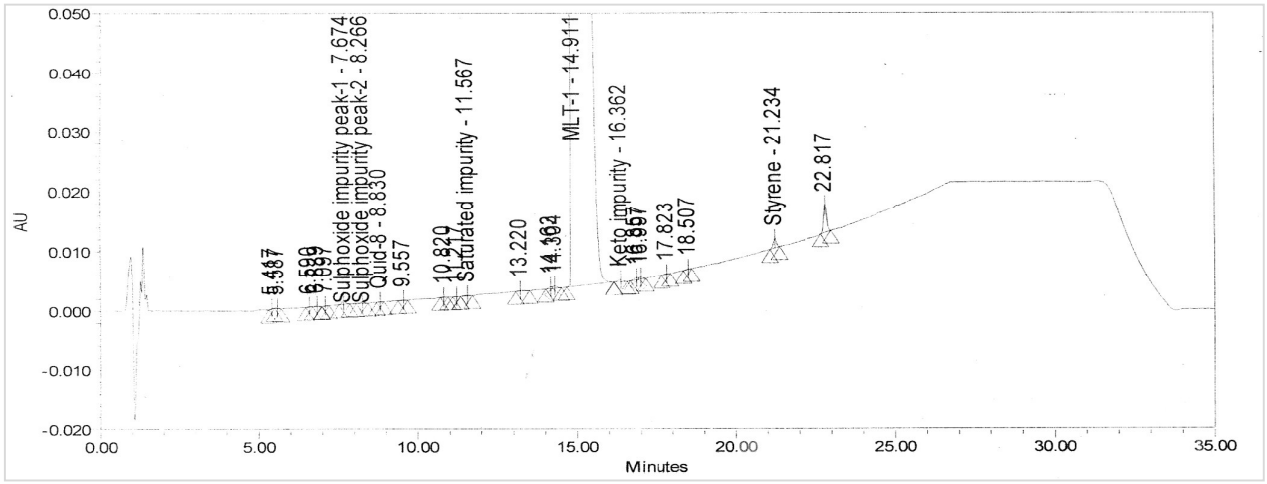

Fig. 3 HPLC chromatogram of montelukast Laboratory Batch

3.2. Synthesis and isolation of impurity by preparative HPLC

Unknown impurity in montelukast base was enriched by exposing about 1.0 g of montelukast base to sun light for about 6 h at ambient temperature in dry conditions. The content of unknown impurity in photolytic degraded sample of montelukast base was found 6.0% when analysed as per related substance chromatographic conditions.

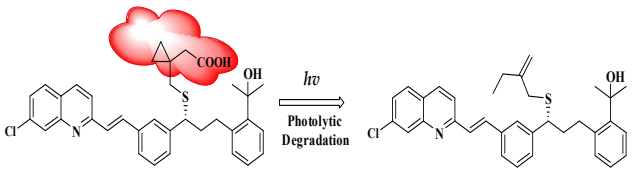

Fig. 4 Synthetic scheme for unknown impurity

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About 1.0 g of photolytic degraded sample of montelukast base was taken in 50 ml volumetric flask and diluted up to the mark with diluent. This solution was loaded into the preparative column using the conditions mentioned in the preparative liquid chromatography section. Fraction of impurity ≥95% were pooled together and concentrated on a rotavapour to remove organic solvent. The aqueous solution was lyophilized using freeze dryer (Virtis Advantage 2XL). The impurity obtained was an yellow powder in description and chromatographic purity was 90.11% determined by the HPLC method.

3.3 Structure elucidation of Montelukast

ESI mass spectrum of the montelukast in positive ion mode exhibited molecule ion peak at m/z 586.2179 [$(MH)^+$] indicating the mass of this compound to be 585.2. The high resolution mass spectrometry for m/z 586.2179 proposed elemental composition $C_{35}H_{36}ClNO_3S$ complies with the structure of montelukast. LC/MS/MS spectrum for mass m/z 586.2179 displayed daughter ion peaks at m/z 568.2, 524.2, 440.2, 422.1, 292.1 and 278.1. Probable fragmentation pattern is displayed in Fig. 5.

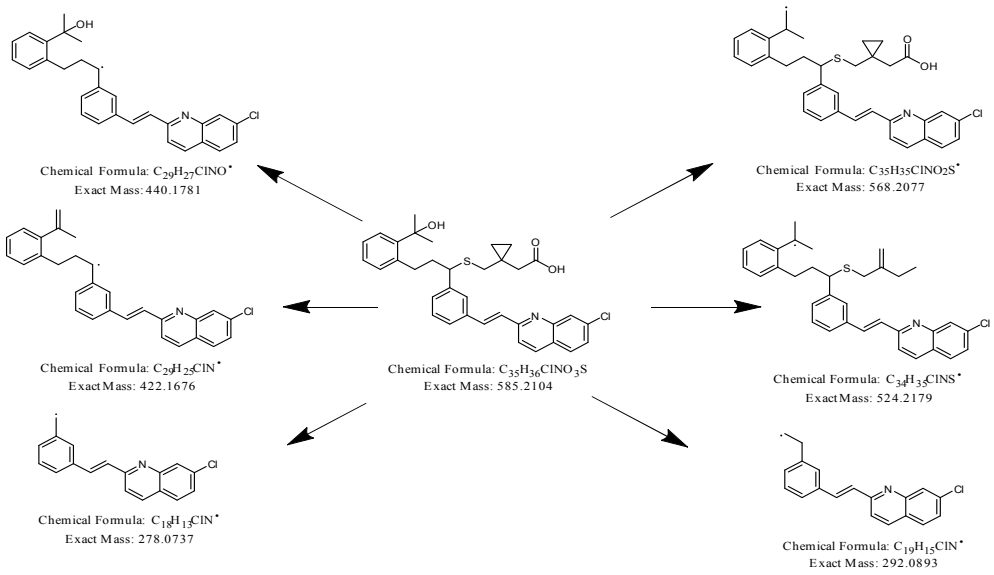

Fig. 5: Probable fragmentation pattern of Montelukast

In ^{1}H NMR spectrum, multiplet signal (δ 0.39) corresponding to cyclopropyl ring and signal at (δ 11.2) corresponding to acid OH were observed. In FT-IR spectrum, a broad band at 3428.98 cm^{-1} corresponding to acid OH, a band at 1633 cm^{-1} corresponding to acid C=O bond and a band at 1131.04 cm^{-1} corresponding to aromatic C–Cl were observed. Based on the above spectral data structure of montelukast was confirmed as (R,E)-2-(1-((1-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-(2-(2-hydroxypropan-2-yl)phenyl)propylthio)methyl)cyclopropyl) acetic acid (Fig. 6). The spectral data of montelukast are given in Table 1.

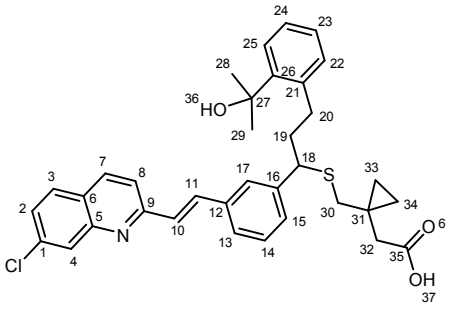

Fig. 6: Structure of Montelukast

3.4 Structure elucidation of Impurity

ESI mass spectrum of the impurity in positive ion mode exhibited molecule ion peak at m/z 542.2280 [$(MH)^+$], which is 44 amu less than that of the montelukast protonated molecular ion. The probable elemental composition proposed by high resolution mass spectrometry for mass m/z 542.2280 is $C_{34}H_{36}ClNOS$. LC/MS/MS spectrum for mass m/z 542.22280 displayed daughter ions peaks at m/z 524.2, 440.2, 422.1, 292.1 and 278.1 which are same as that of montelukast. Probable fragmentation pattern displayed in Fig. 7.

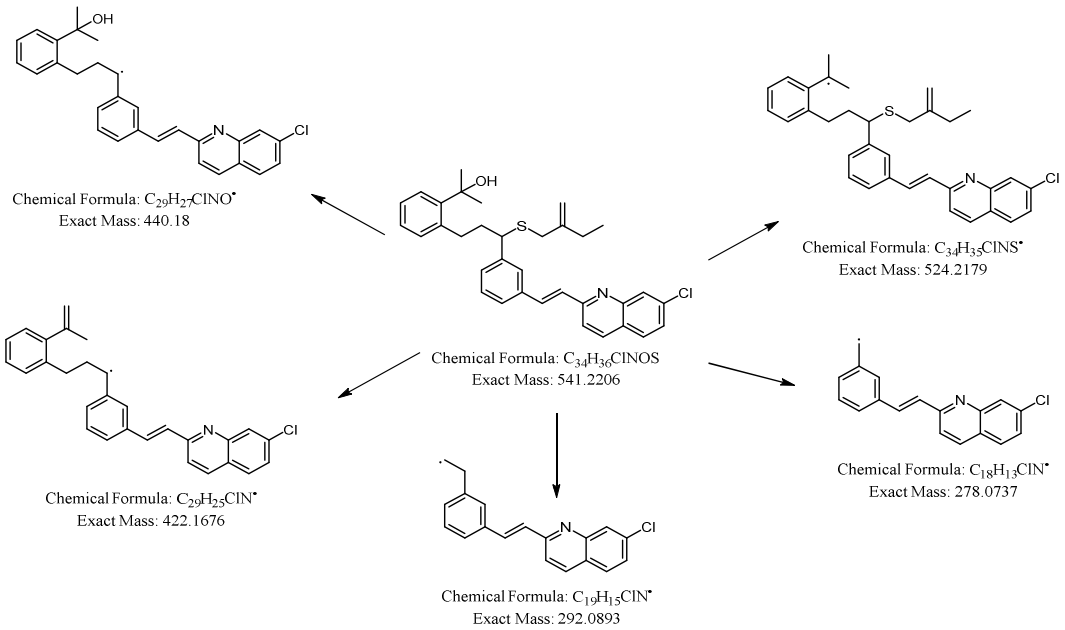

Fig. 7: Probable fragmentation pattern of the impurity

In ^{1}H NMR and ^{13}C NMR spectra of the impurity, chemical shift (δ) values were similar to montelukast except at cyclopropylacetic acid moiety. Moreover, when close inspection of structure of montelukast and impurity (Figs. 6 and 8) with ^{1}H NMR and ^{13}C NMR data (see Table 1) multiplet signal at (δ 0.39) in ^{1}H NMR corresponds to cyclopropyl and signal at (δ 11.2) corresponding to acidic OH of montelukast disappeared in ^{1}H NMR spectrum of the impurity. This observation suggested the cleavage of cyclopropane ring and loss of terminal carboxylic acid. Triplet at (δ 0.93) and quartet at (δ 2.07) in ^{1}H NMR spectrum of impurity indicate cyclopropyl ring opening. Singlet signal at (δ 4.82) in ^{1}H NMR spectrum of impurity corresponds to alkenes further supported by the presence of additional signals at δ 111.561 and δ 146.3 in ^{13}C NMR spectrum. In FT-IR spectrum C–O band at 1129.40 cm^{-1} was absent and a strong alkenes broader band was observed at 1691.52 cm^{-1}. Based on the above spectral data the molecular formula of impurity is confirmed as $C_{35}H_{34}ClNO_2S$ and its chemical name is proposed as (E)-2-(2-(3-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-((2-methylenebutyl)thio)propyl)phenyl)propan-2-ol (Fig. 8).

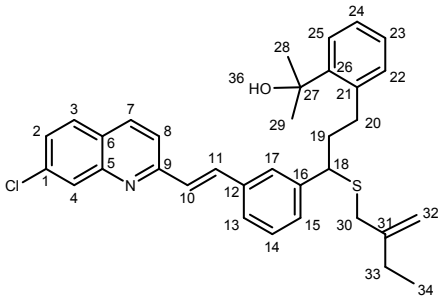

Fig. 8 Structure of the unknown impurity

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Table 1 Comparative 1H, 13C NMR assignments for montelukast and Impurity and gDQCOSY, gHSQC data interpretation

Position	Montelukast		Impurity			
	1H δ (ppm), multiplicity, J_{1-2}	13C δ (ppm)	1H δ (ppm), multiplicity, J_{1-2}	gDQCOSY	13C δ (ppm)	gHSQC
1	----	125.6	----	----	125.8	
2	7.59 (dd, 1H, 8.4 & 2.0)	126.6	7.59 (dd, 1H, 8.4 & 2.0)	3H, 8.02	126.5	2H, 7.40
3	7.98 (d, 1H, 8.4)	129.7	8.02(d, 1H, 8.4)	2H, 7.59	130.1	3H, 8.02
4	8.04(d, 1H, 2.0)	127.2	8.05 (d, 1H, 2.0)	----	127.3	4H, 8.05
5	----	148.0	----	----	146.9	----
6	----	134.3	----	----	135.3	----
7	8.38 (d, 1H, 8.40)	136.5	8.49(d, 1H, 8.40)	8H, 8.0.	138.2	7H, 8.49
8	7.93(d, 1H, 8.40)	120.3	8.01(d, 1H, 8.40)	7H, 8.49	120.4	8H, 8.01
9	----	156.8	----	----	156.4	----
10	7.89 (d, 1H, 18.0)	135.0	7.94(d, 1H, 16.4)	10H, 7.51	136.0	10H, 7.94
11	7.50 (d, 1H, 16.4)	128.4	7.51(d, 1H 16.4)	11H, 7.94	129.1	11H, 7.51
12	----	136.1	----	----	137.0	----
13	7.64 (d, 1H, 7.20)	125.9	7.62(d, 1H, 7.20)	14H, 7.43	127.0	13H, 7.62
14	7.44 (m, 1H)	125.3	7.43 (m, 1H)	13H, 7.61 15H,	125.5	14H, 7.43
15	7.40 (m, 1H)	128.4	7.34 (m, 1H)	14H, 7.43	127.4	15H, 7.34
16	----	143.7	----	----	143.6	----
17	7.76 (s, 1H)	126.8	7.71 (s, 1H)	----	126.9	17H, 7.71
18	4.06 (t, 1H, 6.8)	49.4	3.85(t, 1H 6.8)	19H, 2.20	48.8	18H, 3.85
19	2.20 (m, 2H)	38.5	2.20 (m, 2H)	18H, 3.84, 20,2H	29.2	19H, 2.20
20	3.09 & 2.81 (m, Ha & Hb)	31.9	3.05, 2.74 (m, Ha/Hb)	19H, 2.20	32.1	20H,
21	----	139.8	----	----	139.9	----
22	7.15 (m, 1H)	131.0	7.10 (m, 1H)	23H, 7.10	131.2	22H, 7.10
23	7.14 (m, 1H)	126.4	7.10 (m, 1H)	24H, 7.09, 22H,	126.3	23H, 7.10
24	7.09 (m, 1H)	125.2	7.04 (m, 1H)	25H, 7.35, 23H,	125.4	24H, 7.04
25	7.42 (m, 1H)	128.9	7.35 (m, 1H)	24H, 7.09	129.2	25H, 7.35
26	----	146.7	----	----	146.7	----
27	----	71.6	----	----	71.8	----
28	1.47 (s, 3H)	31.7	1.42 (s, 3H)	----	31.7	28H, 1.42
29	1.47 (s, 3H)	31.8	1.42 (s, 3H)	----	31.8	29H, 1.42
30	2.36 (s, 2H)	40.1	2.04 (s, 2H)	----	40.4	30H,2.04
31	----	16.7	----	----	146.5	----
32	2.57 & 2.53 (d, Ha & Hb, 12.8)	38.9	4.82 (s, 2H)	----	111.5	32H, 4.82
33	0.37 (m, 2H)	11.9	2.07 (q, 2H, 6.8)	34H, 0.93	28.6	33H, 2.07
34	0.45 (m, 2H)	12.1	0.93 (t, 3H, 7.2)	33H, 2.07	12.1	34H,0.93
35	----	173.1	----	----	----	----
36	5.24 (s, OH)	----	5.70 (s, OH)	----	----	----
37	12.09 (s, OH)	----	----	----	----	----

s, singlet; d, doublet; t, triplet, q, quartet; m, multiplet; dd, doublet of doublet; J, coupling constant, Refer the structural formulae given in Figs. 6 and 8 for numbering

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Table 2 FT-IR spectral data for montelukast and Impurity

IR (KBr) absorption bands (Vmax/cm-1)			
Montelukast		Impurity	
3428(b)	OH Stretching	3431(b)	OH Stretching
2971(s)	aromatic C-H	2969(s)	aromatic C-H
2926(s)	aliphatic C-H	2928(s)	aliphatic C-H
1634(s)	C=O stretching		
1610(s)	C=C stretching	1691(b)	C=C stretching
1596(b)	C=N stretching	1594(s)	C=N stretching
1497(s)	aliphatic C-H bending	1497(s)	aliphatic C-H bending
1132(s)	C-O stretching	1129(s)	C-O stretching
1053(s)	C-Cl stretching	1053(s)	C-Cl stretching
837(s)	aromatic C-H bending	824(s)	aromatic C-H bending
697(s)	C-S stretching	719(s)	C-S stretching

4. Conclusion

A new unknown impurity observed in montelukast drug substance resulting from photolytic degradation of montelukast base was identified by HPLC and LC-MS. The impurity was isolated, characterized by various spectroscopic techniques like NMR (^{1}H, ^{13}C, gDQCOSY and gHSQC), LC/MS/MS and FT-IR and the probable structure of impurity is proposed.

5. Acknowledgment

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Intellectual Property Management department (IPM) has given this manuscript the internal publication number IPDO IPM-00479

6. References

1 M. Saravanan, K. Siva kumari, P. Pratap Reddy, M.N. Naidu, J. Moses Babu, Alok Kumar Srivastava, T. Lakshmi Kumar, B.V.V.N. Chandra Sekhar, Bollikonda Satyanarayana , J. Pharm. Biomed. Anal., 4 November 2008Volume 48, Issue 3, Pages 708–715.

2 M. Belley, S. Leger, P. Roy, Y.B. Xiang, M. Labelle, D. Guay, D. European Patent 0480717B1, April 15 1998.

3 M. Labelle, M. Belley, Y. Gareau, J.Y. Gauthier, D. Guay, R. Gordon, S.G. Grossman, T.R. Jones, Y. Leblanc, M. McAuliffe, C. McFarlane, P. Masson, K.M. Metters, N. Ouimet, D.H. Patrick, H. Piechuta, C. Rochette, N. Sawyer, Y.B. Xiang, C.B. Pickett, A.W. Ford-Hutchinson, R.J. Zamboni, R.N. Young, Bioorg. Med. Chem. Lett.,1995, 5, 283–288.

4 International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline. Impurities in New Drug Substances Q3A(R2), step 4 2006.

5 Chandra Sekhar B.V.V.N, Ramesh Kumar N, Kalyan chakravarthy A & Mukkanti K, International Journal of Pharma and Bio Sciences,Jul-Sept 2011,Volume 2, Issue 3, Pages 417–425.

6 P. Pratap Reddy, B. Satyanarayana, S. Alok Kumar, K. Ravi Kumar, J. Rajender Reddy, M.N. Naidu, *US Pat.,* WO2007/012075A2, January 25, 2007.

7 N. Rashmitha, T. Joseph Surender Raj, C.H. Srinivas, N. Srinivas, U.K. Ray, H.K. Sharma and K. Mukkanti, *E-J. Chem*., 2010, 7 (2) 555-563.

8 Hisao O, Naotaka U, Terukazu T, Ken-ichi H, Toshio K., *J Chromatog B*. 1998; 713,409–14.

9 Shajan A, Narayanan N, *Asian J. pharm. Res.,* 2013, 3 (2), 56-59

10 C.B. Reddy, B.Z. Awen, C. Babu Rao, K. Mukkanti, K.C. Bannoth. *Sci Pharm*. 2010, 78, 411-422

11 Aboulkhair M, Nofal A, AL-Mardini M.A, *Int. J. Pharm. Sci. Rev. Res*., Sep-Oct 2013, 22(1),48-50.

12 Essam Ezzeldin, Nisreen F Abo-Talib, Marwa H Tammam and Abdelaaty A Shahat, Chem. Central J. 2014, 8:17.

13 UPS36-NF27 page 3471, pharmacopeia Forum: Volume No. 33(4) page 673

14 G. Srihari, K. Nagaraja Setty, N. Rami Reddy and I.E. Chakravarth. J. Chem. Pharm. Res.,2011, 3(6), 23-27.

15 R.C. Vibhuti, J. Patel. Int. J. Chem. Tech. Res. Oct-Dec 2012, 4 (4), 1402-1407.

16 Yao Huang, Li Ding, Yuan-Yuan Liu, He-Ying Liu, Ai-Dong Wen, Lin Yang., J. Chinese Pharm. Sci., 2009, 18, 261–266.

17 G. Nirupa, A. Siva Kumar, and U.M. Tripathi, J. Chem., 2013, DOI: 10.1155/2013/402723.

18 S. K. Balani, X. Xu, V. Pratha, M. A. Koss, R. D. Amin, C. Dufresne, R. R. Miller, B. H. Arison, G. A. Doss, M. Chiba, A. Freeman, S. D. Holland, J. I. Schwartz, K. C. Lasseter, B. J. Gertz, J. I. Isenberg, J. D. Rogers,J. H. Lin, And T. A. Baillie, *The American Society for Pharmacology and Experimental Therapeutics*, 1997, 25(11), 1282-1289.

19 Vijaya Lakshmi Maddala1, Kishore Kumar Kakumani, Kameswara Rao Chimalakonda1, Srinivasulu Polisetty1, P. C. Ray., *American Journal of Analytical Chemistry, 2013, 4, 56-61*

20 Smita P, Pore VY, kuchekar SB, Aruna MN. Determination of montelukast sodium and bombuterol hydrochloride in tablets using RP HPLC. *Indian J Pharm Sci*. 2009, 71(1),58-61.

21 Radhakrishna TA, Narasaraju M, Ramakrishna, Satyanarayana A. Simultaneous determination of montelukast and loratadine by HPLC and derivative

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ARTICLE

spectrophotometric methods. *J Pharm Biomed Anal.* 2003, 31(2), 359-68.

22 Lida L, Haiyung C, Jamie JZ, Douglas RJ., *J Pharm Biomed Anal*. 1997, 15, 631-638.

23 B. Hanimi Reddy, M. Ravi Kumar, L.K. Garg, D. Venugopal and A. M. Reddy *Int. J. Pharma and Bio Sci.,* Jan-Mar 2012, 3(1), 345-355.

24 Rupali L. Choudekar, Moreshwar P. Mahajan, Sanjay D. Sawant, *Int J Pharm Pharm Sci*, 2012, 4(3), 737-740

25 Sailaja B and Venkateswarlu G., Acta Biomedica Scientia. 2014,1(1),1-5.