Asian Journal of Chemistry; Vol. 23, No. 7 (2011), 3252-3256

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Structure Elucidation of Two Unknown Oxydic Degradation Impurities of Rifaximin

CHAO LIU[1,2], CHANG-QIN HU[2] and SHAO-HONG JIN[2,*]

[1]China Pharmaceutical University, Nanjing 210009, Jiangsu Province, P.R. China
[2]National Institute for the Control of Pharmaceutical and Biological Products, Beijing 100050, P.R. China

*Corresponding author: Tel: +86 10 67095258; E-mail: jinshh@nicpbp.org.cn

(*Received*: 20 December 2010; *Accepted*: 24 March 2011) AJC-9774

Rifaximin is a semi-synthetic rifamycin derivate. Two unknown oxydic degradation impurities (impurity 1 and impurity 2) were prepared from rifaximin based on the degradation mechanism by using manganese dioxide as the oxidant. The structure of the impurities was elucidated by using MS and other modern spectroscopic (NMR and FTIR) techniques and as (2S,20S,21S,22R,23R,24R,25S,26R,27S)-6,21,23-trihydroxy-27-methoxy-2,4,16,20,22,24,26-heptamethyl-11-methylene-1,5,15-trioxo-1,2,5,11-tetrahydro-2,7-(epoxypentadeca[1,11,13]trienoimino)furo[2'',3'':7',8']naphtho[1',2':4,5]imidazo[1,2-a]pyridin-25-yl-acetate and (2S,20S,21S,22R,23R,24R,25S,26R,27S)-5,21,23-trihydroxy-27-methoxy-2,4,16,20,22,24,26-heptamethyl-11-methylene-1,6,15-trioxo-1,2,6,11-tetrahydro-2,7-(epoxypentadeca[1,11,13]trienoimino)furo[2'',3'':7',8']naphtho[1',2':4,5]imidazo[1,2-a]pyridin-25-yl-acetate by IUPAC.

Key Words: Rifaximin structure, Impurity, Antibiotic.

INTRODUCTION

Rifaximin is a semi-synthetic rifamycin derivate and it has been clinically used for the treatments of enterocolitis, travel diarrhea and hepatic coma[1-4]. High performance liquid chromatographic (HPLC) methods have been reported in the literature for the determination of rifaximin in pharmacokinetic research[5-7] and for quality control[8-10]. Eight impurities of rifaximin (impurity A to impurity H) from rifaximin industrial process have been listed in European/British pharmacopoeia[11,12] and the erroneous structure of impurity H in European Pharmacopoeia 6.5 (2009) was corrected which was reported in the cited literature[13].

The HPLC analysis of rifaximin bulk drug has been performed as per the method described in HPLC of the materials and procedures. During this analysis of different batches of rifaximin, two unknown impurities have been detected whose area percentage ranged up to 0.1 % sometimes and have a close relation with an oxydic degradation pathway. Organic impurities can arise during the manufacturing process and storage of the drug substance and criteria for their acceptance upto certain limits are based on pharmaceutical studies, clinical trials or known safety data. As per regulatory guideline, the pharmaceutical studies using the sample of the isolated impurity can be considered for safety assessment[14]. Therefore, it is essential to isolate and characterize the unidentified impurities present in active pharmaceutical ingredients (APIs). This paper aims for the preparation and structure elucidation of the two potential impurities.

EXPERIMENTAL

All reagents and spectroscopic solvents pure grade employed for high performance liquid chromatography and LC/MS were of HPLC grade. The samples of rifaximin were obtained from NICPBP (National Institute for the Control of Pharmaceutical and Biological Products) in China.

Preparation of impurity 1 and impurity 2: Fist, in a 250 mL four-necked flask, 12 g rifaximin was stirred and dissolved by 72 mL chloroform. Then 10 g manganese dioxide was added. Subsequently, it was stirred until no spot of the rifaximin was found in the thin-layer chromatography plate with the mobile phase of dichloromethane:ethylbutyl acetate: methanol:ammonia water (4:2:1:2 drops). And in the reaction process, 2.5 g manganese dioxide was added. After 18 h, the reaction was stopped. After filtration, the residue was immersed and washed by chloroform twice. Then, the 10 g impurity 2 was obtained after evaporation. And the impurity 2 was looked as red powder. The 65 mL ethanol was added into the 15 g impurity 2 in the procession of the circumfluence reaction for 8.5 h. Then it was placed at the room temperature and over night. Then the reaction liquid was frozen for 1.5 h. A spot of ethyl acetate was added into the obtained solid. Then it was filtered and 5.6 g impurity 1 was obtained. The impurity 1 was looked as yellow powder.

HPLC: The analytical HPLC system is Dionex P680 of Dionex USA LIMITED including quaternary pump, ASI-100 auto injector, TCC-100 column container and PDA-100 detector. In order to analyze the two impurities, a CAPCELL PAK C_8 DDS5, 250 mm × 4.6 mm i.d. (5 μm particle size) was employed and methanol-acetonitrile-buffer (0.075 mol/L monopotassium phosphate-0.5 mol/L citric acid (55:10, v/v) (500:90:325,v/v/v) was used at a flow rate of 1 mL/min at 35 ºC. Detector wavelength was set at 254 nm and injection volume is 20 μL. The test solutions of the rifaximin, impurity 1 and impurity 2, were prepared as 40 μg/mL, which were dissolved by the mobile phase.

Mass spectroscopy: LC/DAD/MS analyses were performed by AB-3200 Q trap of applied biosystems USA LIMITED. Methanol was used as the mobile phase. Methanol-dissolved samples were prepared at a concentration of 10 ppm and then these samples were directly injected.

Fourier transform infrared photoacoustic spectroscopy: Fourier transform infrared photoacoustic spectra were collected by Bruker Equinox 55 of BRUKER OPTICS in Germany in the 4000 and 400 cm^{-1} region. Samples, mixed with spectroscopy grade potassium bromide, were pressed and turned into the transparent tablets. These tablets were analyzed by transmittance technique with 26 scansions and 5 cm^{-1} resolution.

Nuclear magnetic resonance spectroscopy: The ^{1}H and ^{13}C NMR and two-dimensional analyses, HMQC and HMBC, were collected by a Bruker Avance 500 of Bruker Equinox 55 of BRUKER OPTICS in Germany at 500 MHz in Institute of Medical Material, Chinese Academy of Medical Sciences & Peking Union Medical College. Chemical shifts were expressed as ppm (δ) from tetramethylsilane. Samples were dissolved in $CDCl_3$.

RESULTS AND DISCUSSION

Detection of impurity by HPLC: Fig. 1 shows that in the HPLC chromatogram of the rifaximin the chromatographic behaviour of the two impurities were found as the same as the preparative impurity 1 and the impurity 2. The normalization content of the impurity 1 or impurity 2 was more than 0.1 %.

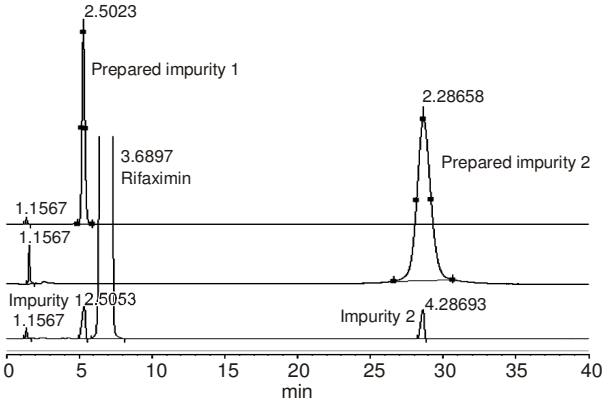

Fig. 1. Chromatographic behaviour of the rifaximin raw material compared with those of prepared impurity 1 and impurity 2 in HPLC

Structural elucidation by mass spectrometry: According to the results of the HPLC, these two samples were directly analyzed by MS in order to confirm the molecular weights (Table-1). From the mass spectra, both the molecular ion peaks of the two unknown impurities were 784. The molecular weights of the two unknown impurities were 783. According to obtain detailed fragments, the 784 as the molecular ion peaks of the impurity 1 and impurity 2 was selected for the MS2. Fig. 2 shows that the possible structure of impurity 1 and impurity 2 which were the derivants from rifaximin without two hydrogen atoms and the two impurities were isomers.

Structural confirmation by IR and NMR: The infrared spectrum of the impurity 1 and impurity 2 showed that the main absorption bands were at 3,440, 1,728, 1,650 cm^{-1}, suggesting a strong structural relation with rifaximin. Fig. 3 shows that the bands at 3,083, 2,972 cm^{-1} of the infrared spectrum of impurity 1 and the bands at 3,056, 2,962 cm^{-1} of the infrared spectrum of impurity 2 were due to the 8'CH_2 as the terminal methylene group's stretching vibration, respectively and 885 cm^{-1} of the infrared spectrum of impurity 1 and 883 cm^{-1} of the infrared spectrum of impurity 2 were the 8'CH_2 bending

TABLE-1
MULTI-LEVEL MS DATA OBTAINED FROM LCMS

Scan type	Impurity 1, fragment ion peak (m/z) and ion style	Impurity 2, fragment ion peak (m/z) and ion style
MS1	751 $C_{42}H_{45}N_3O_{10}[C_{43}H_{49}N_3O_{11}–CH_3OH]^+$ 346 $C_{20}H_{14}N_2O_4[[C_{43}H_{49}N_3O_{11}–C_{23}H_{35}NO_7]^+$ 580 $C_{34}H_{32}N_2O_7[C_{43}H_{49}N_3O_{11}–C_9H_{17}NO_4]^+$ 299 $C_{20}H_{27}O_2[C_{43}H_{49}N_3O_{11}–C_{23}H_{22}N_3O_7]^+$	784 $C_{43}H_{50}N_3O_{11}[C_{43}H_{49}N_3O_{11}+H]^+$ 806 $C_{43}H_{49}N_3O_{11}Na[C_{43}H_{49}N_3O_{11}+Na]^+$ 751 $C_{42}H_{45}N_3O_{10}[C_{43}H_{49}N_3O_{11}–CH_3OH]^+$ 420 $C_{24}H_{36}O_6[C_{43}H_{49}N_3O_{11}–C_{19}H_{13}N_3O_5]^+$ 395$C_{22}H_{34}O_6[C_{43}H_{49}N_3O_{11}–C_{19}H_{13}N_3O–C_2H_2]^+$ 329$C_{21}H_{29}O_3[C_{43}H_{49}N_3O_{11}–C_{19}H_{13}N_3O–C_2H_2–2H_2O–CH_3OH]^+$ 302 $C_{19}H_{26}O_3[C_{43}H_{49}N_3O_{11}–C_{19}H_{13}N_3O–C_2H_2–2H_2O–CH_3OH–C_2H_4]^+$
MS2	751 $C_{42}H_{45}N_3O_{10}[C_{43}H_{49}N_3O_{11}–CH_3OH]^+$ 766 $C_{43}H_{47}N_3O_{10}$ $[C_{43}H_{49}N_3O_{11}–H_2O]^+$ 722 $C_{42}H_{43}N_3O_9[C_{43}H_{49}N_3O_{11}–H_2O–CH_3CHO]^+$ 690 $C_{41}H_{40}N_3O_8[C_{43}H_{49}N_3O_{11}–H_2O–CH_3CHO–CH_3OH]^+$ 540 $C_{34}H_{39}N_2O_6[C_{43}H_{49}N_3O_{11}–C_9H_{10}NO_5]^+$ 512$C_{32}H_{35}N_2O_6[C_{43}H_{49}N_3O_{11}–C_9H_{10}NO_5–C_2H_4]^+$ 484 $C_{30}H_{31}N_2O_6[C_{43}H_{49}N_3O_{11}–C_9H_{10}NO_5–C_2H_4–C_2H_4]^+$ 345 $C_{21}H_{31}NO_3[C_{43}H_{49}N_3O_{11}–C_{22}H_{18}N_2O_8]^+$ 346 $C_{21}H_{32}NO_3[C_{43}H_{49}N_3O_{11}–C_{22}H_{17}N_2O_8]^+$	751 $C_{42}H_{45}N_3O_{10}[C_{43}H_{49}N_3O_{11}–CH_3OH]^+$ 736 $C_{42}H_{43}N_3O_9[C_{43}H_{49}N_3O_{11}–CH_3OH–H_2O]^+$ 766 $C_{43}H_{47}N_3O_{10}[C_{43}H_{49}N_3O_{11}–H_2O]^+$ 724 $C_{41}H_{43}N_3O_9[C_{43}H_{49}N_3O_{11}–H_2O–C_2H_4O]^+$ 706 $C_{41}H_{41}N_3O_8[C_{43}H_{49}N_3O_{11}–H_2O–C_2H_4O–H_2O]^+$ 688 $C_{41}H_{39}N_3O_7[C_{43}H_{49}N_3O_{11}–H_2O–C_2H_4O–H_2O–H_2O]^+$ 540 $C_{36}H_{33}N_2O_3[C_{43}H_{49}N_3O_{11}–C_7H_{16}NO_8]^+$ 510 $C_{34}H_{27}N_2O_3[C_{43}H_{49}N_3O_{11}–C_9H_{22}NO_8]^+$ 484 $C_{32}H_{25}N_2O_3[C_{43}H_{49}N_3O_{11}–C_9H_{22}NO_8–C_2H_2]^+$ 456 $C_{30}H_{21}N_2O_3[C_{43}H_{49}N_3O_{11}–C_9H_{22}NO_8–C_2H_2–C_2H_4]^+$ 345 $C_{21}H_{31}NO_3[C_{43}H_{49}N_3O_{11}–C_{22}H_{18}N_2O_8]^+$ 346 $C_{21}H_{32}NO_3[C_{43}H_{49}N_3O_{11}–C_{22}H_{17}N_2O_8]^+$

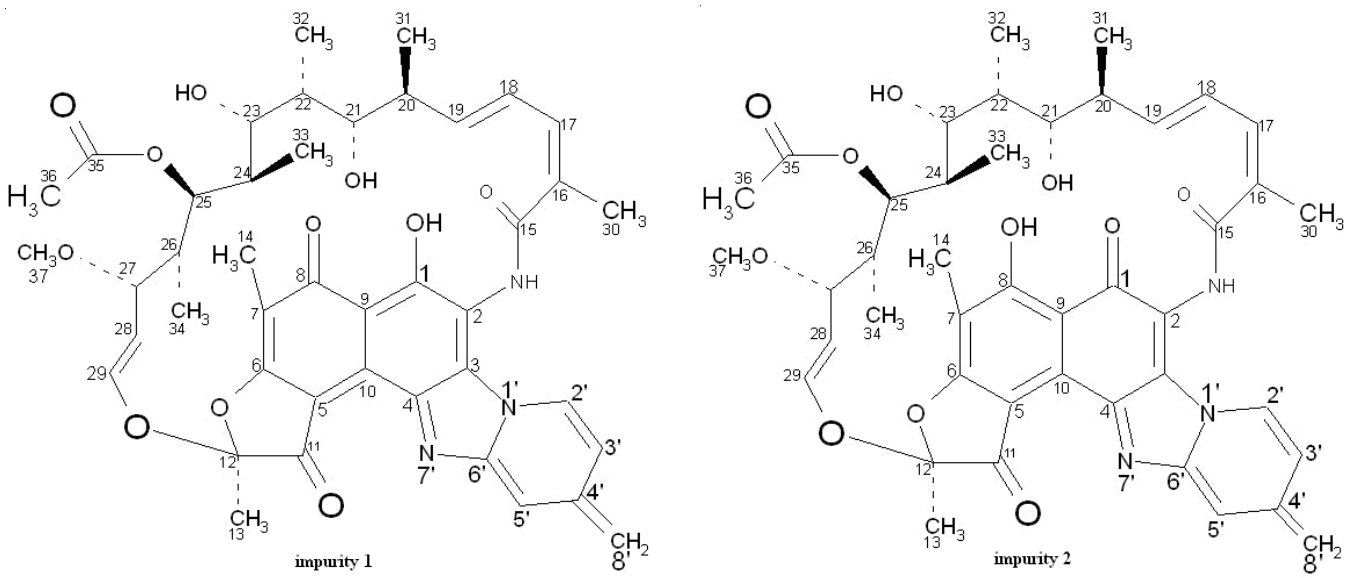

Fig. 2. Structures of the impurity 1 and impurity 2

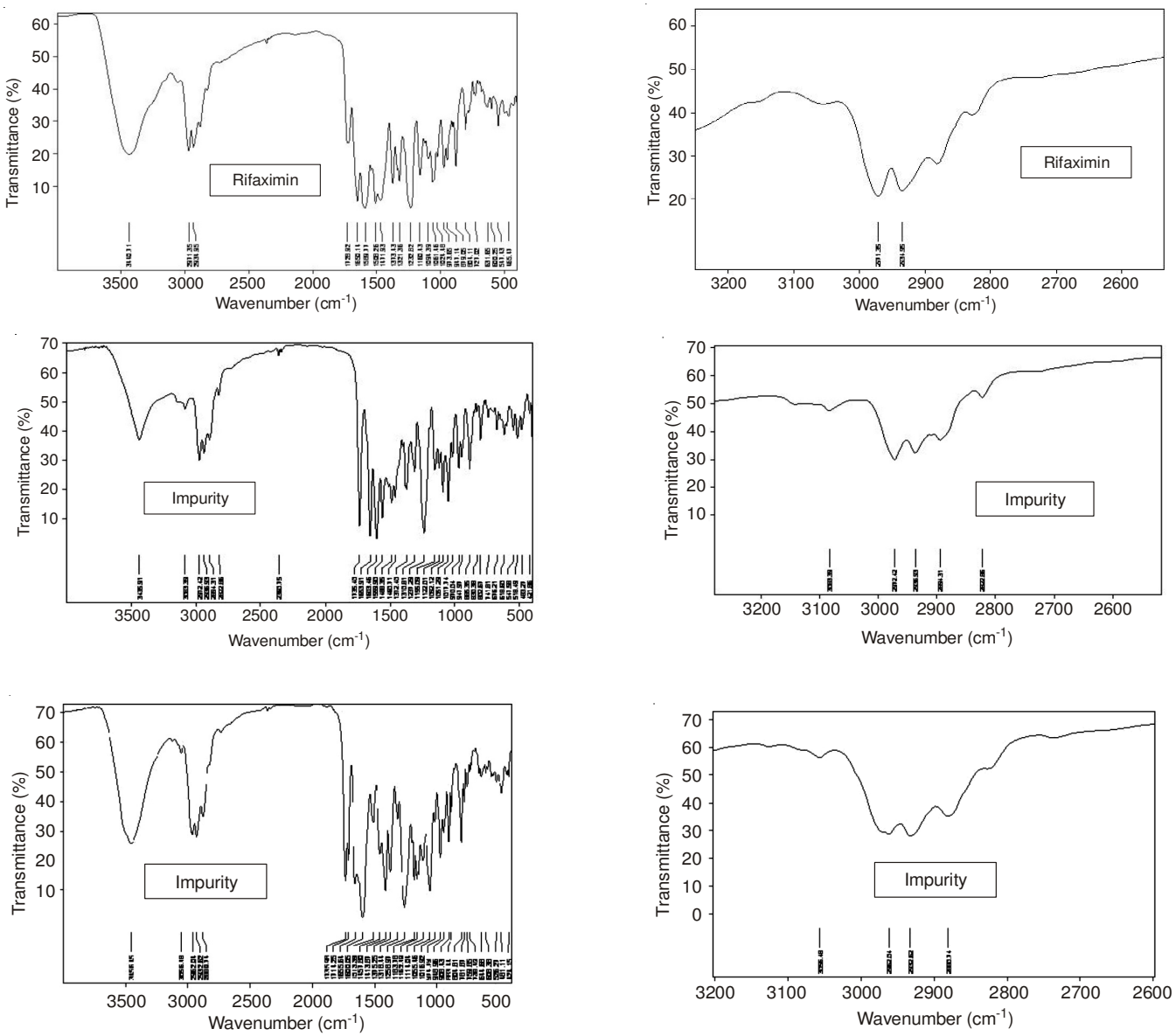

Fig. 3. FT-IR spectra of rifaximin, impurity 1 and impurity 2

vibration, respectively. These data suggested the existence of the terminal methylene group in impurity 1 and impurity 2 authentically.

Table-2 shows the ^{1}H NMR chemical shifts of the rifaximin, impurity 1 and impurity 2. There were similar chemical shifts in the CH_3(13,14,30,31,32,33,34,36,37). Table-3 shows the ^{13}C NMR chemical shifts of the rifaximin, impurity 1 and impurity 2. Moreover, the lack of the 8'CH_3 was the most remarkable difference among the impurity 1, impurity 2 and rifaximin. Furthermore, the chemical shifts of the 8'CH_2 ethylenic bond in impurity 1 and impurity 2 were 3.442 and 3.979, respectively. In the ^{1}H NMR spectrum of rifaximin, chemical shift of hydroxyl of 1st position was 17.908 and the chemical shift of hydroxyl of 8th position was 14.908. This was significantly different from the literature of the rifaximin when compared the chemical shifts, probably because vivacious hydrogen atoms are replaced by the $CDCl_3$ and the apparatuses were used differently. In the ^{13}C NMR chemical shifts of 8'CH_2 are less than 80 mainly because of the impact of the irregular electron clouds of the two nitrogen atoms in the heterocyclic ring. So the chemical shifts of 8'CH_2 move to the high field.

TABLE-2
^{1}H NMR δ OF RIFAXIMIN, IMPURITY 1 AND IMPURITY 2 AT 500 MHz

Rifaximin		Impurity 1		Impurity 2	
Proton (s)	δ (ppm)	Proton (s)	δ (ppm)	Proton (s)	δ (ppm)
CH_3 (13)	1.98	CH_3 (13)	1.847	CH_3 (13)	1.750
CH_3 (14)	2.10	CH_3 (14)	1.994	CH_3 (14)	2.231
CH (17)	6.30	CH (17)	6.252	CH (17)	6.490
CH (18)	6.74	CH (18)	7.082	CH (18)	6.645
CH (19)	6.18	CH (19)	5.626	CH (19)	6.299
CH (20)	2.37	CH (20)	0.845	CH (20)	2.878
CH (21)	3.68	CH (21)	3.741	CH (21)	3.565
CH (22)	1.53	CH (22)	1.333	CH (22)	1.257
CH (23)	2.95	CH (23)	2.872	CH (23)	2.945
CH (24)	1.43	CH (24)	1.208	CH (24)	1.377
CH (25)	4.95	CH (25)	4.708	CH (25)	4.808
CH (26)	1.31	CH (26)	2.916	CH (26)	0.847
CH (27)	3.38	CH (27)	4.069	CH (27)	3.424
CH (28)	5.08	CH (28)	5.158	CH (28)	5.340
CH (29)	6.15	CH (29)	5.991	CH (29)	4.119
CH_3 (30)	2.33	CH_3 (30)	2.572	CH_3 (30)	2.513
CH_3 (31)	0.82	CH_3 (31)	0.963	CH_3 (31)	2.264
CH_3 (32)	1.02	CH_3 (32)	1.021	CH_3 (32)	0.902
CH_3 (33)	0.30	CH_3 (33)	0.644	CH_3 (33)	0.526
CH_3 (34)	-0.44	CH_3 (34)	-0.099	CH_3 (34)	-0.020
CH_3 (36)	2.07	CH_3 (36)	2.083	CH_3 (36)	2.036
CH_3 (37)	3.08	CH_3 (37)	3.020	CH_3 (37)	3.078
CH (2')	8.45	CH (2')	7.960	CH (2')	9.296
CH (3')	7.36	CH (3')	7.303	CH (3')	7.779
CH (5')	7.10	CH (5')	7.640	CH (5')	6.973
CH_3 (8')	**2.62**	**CH_2 (8')**	**3.442**	**CH_2 (8')**	**3.979**
		OH (1)	**14.405**	**OH (8)**	**13.228**

TABLE-3
^{13}C NMR δ OF RIFAXIMIN, IMPURITY 1 AND IMPURITY 2 AT 500 MHz

Rifaximin		Impurity 1		Impurity 2	
Proton (s)	δ (ppm)	Proton (s)	δ (ppm)	Proton (s)	δ (ppm)
1	171.89	1	165.476	1	180.573
2	112.03	2	112.220	2	127.118
3	122.99	3	117.888	3	120.774
4	128.14	4	124.701	4	128.408
5	115.51	5	117.201	5	111.245
6	171.89	6	170.956	6	170.960
7	104.09	7	105.778	7	109.791
8	182.19	8	191.323	8	132.704
9	97.83	9	99.920	9	110.429
10	155.10	10	162.717	10	146.415
11	188.84	11	169.965	11	192.960
12	108.97	12	107.873	12	108.191
13	20.78	13	20.500	13	20.699
14	21.44	14	20.873	14	21.404
15	171.89	15	172.970	15	181.854
16	130.00	16	127.908	16	138.596
17	136.21	17	138.594	17	139.707
18	125.35	18	118.836	18	125.111
19	142.10	19	138.943	19	142.445
20	37.78	20	44.015	20	36.466
21	72.65	21	72.254	21	76.904
22	32.91	22	32.145	22	31.551
23	76.75	23	75.333	23	76.999
24	36.93	24	45.441	24	34.273
25	73.91	25	76.745	25	73.989
26	38.59	26	46.552	26	39.811
27	77.86	27	60.186	27	76.745
28	115.52	28	74.078	28	78.365
29	142.10	29	149.436	29	149.865
30	20.43	30	22.139	30	21.788
31	17.56	31	9.367	31	14.199
32	10.76	32	14.338	32	10.726
33	8.21	33	15.746	33	14.079
34	8.06	34	9.022	34	8.207
35	171.89	35	179.179	35	172.540
36	6.98	36	16.918	36	9.551
37	56.99	37	55.691	37	57.283
2a	128.96	2a	126.452	2a	118.241
3a	117.61	3a	126.205	3a	117.622
4a	141.75	4a	148.029	4a	149.865
5a	109.99	5a	110.898	5a	118.856
6a	142.1	6a	139.105	6a	142.023
8a	22.35	8a	76.391	8a	76.189

Conclusion

Preparation of two potential oxydic degradation impurities of rifaximin has been carried out based on the degradation mechanism by using manganese dioxide as the oxidant. The structural characterization of the impurities was elucidated by using MS and other modern spectroscopic (NMR and FTIR) techniques. The combined result of MS, NMR and FTIR confirmed the structure of unknown impurities as (2S, 20S, 21S,22R,23R,24R,25S,26R,27S)-6,21,23-trihydroxy-27-methoxy-2,4,16,20,22,24,26-heptamethyl-11-methylene-1,5,15-trioxo-1,2,5,11-tetrahydro-2,7-(epoxypentadeca[1,11,13]trienoimino)furo[2",3":7',8']naphtho[1',2':4,5]imidazo[1,2-a]pyridin-25-yl-acetate and (2S,20S,21S,22R,23R,24R,25S,26R,27S)-5,21,23-trihydroxy-27-methoxy-2,4,16,20,22,24,26-heptamethyl-11-methylene-1,6,15-trioxo-1,2,6,11-tetrahydro-2,7-(epoxypentadeca[1,11,13]trienoimino)furo[2",3":7',8']naphtho[1',2':4,5]imidazo[1,2-a]pyridin-25-yl-acetate (Fig. 2).

REFERENCES

1. J.C. Gillis and R.N. Brogden, *Drugs*, **49**, 467 (1995).
2. V. Ojetti, E.C. Lauritano, F. Barbaro, A. Migneco, M.E. Ainora, L. Fontana, M. Gabrielli and A. Gasbarrini, *Exp. Opin. Drug Metab. Toxicol.*, **5**, 675 (2009).
3. A. Tursi and S. Papagrigoriadis, *Aliment Pharmacol. Therap.*, **30**, 532 (2009).
4. L. Gerard, K.W. Garey and H.L. DuPont, *Exp. Rev. Antiinfect. Therap*., **3**, 201 (2005).
5. J.J. Descombe, D. Dubourg, M. Picard and E. Palazzini, *Int. J. Clin. Pharmacol. Res*., **14**, 51 (1994).
6. X. Zhang, J. Duan, K. Li, L. Zhou and S. Zhai, *J. Chromatogr. B; Anal. Technol. Biomed. Life Sci.*, **850**, 348 (2007).
7. R.N. Rao, D.D. Shinde and S.B. Agawane, *Biomed. Chromatogr.*, **23**, 563 (2009).
8. T. Sudha, P.V. Heamlatha, V.R. Ravikumar, R. Jothi and M. Radhakrishnan, *Asian J. Pharm. Clin. Res.*, **2**, 112 (2009).
9. C.-R. Qin and R.-Z. Lu, *Chin. J. Antibiotics*, **2**, 89 (2007).
10. Z.-B. Zhang, Z.-W. Li and C.-Y. Li, *West Chin. J. Pharm. Sci.*, **4**, 281 (2003).
11. European Pharmacopoeia (2010).
12. European Pharmacopoeia 6.5 (2009).
13. R. Stradi, D. Nava, M. Nebuloni, B. Pastura and E. Pini, *J. Pharm. Biomed. Anal.*, **51**, 858 (2009).
14. WHO, Impurities in New Drug Substances Q3A (R2) in ICH Guideline, http://www.ich.org/cache/compo/363-272-1.html#Q3A(R). October (2006).