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Short communication

Structural identification and characterization of impurities in moxifloxacin

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Abstract

In the synthesis of Moxifloxacin four prominent impurities were detected in HPLC analysis. These impurities were detected in gradient HPLC method. They were isolated from enriched mother liquors and were characterized as 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(*S*,*S*)-*N*-methyl-2,8-diazabicyclo (4,3,0) non-8yl]-4-oxo-3-quinoline carboxylic acid (Impurity-1), methyl-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(*S*,*S*)-2,8-diazabicyclo(4,3,0)non-8-yl]-4-oxo-3-quinoline carboxylate (impurity-2), and 1-cyclopropyl-6-fluoro-1,4 dihydro-8-hydroxy-7-[(*S*,*S*)-2,8-diazobicyclo(4,3,0)non-8-yl]-4-oxo-3-quinoline carboxylicacid (impurity-3), 1-cyclopropyl-6,7-difluoro-8-hydroxy-4-oxo-1,4 dihydro-3-quinoline carboxylicacid (impurity-4) by means of ^{1}H, ^{13}C NMR, DEPT, IR and mass spectral data. Structural elucidation by spectral data was discussed.

Keywords: Moxifloxacin; Characterization; Spectroscopy; Structure elucidation

1. Introduction

Moxifloxacin is a new 8-methoxy quinolone with enhanced anti-gram positive activity in vitro compared with ciprofloxacin and ofloxacin [1–3]. Moxifloxacin differs from other quinolones in that it has a methoxy function at the 8-position and a diazabicyclononyl moiety with *S*,*S*-configuration at the 7-position. The results of well-designed, prospective and comparative clinical trials proved moxifloxacin to be safe and efficacious in treating community-acquired respiratory tract infection, sinusitis, acute exacerbation's of chronic bronchitis and pneumonia.

The analysis of moxifloxacin bulk drug revealed the presence of four impurities which were up to 0.1%. As per the stringent regulatory requirements the impurity profile study has to be carryout for any final product to identify and characterize all the unknown impurities that are present at a level of >0.1%. This paper describes the isolation and characterization of impurities present in the bulk drug of moxifloxacin. Though chiral separation of moxifloxacin intermediate diazabicyclo compound was cited in the literature [4], the impurity profile study of moxifloxacin was not reported to the best of our knowledge.

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2. Experimental

2.1. Samples

The investigated samples were obtained from synthetic R&D laboratory of Dr. Reddy's Laboratories Ltd., Bulk Actives, Unit-III, Hyderabad, India. The impurities were synthesized from the same laboratory.

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. The analysis was carried out on ACE 5 C18 column, 250 mm × 4.0 mm i.d., 5 μm particle size (advanced chromatography technologies ACT, Scotland) with a mobile phase consisting of A: 10 ml H_3PO_4 + 1 ml trifluoroacetic acid in 1000 ml water (pH adjusted to 2.2 with triethylamine) and methanol in the ratio of 85:15. B: methanol and water in the ratio of 80:20. Program gradient elution (T/%B = 0/20, 18/20, 45/80) was used with UV detection at 295 nm at a flow rate of 1.0 ml/min. The column temperature was maintained at 45 °C. The data was recorded using Waters Millennium software.

2.3. High performance liquid chromatography (preparative)

A Waters delta 4000 preparative chromatography system equipped with Waters 2487 UV-Vis detector, fraction collector model Waters FCM-II and Rheodyne Injector Model 7725I with 1.0 ml loop was used. A 250 mm × 20 mm i.d. column packed with 5 μm Inertsil ODS (GL sciences Inc., Japan) was employed for separation. The mobile phase consisted of 0.01 M CH_3COONH_4 (pH = 3.5 with CH_3COOH):CH_3CN in the ration of 80:20 (v/v). The flow rate was set at 10.0 ml/min. Detection was carried out at 295 nm.

2.4. NMR spectroscopy

The ^1H, ^{13}C and DEPT spectra were recorded on Varian 200 Gemini spectrometer. The ^1H (200 MHz) and ^{13}C NMR (50 MHz) were recorded using TMS and DMSO-d_6 as internal standards, respectively.

2.5. Mass spectrometry

EI (70 eV) and CI mass spectra were recorded on HP5989A mass spectrometer. The samples were introduced with particle beam interface using LC and reodyne injector. The source manifold and quadrupole temperatures were maintained at 250 and 100 °C, respectively. Isobutane was used as a reagent gas for chemical ionization (CI) mode. The APCI and ESI mass spectra were recorded on Shimadzu LC–mass spectrometer model QP-8000α.

2.6. FT-IR spectroscopy

FT-IR spectra were recorded on Perkin-Elmer model Spectrum GX series FT-IR as KBr pellet.

3. Synthesis of moxifloxacin

The scheme for the synthesis of moxifloxacin [5] was shown in the Fig. 1

4. Results and discussion

4.1. Detection of impurities 1, 2, 3 and 4

A typical LC-chromatogram of moxifloxacin bulk drug was recorded using the LC- method as described in Section 2.2.The target impurities under study were marked as impurity-1, impurity-2, impurity-3, and impurity-4. Retention times and structures of these impurities and moxifloxacin are shown in Table 1.

4.2. Isolation of impurities by preparative HPLC

An isocratic reverse phased solvent system discussed under Section 2.3 was used for the isolation of these impurities. All the fractions of impurities isolated were concentrated and extracted with chloroform. The isolated solids obtained from the concentrated fractions of impurities were used to generate spectral data. The details of the elucidation of structures of these impurities are presented in the following sections.

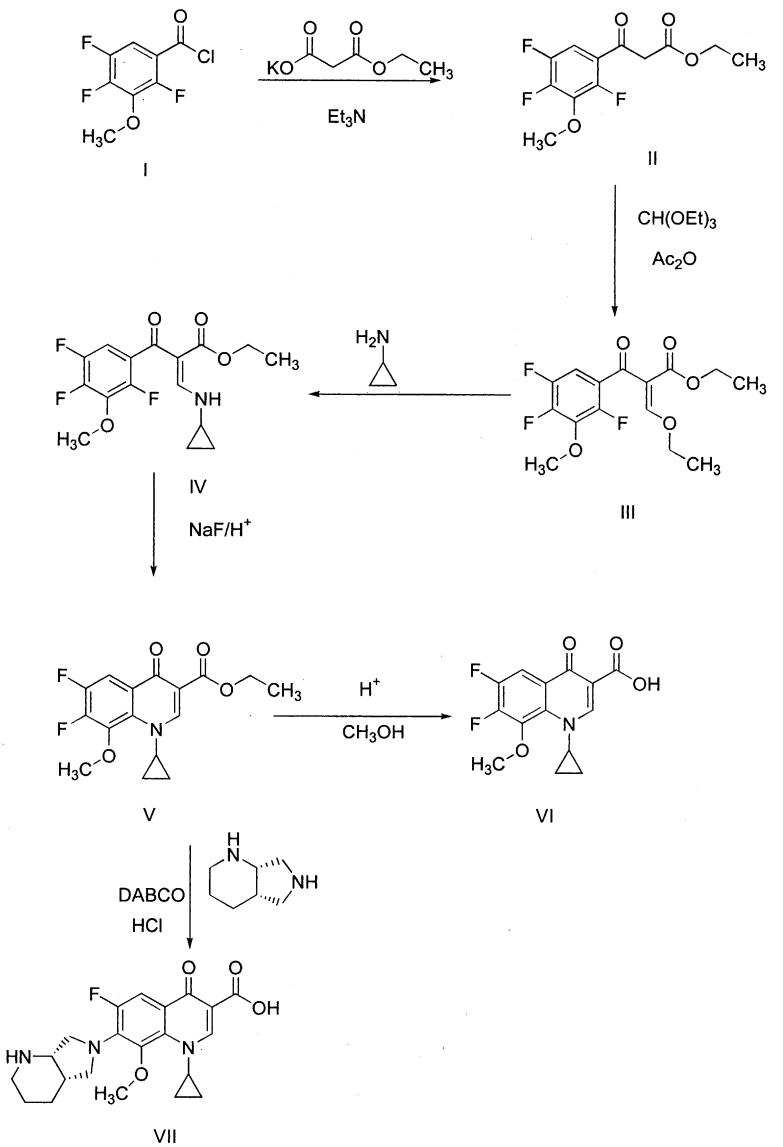


Fig. 1. Scheme for the synthesis of moxifloxacin.

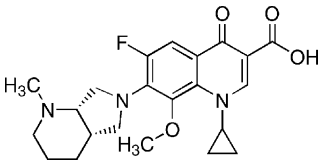
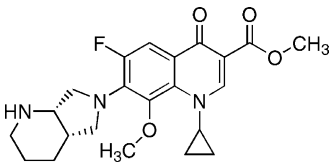
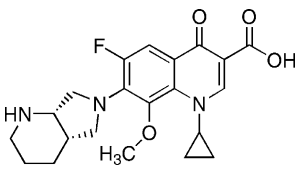
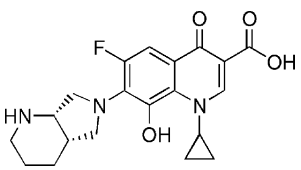
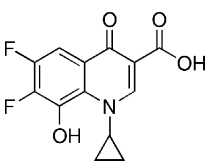
4.3. Structural elucidation

4.3.1. Structure elucidation of impurity-1

The EI mass spectrum of impurity-1 was displayed the molecular ion peak at *m/z* 415. The CI and APCI (+ve) mass spectra further confirmed this with the presence of protonated molecular ion peak as base peak at *m/z* 416, which is 14 mass units higher than that of moxifloxacin. This can be attributed to the methyl incorporation in moxifloxacin. In the IR spectrum of impurity-1, the absence of stretching frequency at 3394 cm^{-1} indicated the absence of NH. The methyl substitution on azabicyclo moiety was further confirmed from the additional singlet signal at δ, 2.17 (3H) in ^{1}H NMR spectrum.

In the ^{13}C NMR spectrum the presence of an additional signal presence at 44.1 ppm further lends support to the *N*-methyl substitution on moxifloxacin.

Table 1

S. No.	Retention time (min)	Compound	Structure	Nature
1	13.2	Impurity-1		Process related
2	14.5	Impurity-2		Process related
3	16.7	Moxifloxacin		Moxifloxacin
4	25.5	Impurity-3		Process related
5	32.3	Impurity-4		Process related

Based on this data the structure of impurity-1 was confirmed as 1-cyclopropyl-6-fluoro-1,4 dihydro-8-methoxy-7-[(*S*,*S*)-*N*-methyl-2,8-diazabicyclo (4,3,0) non-8yl]-4-oxo-3-quinoline carboxylic acid.

4.3.2. Structural elucidation of impurity-2

The mass spectra of impurity-2 also displayed the molecular ion at $m/z = 415$ like in the case of impurity-1. The possibility of ester formation fulfills the another methyl incorporation. In the ^{1}H NMR spectrum the OCH_3 singlet appeared at δ, 3.72. ^{13}C NMR spectrum with methoxy carbon signal at 56.0 ppm is complimentary to ^{1}H NMR spectrum. Based on this data the structure of impurity-2 was confirmed as methyl-1-cyclopropyl-6-fluoro-1,4 dihydro-8-methoxy-7-[(*S*,*S*)-2,8-diazabicyclo (4,3,0) non-8-yl]-4-oxo-3-quinoline carboxylate.

4.3.3. Structural elucidation of impurity-3

The mass spectra of impurity-3 confirmed the molecular ion at $m/z = 387$ which was 14 mass units less than that of moxifloxacin. The absence of singlet at 3.60 ppm in ^{1}H NMR and corresponding carbon signal in ^{13}C NMR indicated the hydroxy substituted moxifloxacin structure for impurity-3.

Based on this data the structure of impurity-1 was confirmed as $C_{20}H_{22}FN_3O_4$ and the corresponding structure as 1-cyclopropyl-6-fluro-1,4 dihydro-8-hydroxy-7- [(*S*,*S*)-2,8-diazobicyclo (4,3,0) non-8 yl]-4-oxo-3-quinoline carboxylic acid.

4.3.4. Structure elucidation of impurity-4

The EI mass spectrum of impurity-4 displayed the molecular ion peak at $m/z = 281$. The electrospray positive mass spectrum of impurity-4 displayed the

protonated molecular ion peak as base peak at $m/z = 282$ which further confirmed the molecular ion. The observation in ^{1}H NMR spectrum particularly in aromatic region derived the structure of impurity-4. The signal for the proton adjacent to fluorine on quinazoline moiety which was doublet in moxifloxacin appeared as triplet in impurity-4. This was attributed to the fluorine substitution in place of diazabicyclo moiety in impurity-4. Further, the corresponding carbon signal, which was shifted, to 100.4 ppm from 106.5 ppm as broad doublet lends support to the fluorine substitution. The absence of methyl signal at 3.60 ppm also indicated the hydroxy group presence.

Based on the above data the structure of impurity-4 was confirmed as 1-cyclopropyl-6,7-difluoro-8-hydroxy-4-oxo 1,4 dihydro-3-quinoline carboxylic acid.

The spectral data for the synthesized and isolated impurities were found to be identical. The synthetic standards of impurities 1,2,3,4 were coeluted on LC with moxifloxacin. The ^{13}C NMR assignments of moxifloxacin were confirmed with HETCOR experiment without any ambiguity.

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References

[1] C.M. Tobin, J. Sunderland, L.O. White, A.P. MacGowan, D.S. Reeves, J. Antimicrob. Chemother. 42 (1998) 278–279.
[2] J.M. Woodcock, J.M. Andrews, F.J. Boswell, N.P. Brenwald, R. Wise, J. Antimicrob. Chemother. 41 (1997) 101–106.
[3] K.E. Aldrige, D.S. Ashcraft, J. Antimicrob. Chemother. 41 (1997) 709–711.
[4] T.R. Krishna, D.S. Rao, K. Vyas, G.O. Reddy, J. Pharm. Biomed. Anal. 22 (2000) 691–697.
[5] A.M. Martel, P.A. Leeson, J. Castaner, Drugs Fut. 22 (1997) 109–110.