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Isolation and Characterization of Fesoterodine Fumarate Related Impurity

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During analytical method for the determination of related substances of fesoterodine fumarate using RP-HPLC, one unknown impurity was observed more than 0.1 % level and also keeps on increasing in forced degradation study. Hence it was desirable to isolate, identify and characterize this impurity using advanced analytical techniques. A simple, sensitive and specific RP-HPLC method was developed for the quantification of related impurities of fesoterodine fumarate. The chromatographic separation was accomplished on a YMC Pak ODS-A (150 mm × 4.6 mm), 5 µ column using mobile phase system with gradient elution of solvent-A (0.1 % trifluro acetic acid in water and methanol in the ratio of 70:30 v/v) and solvent-B (acetonitrile). The analytes were detected at 215 nm using photo diode-array (PDA) detector. The investigated impurity has been isolated using preparative RP-HPLC method using YMC Pak ODS-A (250 mm × 50 mm) 12 µ. Then the isolated impurity was identified and characterized.

Key Words: Fesoterodine fumarate, Preparative RP-HPLC.

INTRODUCTION

A comprehensive study has been made to isolate and characterize these impurities by spectroscopic techniques[1-4]. The impurity profile study has to be carried out from any final drug substances to identify and characterize the unknown impurities. The requirement of identification and characterization of the impurity in the final drug substances is extremely necessary to meet the regulatory requirements[5]. However exhaustive literature survey revealed that none of the most recognized pharmacopoeias or any journals includes the isolation, identification and characterization of an investigated compound.

EXPERIMENTAL

Fesoteroine fumarate active pharmaceutical ingredient (API) test sample were kindly supplied by research and Development Centre, Wockhardt Laboratories Limited, Aurangabad, India. Trifluoroacetic acid, HPLC grade acetonitrile and methanol were obtained from Merck limited, Mumbai, India. High purity deionised water were obtained from Millipore, Milli-Q (Bedford, MA, USA) purification system.

The experiments were performed using a commercial LCQ ion trap mass spectrometer (Quattro II, Salt Lake City, Utah, USA), equipped with an ESI source. The data acquisition was carried out using Mass lynx software. The typical source conditions were: spray voltage, 27 V; capillary voltage, 3.6 kV; heated capillary temperature, 150 ºC; drying and nebulizing gas (N_2) with flow rate of 15 units. ^{1}H NMR measurements were performed on a Varian Gemini 2000 model 400 MHz instrument at 25 ºC. The ^{1}H NMR chemical shift values were reported on the δ scale in ppm, relative to TMS (δ = 0.00) as internal standard. The Fourier transfer infrared spectroscopy spectra of the impurity were recorded in the solid state as KBr dispersion using Perkin-Elmer 1650 FT-IR spectrophotometer.

High performance liquid chromatography (analytical): HPLC system (Waters Milford, USA) equipped with inbuilt auto sampler and quaternary gradient pump with an on-line degasser was used. The column compartment having temperature control and photodiode array detector (PDA) was employed throughout analysis. Chromatographic data was acquired using empower software.

High performance liquid chromatography (preparative): HPLC system (Shimadzu, Japan) equipped with UV detector and binary gradient pump. The column compartment having temperature control was employed throughout analysis. Chromatographic data was acquired using class VP software.

Chromatographic conditions for HPLC (analytical): YMC Pak ODS-A (150 mm × 4.6 mm), 5 µ, (YMC, Japan) column was used as stationary phase maintained at 30 ºC. The mobile phase involve a variable composition of solvent A

(0.1 % TFA in water and methanol in the ratio of 70:30 v/v) and solvent-B (acetonitrile). The mobile phase was pumped through the column at a flow rate of 1 mL min^{-1}. The optimum wavelength of 215 nm, which represents the wavelength of maximum response for investigated impurity.

Chromatographic conditions for HPLC (preparative): YMC Pak ODS-A (250 mm × 50 mm), 12 µ, (YMC, Japan) column was used as stationary phase maintained at 30 ºC. The mobile phase involve a variable composition of solvent A (0.1 % TFA in water and methanol in the ratio of 70:30 v/v) and solvent-B (acetonitrile). The mobile phase was pumped through the column at a flow rate of 70 mL min^{-1}. The optimum wavelength of 215 nm, which represents the wavelength of maximum response for investigated impurity.

Sample solution preparation: About 200 mg of fesoterodine fumarate sample was dissolved in 10 mL of acetonitrile and methanol in the ratio of 1:1(v/v).

RESULTS AND DISCUSSION

Detection of impurity: A typical analytical LC chromatogram of fesoterodine fumarate was recorded 215 nm. The target impurity under investigation is eluting at retention time about 17 min. The impurity was isolated from enriched impurity sample of fesoterodine fumarate on preparative LC. Attempts were also made to synthesize the impurity.

Isolation of impurity by preparative HPLC: A reversed phase solvent system was used for the isolation of impurity. The enriched impurity sample was dissolved in acetonitrile and methanol in the ratio of 1:1 (v/v) and loaded on the preparative column and the fraction collected were pooled together and analyzed using analytical HPLC to confirm the RRT and purity of the isolated impurity[6]. The pooled fraction was concentrated under high vacuum Buchi Rotavapour R-124 with bath temperature at 45 ºC to distill out the acetonitrile and methanol. The remaining aqueous layer is subjected to Lyophilization in Vertis 6 L lyophilizer to get a pure white solid compound. The chromatographic purity of the impurity is tested by analytical LC method separately before and after concentration. The isolated fluffy solid mass used for spectral studies. The HPLC purity of lyophilized impurity was found to be 95.26 % by area normalization.

Structure elucidation of compound: The FT-IR ESI-MS/MS and NMR methods were used to characterize the structure of unknown impurity. The FT-IR spectra confirms the aromatic C=C, C=O and OH stretching vibrations (Table-1), In MS full scan for compound, the major ion detected was protonated molecular ion $[M + H]^+$ m/z 510 confirms the molecular weight of compound *i.e.*, 509. The mass, ^1H NMR (Table-2) ^{13}C NMR (Table-3) spectral data (Fig. 1) confirmed the proposed structure of impurity (Fig. 2). The absence of peak at about 10.4 ppm δ value in D_2O exchanges ^1H NMR analysis indicates the presence of OH group. It is also clear from ^1H-^1H COSY that there is a correlation peak between protons of 2nd position and 3rd position as well as between 2nd position and 1st position which indicates the presence of - C^3-*C^2-C^1-X group in the investigated compound. The fragmentation pattern of the molecular ion in mass spectrometer shows the possible fragment ions of the investigated compound.

TABLE-1
FTIR SPECTRAL DATA INTERPRETATION FOR FESOTERODINE FUMARATE RELATED IMPURITY

Assignment	Mode of vibration	Wave number (cm^{-1})
OH	Stretching	2992.30
Aromatic C=C	Stretching	1659.53
C=O	Stretching	1754.59, 1722.79

TABLE-2
^1H NMR SPECTRAL DATA INTERPRETATION FOR FESOTERODINE FUMARATE RELATED IMPURITY

^1H Position (ppm)	No. of protons	Multiplicity	^1H Chemical Shift
5, 6,8,9,24,25	18H	m	1.24-1.35
26	2H	q	5.14-5.23
3	2H	t	3.56-3.59
2	2H	m	2.58-2.73
1	1H	t	3.97-4.01
4,7,23	3H	m	2.75-2.88
28,29	2H	m	6.83-7.03
11,12,13,14,1,5,16,18,19	8H	m	7.20-7.31
31	1H	s	10.4

Proton No. 31 is an exchangeable proton. m-multiple t, s-singlet, t-triplet, q-quartet.

TABLE-3
^{13}C NMR SPECTRAL DATA INTERPRETATION FOR FESOTERODINE FUMARATE RELATED IMPURITY

^{13}C Position	Type of carbon	^{13}C Chemical shift (ppm)
5,6,8,9,24,25	CH_3	–
17.07, 17.20, 18.53, 18.70, 19.15, 19.30		
26, 3, 2	CH_2	66.38, 46.37, 31.76
4, 7, 23, 1	CH	55.04, 41.71, 34.35
10, 21, 20, 17	C	134.29
135.38, 141.90, 148.87		
22, 27, 30	–	164.97, 167.0, 175.92
11, 12, 13, 14, 15	CH-Ar, C=C	
123.25, 127.33, 127.57, 128.08, 128.27		
16, 18, 19, 28, 29	–	129.12, 133.28, 135.09

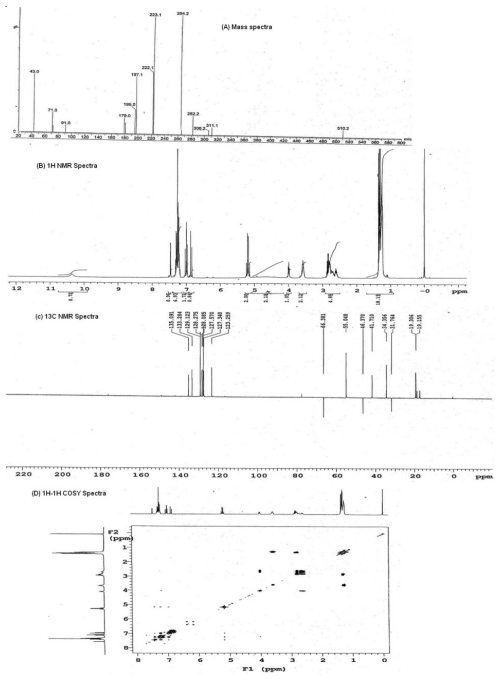

Fig. 1. Spectral representation of isolated fesoterodine fumarate related impurity (A) mass spectra (B) ^1H NMR spectra (C) ^{13}C NMR spectra, (D) ^1H-^1H COSY spectra

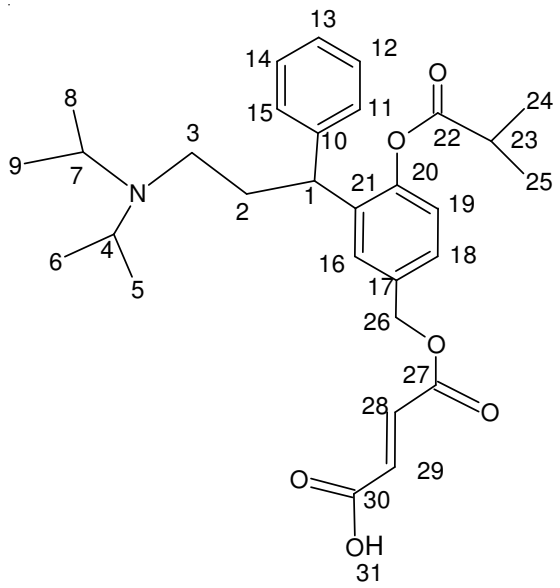

Fig. 2. Chemical structure of the isolated fesoterodine fumarate related impurity

Conclusion

Fesoterodine fumarate related impurity was isolated using preparative HPLC and the same was characterized using ^{1}H NMR, NMR with D_2O exchange analysis, ^{13}C NMR, ^{1}H-^{1}H COSY and mass spectrophotometer. All the data obtained from the spectral analysis confirmed the proposed structure of the impurity under the investigation.

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