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Spectral characterization of rupatadine fumarate and its potential impurities

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ABSTRACT

During the process development of rupatadine fumarate (1). Two impurities (2,3) with respect to rupatadine fumarate were detected by in-house method of simple reverse phase high-performance liquid chromatography (HPLC). Three (1-3) have been prepared by the known synthetic method. To the best of our knowledge, compounds (1-3) structure elucidation had not been reported until now. The ¹H and ¹³C NMR data of impurities (2,3) and rupatadine fumarate (1) were reported in this paper for the first time. Based on the spectral data, the structure of these compounds 1-3 were characterized as 8-Chloro-6, 11-dihydro-11-[1-[(5-methyl-3-pyridinyl)methyl]-4-piperidinylidene]-5Hbenzo[5, 6] cyclohepta [1, 2-b] pyridine fumarate (1), 8-Chloro-11-(4-piperidinylidene)-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridine (2), 3-(Chloromethyl)-5-methyl pyridine (3). The HPLC analysis and characterization of these drug and impurities were discussed. © 2012 Trade Science Inc. - INDIA

KEYWORDS

Rupatadine;
 Impurities;
 Desloratadine;
 Characterization;
 HPLC analysis

INTRODUCTION

Rupatadine fumarate (RUPA) is a non-sedating H₁-antihistamine (second generation) and platelet-activating factor inhibitor. Chemically it is 8-Chloro-6, 11-dihydro-11-[1-[(5-methyl-3-pyridinyl) methyl]-4-piperidinylidene]-5Hbenzo[5, 6] cyclohepta [1, 2-b] pyridine fumarate. It was discovered and developed by J. Uriach y Cia, S. A.^[1] and further blocks the receptors of the platelet-activating factor (PAF) according to in vitro and in vivo studies^[2]. Rupatadine possesses anti-allergic properties such as the inhibition of the degranulation of mast cells induced by immunological and non-immunological stimuli, and inhibition of the release of cytokines, particularly of the TNF in human mast cells and monocytes^[3]. Rupatadine fumarate (RUPA) is a

non-sedating H₁-antihistamine (second generation) and platelet-activating factor inhibitor. Chemically it is 8-Chloro-6, 11-dihydro-11-[1-[(5-methyl-3-pyridinyl) methyl]-4-piperidinylidene]-5Hbenzo[5, 6] cyclohepta [1, 2-b] pyridine fumarate. The structure of RUPA is shown in Figure 1. The drug is not official reported in pharmacopoeia. It is off white to pinkish crystalline powder that is soluble in soluble in methanol and ethanol, very slightly soluble in chloroform and insoluble in water. Rupatadine fumarate belongs to a class of medications called Antiallergic, Antihistaminic. It is potent and orally active that was developed as a therapeutic agent for the treatment of seasonal allergic rhinitis and chronic idiopathic urticarial^[4,5].

The presence of impurities or its related compounds in a drug substance can have a significant impact on the

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quality and safety of the drug product. During the process development of rupatadine fumarate, two impurities were observed in the range of 0.05-0.15% level along with the main product peak in the HPLC analysis. As per the general guidelines recommended by ICH^[6] 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. Hence, a comprehensive study was undertaken to synthesize and characterize these two impurities of rupatadine fumarate. The ¹H, ¹³C NMR, IR and mass data for characterization of impurities (2,3) and rupatadine fumarate (1) were reported in this paper for the first time.

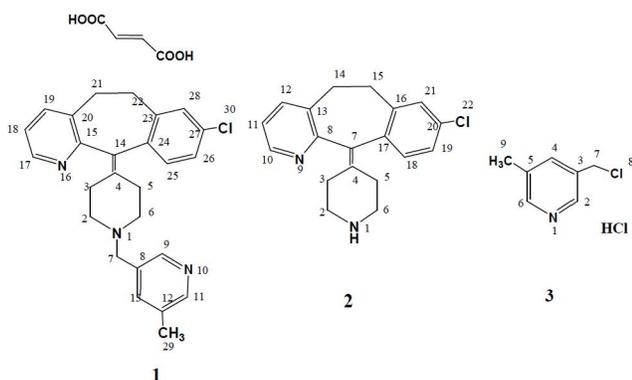


Figure 1

EXPERIMENTAL

Samples and chemicals

The investigated samples of rupatadine fumarate (1) and impurities (2,3) were synthesized after identification by in-house HPLC method. HPLC grade acetonitrile and acetic acid were obtained from Merck, Mumbai, India. AR grade sodium dihydrogen phosphate, phosphoric acid and triethylamine were obtained from SD Fine Chemicals Limited, Mumbai, India. Water used for the preparation of mobile phase was purified using Millipore Milli-Q plus (Milford, MA, USA) purification system. Chloroform-d and dimethylsulfoxide-d₆ were purchased from Aldrich Chemicals Co., USA.

High-performance liquid chromatography (HPLC)

An in-house LC gradient method was developed for the separation of all possible related substances (impurities) of rupatadine fumarate. SHIMADZU make HPLC

system equipped with 436 pumps and UV detector was used for better separation and quantification of impurities. The buffer solution used for the preparation of mobile phases A and B consists of 0.01M aqueous potassium dihydrogen ortho phosphate and its pH was adjusted to 3.0 ± 0.05 with diluted ortho phosphoric acid. Mobile phase A is buffer only; mobile phase B is acetonitrile only. Inertsil ODS – 3V, 250 X 4.6 mm, 5 μm particle size column was used with a time gradient programme of T/ % B: 0/25, 20/40, 30/70, 35/70, 40/25, 45/25 of 45 min. The column oven temperature was 30°C and column eluent was monitored by UV detector at 250 nm. This LC method was able to separate all the process-related substances with good resolution.

Mass spectrometry

The electrospray ionization and MS-MS studies were performed on a triple quadrupole mass spectrometer PE Sciex model API 3000. The positive and negative electrospray MS data was obtained by switching the capillary voltage between +5000 and –4500 V respectively. The MS-MS data was generated with the collision energy ramping from 30 to 60 V in nitrogen atmosphere.

NMR spectroscopy

The ¹H, ¹³C, DEPT and 2D experiments for rupatadine fumarate and impurities were done on Varian Mercury plus 400 MHz FT NMR spectrometer. The solvents used for rupatadine fumarate, impurities were in DMSO and CDCl₃. The ¹H chemical shift values were reported on δ scale in ppm, relative to TMS (δ=0.00 ppm) and in the ¹³C NMR the chemical shift values were reported relative to CDCl₃ (δ=77.00 ppm) as internal standard. DEPT spectra revealed the presence of methyl and methine groups as positive peaks and methylenes as negative peaks.

FT-IR spectroscopy

The IR spectra were recorded in the solid state as KBr dispersion medium using PerkinElmer 1600 series FT-IR spectrophotometer.

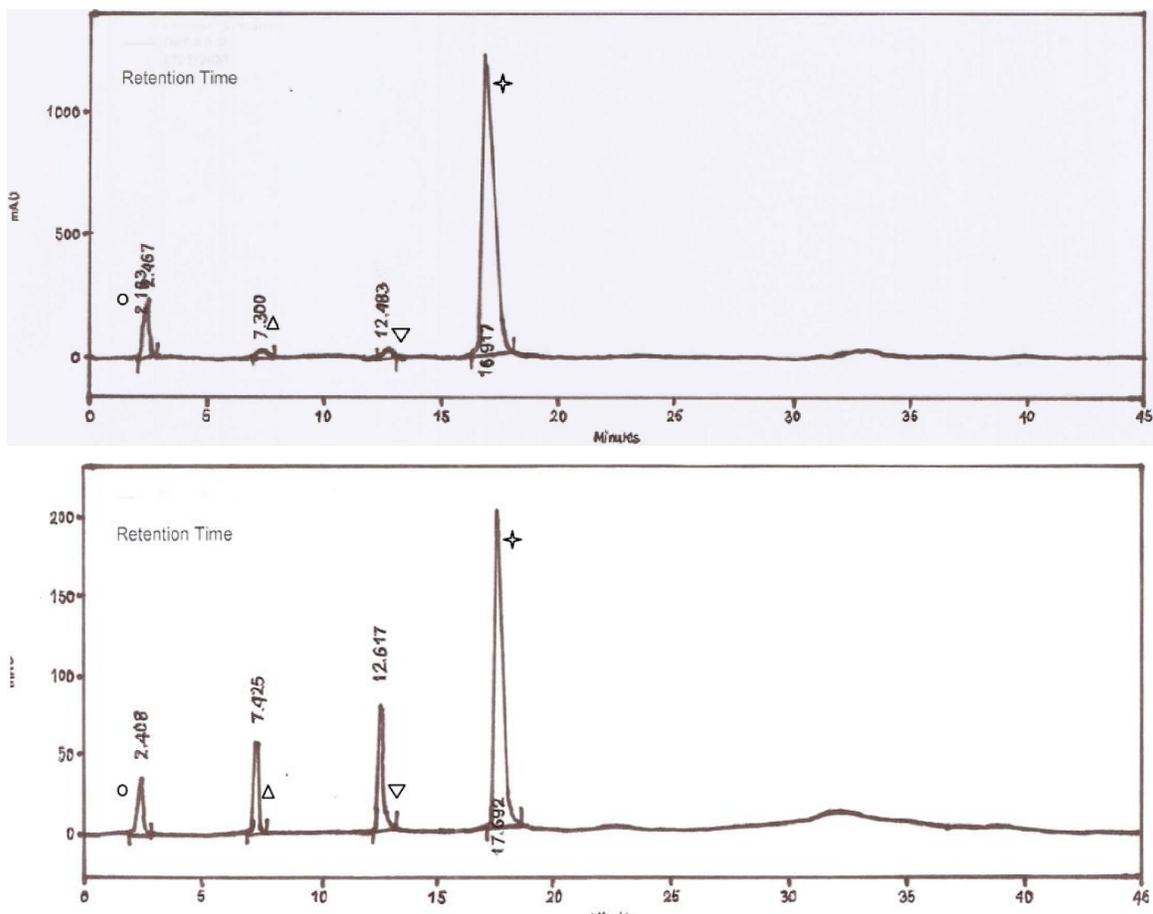
RESULTS AND DISCUSSIONS

Detection of impurities 2 and 3

A typical analytical LC chromatogram of a labora-

tory batch of rupatidine fumarate bulk drug recorded using the LC method as described in section 2.2 is shown in Figure 2a. The target impurities under study

are marked as IMP-2, and IMP-3 are stereoisomers recorded using the LC method as described in section 2.2 is shown in Figure 2b.



♦ Rupatidine Base; ○ Fumaric acid; Δ Impurity-3; ▽ Impurity-2

Figure 2 : (a) A typical analytical LC chromatogram of a laboratory batch of rupatidine fumarate bulk drug; (b) The LC chromatogram of co injection of the synthetic standard impurity-2 and impurity-3 with rupatidine fumarate.

Structural elucidation of rupatidine fumarate (1)

Sample was analyzed by HPLC and its purity was found to be 99.68%, molecular weight of rupatidine base is 415.95. The EI mass spectrum of rupatidine gave a molecular ion at m/z 416 and, IR spectrum displayed characteristic absorptions at 3398.89 & 2982.02, 2934.06 cm^{-1} corresponding to $>\text{CH}$ and aromatic $>\text{CH}$ stretching. The peaks at 1509.95 & 1452.92 cm^{-1} in IR spectrum is indicative of $>\text{C}=\text{C}<$ ring stretching, The ^{13}C NMR spectrum displayed signals due to the presence of twenty three carbons. The DEPT spectrum displayed seven negative signals due to seven methylene groups and twelve positive signals due to the presence of twelve methine groups (three in the aliphatic and the rest in aromatic region). The FT-

IR spectrum displayed a characteristic absorption band at 1685 cm^{-1} indicating the presence of carbonyl functional group, which was supported by the appearance of quaternary carbon signal due to carbonyl functional group of fumaric acid in ^{13}C NMR spectrum. Based on the above spectral data and molecular formula of rupatidine could be $\text{C}_{26}\text{H}_{26}\text{N}_3\text{Cl}$. This molecular formula matched well with the molecular ion observed at 416 amu in the EI mass spectrum. Molecular Formula: $\text{C}_{26}\text{H}_{26}\text{N}_3\text{Cl} \cdot \text{C}_4\text{H}_4\text{O}_4$ Molecular Weight: 532.03.

Structural elucidation of desloratadine (2)

Sample was analyzed by HPLC and its purity was found to be 99.20%, molecular weight of desloratadine is 310.5. The EI mass spectrum of desloratadine gave a protonated molecular ion at m/z 311.0 and, IR spec-

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trum displayed characteristic absorptions at 3399 & 2978,2936 cm⁻¹ corresponding to >CH and aromatic >CH stretching. The peaks at 1512 & 1462 cm⁻¹ in IR spectrum is indicative of >C=C< ring stretching. The ¹³C NMR spectrum displayed signals due to the presence of nineteen carbons. The DEPT spectrum displayed six negative signals due to six methylene groups and six positive signals due to the presence of six methine groups in aromatic region. Based on the above spectral data and molecular formula of desloratadine could be C₁₉H₁₉N₂Cl. This molecular formula matched well with the molecular ion observed at 311 amu in the EI mass spectrum.

TABLE 1 : ¹H NMR assignments of rupatadine fumarate (1).

| Position | 1H | J (ppm) | 13C | DEPT |
|--------------|----|---------|-------|-----------------|
| 1 | | | | |
| 2 | 2H | 2.65 m | 53.5 | CH ₂ |
| 3 | 2H | 2.25 m | 31.0 | CH ₂ |
| 4 | | | 131.4 | |
| 5 | 2H | 2.25 m | 30.6 | CH ₂ |
| 6 | 2H | 2.35 m | 53.5 | CH ₂ |
| 7 | 2H | 3.52 s | 58.3 | CH ₂ |
| 8 | | | 132.5 | |
| 9 | 1H | 8.26 s | 148.3 | CH |
| 10 | | | | |
| 11 | 1H | 8.30 s | 147.3 | CH- |
| 12 | | | 132.6 | |
| 13 | 1H | 7.52 s | 137.3 | CH |
| 14 | | | 139.9 | |
| 15 | | | 156.8 | |
| 16 | - | | | - |
| 17 | 1H | 8.35 d | 146.1 | CH |
| 18 | 1H | 7.22 m | 122.2 | CH |
| 19 | 1H | 7.57 d | 137.4 | CH |
| 20 | | | 131.6 | |
| 21 | 2H | 3.30 m | 29.7 | CH ₂ |
| 22 | 2H | 2.83 m | 29.5 | CH ₂ |
| 23 | | | 137.6 | |
| 24 | | | 136.7 | - |
| 25 | 1H | 7.28 d | 128.8 | CH |
| 26 | 1H | 7.08 d | 125.5 | CH |
| 27 | | | 133.2 | - |
| 28 | 1H | 7.18 s | 130.5 | CH |
| 29 | 3H | 2.28 s | 17.7 | CH ₃ |
| Fumaric acid | 2H | 6.6 s | 134.2 | 2CH |
| | | | 166.4 | 2Carbonyl |

TABLE 2 : ¹H NMR assignments of desloratadine (2).

| Position | 1H | J (ppm) | 13C | DEPT |
|----------|----|----------|-------|-----------------|
| 1 | | | | |
| 2 | 2H | 2.80 m | 48.0 | CH ₂ |
| 3 | 2H | 2.30 m | 32.6 | CH ₂ |
| 4 | | | 132.3 | |
| 5 | 2H | 2.40 m | 32.4 | CH ₂ |
| 6 | 2H | 2.67 m | 48.0 | CH ₂ |
| 7 | | - | 139.4 | |
| 8 | | | 157.4 | |
| 9 | | | | |
| 10 | 1H | 8.40 d | 146.5 | CH |
| 11 | 1H | 7.13 d | 121.9 | CH |
| 12 | 1H | 7.42 brd | 137.2 | CH |
| 13 | | | 132.4 | |
| 14 | 2H | 3.40 m | 31.6 | CH ₂ |
| 15 | 2H | 3.05 m | 31.2 | CH ₂ |
| 16 | - | | 139.3 | - |
| 17 | | | 137.7 | CH ₂ |
| 18 | 1H | 7.15 d | 128.8 | CH |
| 19 | 1H | 7.08 m | 125.9 | CH |
| 20 | | | 133.2 | |
| 21 | 1H | 7.13 s | 130.2 | CH |

TABLE 3 : ¹H NMR assignments of 3-(Chloromethyl)-5-methyl pyridine (3).

| Position | 1H | (ppm) J | 13C | DEPT |
|----------|----|---------|-------|-----------------|
| 1 | | | | |
| 2 | 1H | 8.8 s | 139.9 | CH |
| 3 | | | 137.5 | |
| 4 | 1H | 8.3 s | 138.3 | CH |
| 5 | | | 137.8 | |
| 6 | 1H | 8.7 s | 145.8 | CH |
| 7 | 2H | 4.75 s | 40.8 | CH ₂ |
| 8 | | | | |
| 9 | 3H | 2.6 s | 18.4 | CH ₃ |

Structural elucidation of 3-(Chloromethyl)-5-methyl pyridine (3)

Sample was analyzed by HPLC and its purity was found to be 96.48%, molecular weight of 3-(Chloromethyl)-5-methyl pyridine (3) is 141.0. The EI mass spectrum of 3 gave a protonated molecular ion at *m/z* 142.0 and, IR spectrum displayed characteristic absorptions at 3399 & 2978,2936 cm⁻¹ correspond-

ing to >CH and aromatic >CH stretching. The peaks at 1512 & 1462 cm⁻¹ in IR spectrum is indicative of >C=C< ring stretching, The ¹³C NMR spectrum displayed signals due to the presence of seven carbons. The DEPT spectrum displayed one negative signal due to one methylene group and four positive signals due to the presence of three methine groups in aromatic region and one methyl in the aliphatic region. Based on the above spectral data and molecular formula of 3-(Chloromethyl)-5-methyl pyridine could be C₇H₈NCl. This molecular formula matched well with the molecular ion observed at 142 amu in the EI mass spectrum.

CONCLUSION

This research paper describes the structure elucidation of rupatadine fumarate and process related impurities. The synthesized impurities were characterized using spectroscopic techniques. The ¹H, ¹³C NMR, IR and mass data of impurities (2,3) and rupatadine fumarate (1) were reported in literature for the first time.

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