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Short communication

Identification, isolation and characterization of process-related impurities in Rizatriptan benzoate

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ABSTRACT

Three process-related impurities were observed in routine monitoring of the samples by HPLC. These impurities were identified by LC–MS. One of the impurities, Imp-3 [rizatriptan-2,5-dimer] was reported in literature. Other two impurities were isolated by preparative HPLC and characterized by NMR, Mass and IR. Pure impurities obtained by isolation were co-injected with Rizatriptan benzoate sample to confirm the retention times in HPLC. Structure elucidation of these impurities by spectral data has been discussed in detail. These impurities were identified as 4-(5-((1H-1,2,4-triazol-1-yl)methyl)-3-(2-(dimethylamino)ethyl)-1H-indol-1-yl)-4-(5-((1H-1,2,4-triazol-1-yl)methyl)-3-(2-(dimethylamino)ethyl)-1H-indol-2-yl)-*N*,*N*-dimethylbutan-1-amine [rizatriptan-1,2-dimer] and [4,4-bis-(5-((1H-1,2,4-triazol-1-yl)methyl)-3-(2-(dimethylamino)-ethyl)-1H-indol-2-yl)-*N*,*N*-dimethylbutan-1-amine [rizatriptan-2,2-dimer].

1. Introduction

Rizatriptan benzoate, chemically known as *N*,*N*-dimethyl-5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indole-3-ethanamine monobenzoate, is a selective 5-hydroxytryptamine$_{1B/1D}$ (5-HT$_{1B/1D}$) receptor agonist. Rizatriptan benzoate (brand name: MAXALT®, Merck&Co., Inc., USA) is used for treatment of migraine headache [1]. Rizatriptan benzoate stimulates the 5-HT$_{1B/1D}$ receptor blocking neuronal transmission of vasoactive neuropeptides and reverse dilation of cranial vassels associated with migraine [2].

Literature available was mainly regarding the determination of rizatriptan in human plasma and serum by liquid chromatography [3,4], and liquid chromatography/mass spectrometry [5–7]. Quantitative analysis of formulated drug product by microemulsion electrokinetic capillary chromatography (MEEK) was also reported [8]. The reversed-phase liquid-chromatography behavior of rizatriptan and its potential process impurities [9], development and validation of a specific stability indicating HPLC method for rizatriptan benzoate [10] was also reported. Till date, no mention is available regarding impurities rizatriptan-1,2-dimer and rizatriptan-2,2-dimer in literature to the best of our knowledge.

The daily dose of this drug should not exceed 30 mg. As per International Conference on Harmonisation (ICH) guidelines for impurities in new drug substances, reporting threshold is 0.05% and identification threshold is 0.1% for maximum daily dose ≤ 2 g/day [11]. During analysis of different laboratory batches of rizatriptan benzoate, three impurities were detected in the range of 0.05–0.1%. Hence, these impurities need to be identified, and characterized. A thorough study has been undertaken to characterize these impurities, by spectroscopic techniques. Three impurities were reported in literature [10,12] (Fig. 1), *Imp-1* {1-(4-hydrazinophenyl) methyl-1,2,4-triazole. Dihydrochloride}, *Imp-2* {*N*,*N*-dimethyl-5-(1H-1,2,4-triazol-1-yl-methyl)-1H-indole-3-ethanamine. *N*-oxide} and *Imp-3* {*N*1,*N*1-dimethyl-2-(2-(3-(2-dimethyl aminoethyl)-1H-4-indolyl methyl)-5-(1H-1,2,4-triazol-1-yl-methyl)-1H-3-indolyl)-1-ethanamine} [rizatriptan-2,5-dimer]. Imp-3 was one of the impurities detected. Present work deals with identification, isolation, characterization and also the formation of other two impurities.

2. Experimental

2.1. Sample, chemicals and reagents

Rizatriptan benzoate was synthesized in Chemical Research Department of APL Research Centre (A Division of Aurobindo

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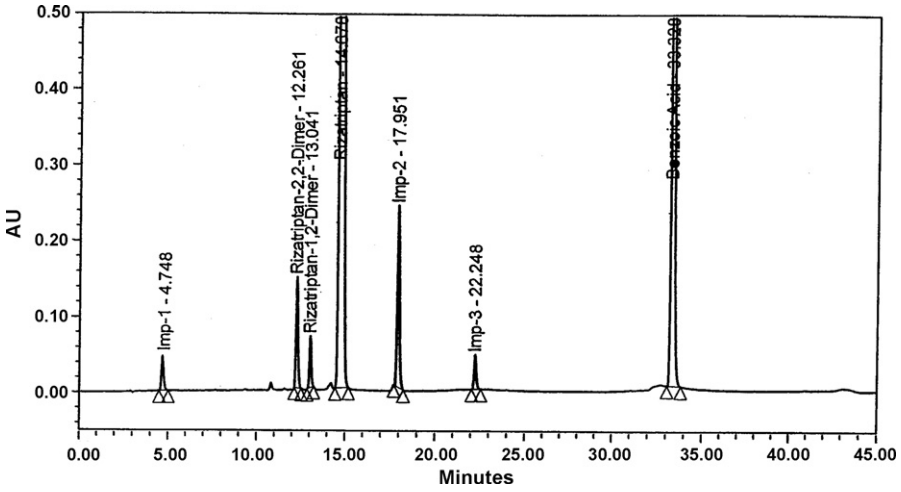


Fig. 1. Representative HPLC chromatogram of rizatriptan benzoate spiked with impurities.

Pharma Ltd.) [Bachupally, Quthubullapur, Hyderabad, India]. Ammonium formate (GR grade), ammonium dihydrogen orthophosphate (AR grade), triethyl amine (AR grade), orthophosphoric acid (~85%, w/w, AR grade), glacial acetic acid (HPLC grade), formic acid (HPLC grade) and acetonitrile (HPLC grade) were obtained from [E. Merck Limited, Mumbai, India]. Distilled water was purified by using Milli-Q water purification system [Millipore, Bedford, MA].

2.2. Analytical LC conditions

Chromatographic separations were performed on high performance liquid chromatograph system with Waters alliance 2695 separations module equipped with 2996 photodiode array detector with *Empower pro* data handling system [Waters Corporation, MILFORD, USA]. Separations were achieved on YMC Pack C8 column with dimensions of 250 mm × 4.6 mm i.d., 5 μm particle size maintained at 45 °C. The mobile phase consisted of 20 mM ammonium dihydrogen orthophosphate solution with 2 ml triethyl amine, pH adjusted to 2.0 ± 0.05 with orthophosphoric acid (A) and acetonitrile (B). Flow rate was kept at 1.0 ml/min and the column eluent was monitored at 225 nm. Pump mode was gradient and was as follows, time (min)/A (v/v): B (v/v); $T_{0.01}$/98:2, T_{15}/90:10, T_{25}/85:15, T_{30}/70:30, T_{40}/60:40, T_{41}/98:2, T_{55}/98:2.

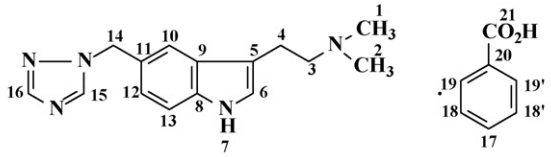
2.3. LC–MS conditions

ESI mass spectra were recorded on PerkinElmer triple quadrupole mass spectrometer [API 2000, PE SCIEX, Foster city, CA] coupled with Shimadzu HPLC [Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan] equipped with SPD 10 A VP UV–vis detector and LC 10 AT VP pumps. Analyst software was used for data acquisition and data processing. The turbo ion spray voltage was maintained at 5.5 kV and temperature was set at 375 °C. The auxiliary gas and sheath gas used was high pure Nitrogen. Zero air was used as Nebuliser gas. LC–MS spectra were acquired from

Table 1
Method validation data for identified impurities.

Validation parameter	Results					
	Rizatriptan-1,2-dimer			Rizatriptan-2,2-dimer		
Repeatability (n = 6, %R.S.D.)						
Method precision	0.8			0.6		
Intermediate precision (n = 6, %R.S.D.)						
Method precision	0.8			0.6		
LOD-LOQ						
Limit of detection (%w/w)	0.005			0.003		
Limit of quantification (%w/w)	0.019			0.015		
Precision at LOQ (%R.S.D.)	2.6			3.3		
Linearity						
Calibration range (μg/mL)	0.077–0.761			0.075–0.760		
Calibration Points	7			7		
Slope	78,300			95,869		
Intercept	144			−57		
Residual Sum of squares	174			135		
Correlation coefficient	0.9999			0.9999		
Accuracy	50% level	100% level	150% level	50% level	100% level	150% level
Added (%w/w)	0.051	0.101	0.151	0.051	0.101	0.151
Recovered (%w/w)	0.051	0.101	0.155	0.051	0.104	0.154
%Recovery	100.0	100.0	102.6	100.0	103.0	102.0
%R.S.D.	2.0	1.0	3.0	1.2	0.6	0.4

RIZATRIPTAN BENZOATE



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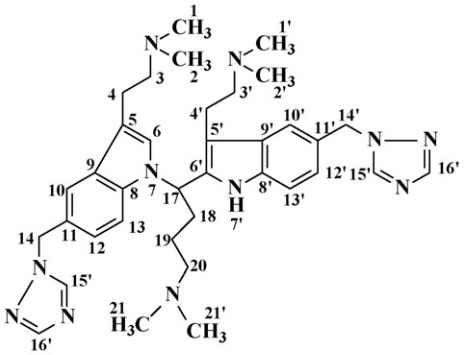

RIZATRIPTAN-2,2-DIMER

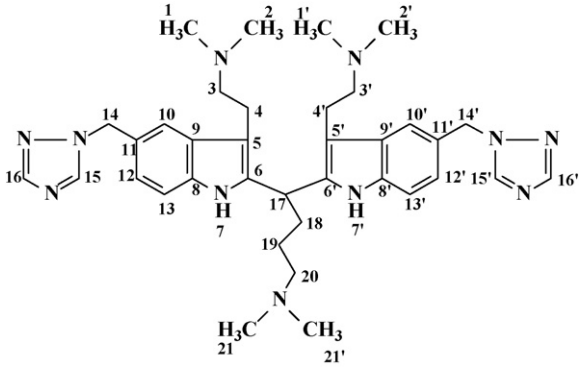

Fig. 2. Chemical structures of rizatriptan benzoate and impurities.

m/*z* 80–1000 in 0.1 amu steps with 2.0 s dwell time. LC–MS analysis of the crude sample was carried out using YMC Pack ODS-A column with dimensions of 250 mm × 4.6 mm, 5.0 μm particle size. The mobile phase consisted of 10 mM ammonium formate solution, pH adjusted to 2.70 with formic acid (A) and acetonitrile (B). Flow rate was 1.0 ml/min. Pump mode was gradient and was as follows, time (min)/A (v/v): B (v/v); $T_{0.01}$/98:2, T_{25}/85:15, T_{35}/70:30, T_{50}/40:60, T_{52}/98:2, T_{60}/98:2.

2.4. Preparative LC conditions

A Shimadzu LC-8A preparative liquid chromatograph equipped with SPD-10A VP, UV–vis detector [Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan] was used.

For isolation of rizatriptan 1,2-dimer, Inertsil ODS (250 mm long × 20 mm i.d.) preparative column packed with 8 μm particle size was employed. The mobile phase consisted of 0.2% glacial acetic acid (A) and acetonitrile (B). Flow rate was 30 ml/min and detection was carried out at 225 nm. Pump mode was gradient and was as follows, time (min)/A (v/v): B (v/v); $T_{0.01}$/100:0, T_{60}/95:05, T_{75}/50:50. The reloading of collected fractions was performed on Hyperprep HS C18 (500 mm long × 30 mm i.d.) preparative column packed with 10 μm particle size. The mobile phase was 0.2% glacial acetic acid:acetonitrile (98:02). Flow rate was 25 ml/min and detection was carried out at 225 nm.

For isolation of rizatriptan 2,2-dimer, Hypersil C-18 (250 mm long × 21.20 mm i.d.) preparative column packed with 8 μm particle size was employed. The mobile phase consisted of 0.2% glacial acetic acid (A) and acetonitrile (B). Flow rate was 30 ml/min and detection was carried out at 225 nm. Pump mode was gradient and was as follows, time (min)/A (v/v): B (v/v); $T_{0.01}$/100:0, T_{60}/95:05, T_{75}/50:50. The reloading of collected fractions was performed on Hypersil C18 (500 mm long × 30 mm i.d.) preparative column packed with 10 μm particle. The mobile phase was water:acetonitrile: 70:30. Flow rate was 25 ml/min and detection was carried out at 225 nm.

2.5. NMR spectroscopy

The ^{1}H and ^{13}C experiments were performed on a Bruker Avance DPX-300 MHz NMR spectrometer [Bruker AG Industries, Faellanden, Switzerland] using deuterated dimethylsulfoxide (DMSO-d_6) as solvent and tetramethylsilane (TMS) as internal standard.

2.6. IR spectroscopy

The IR spectra were recorded in the solid state as KBr pellet using PerkinElmer instrument, model-spectrum one [PerkinElmer Ltd., Beaconsfield, UK].

3. Results and discussion

3.1. Method development and validation

Method development for quantification of related substances was initiated with impurities mentioned in literature [10,12], i.e., Imp-1, Imp-2 and Imp-3. Rizatriptan benzoate samples, spiked with these impurities were co-eluted by gradient elution using different combinations of following chromatographic parameters:

(a) Different stationary phases like C18, C8 and phenyl.
(b) Different buffers like phosphate, sodium and acetate with different pH (2–7).
(c) Different organic modifiers like acetonitrile and methanol.

Satisfactory separations were achieved on YMC Pack C8 column with dimensions of 250 mm × 4.6 mm i.d., 5 μm particle size maintained at 45 °C, using linear gradient programme, 20 mM ammonium dihydrogen orthophosphate solution, pH adjusted to 2.0 ± 0.05 with orthophosphoric acid (A) and acetonitrile (B). When crude samples were analysed, further unknown impurities apart from known impurities were observed with poor resolutions and peak shapes. Addition of triethyl amine to mobile phase improved the peak shapes. Gradient elution, time (min)/A (v/v): B (v/v); $T_{0.01}$/98:2, T_{15}/90:10, T_{25}/85:15, T_{30}/70:30, T_{40}/60:40, T_{41}/98:2, T_{55}/98:2 was optimized for separations. In the optimized conditions, all impurities were separated from each other with resolution greater than 3 and typical relative retention times of Imp-1, rizatriptan-2,2-dimer, rizatriptan-1,2-dimer, Imp-2, Imp-3, and benzoic acid were about 0.33, 0.84, 0.89, 1.22, 1.51, and 2.22, respectively (Rizatriptan retention time at about 14.7 min). A representative HPLC chromatogram of rizatriptan benzoate spiked with

Table 2
Comparative ^{1}H and ^{13}C NMR assignments for Rizatriptan benzoate and its impurities.

Position[a]	Rizatriptan benzoate			Position[a]	Rizatriptan 1,2-dimer			Rizatriptan 2,2-dimer		
	^{1}H ((ppm), multiplicity	^{13}C ((ppm)	DEPT		^{1}H ((ppm), multiplicity	^{13}C ((ppm)	DEPT	^{1}H ((ppm), multiplicity	^{13}C ((ppm)	DEPT
1	2.36 (s, 6H)	44.3	2 × CH_3	1, 1′	2.18 (s, 12H)	45.9, 46.0	4 × CH_3	2.20 (s, 12H)	46.0	4 × CH_3
2				2, 2′						
3	2.69 and 2.88	59.1	CH_2	3, 3′	2.73 (m, 4H)	60.7,60.8	2 × CH_2	2.79 (m, 4H)	61.1	2 × CH_2
4	(2m, 4H)	22.4	CH_2	4, 4′	2.08 (m, 4H)	24.8, 22.9	2 × CH_2	2.26 (m, 4H)	23.1	2 × CH_2
5	–	112.5	–	5, 5′	–	111.2, 114.1	–	–	109.5	–
6	7.53 (s, 1H)	124.5	CH	6, 6′	7.49 (s, 1H)	124.5, 127.2	CH and –	–	126.9	–
7	10.90 (brs, 1H)	–	–	7, 7′	11.18 (s, 1H)	–	–	10.74 (s, 2H)	–	–
8	–	136.7	–	8, 8′	–	136.3	–	–	138.0	–
9	–	126.8	–	9, 9′	–	128.1,128.2	–	–	128.6	–
10	7.19 (s, 1H)	122.3	CH	10, 10′	7.38 (s, 2H)	122.3,122.4	2 × CH	7.38 (s, 2H)	121.7	2 × CH
11	–	135.4	–	11, 11′	–	135.7,136.0	–	–	135.9	–
12	7.03 (d, 1H)	119.3	CH	12, 12′	7.02 and 7.08 (2d, 2H)	119.1,119.5	2 × CH	6.97 (d, 2H)	118.6	2 × CH
13	7.31 (d, 1H)	112.3	CH	13, 13′	7.56 and 7.32 (2d, 2H)	110.7,112.4	2 × CH	7.26 (d, 2H)	111.9	2 × CH
14	5.43 (s, 2H)	54.0	CH_2	14, 14′	5.42 (d, 4H)	53.7,53.8	2 × CH_2	5.41 (s, 4H)	53.9	2 × CH_2
15	7.94 and 8.61	144.6	CH	15, 15′	8.59 (2s, 2H)	144.6	2 × CH	8.58 (s, 2H)	144.5	2 × CH
16	(2s, 2H)	152.3	CH	16, 16′	7.92 (s, 2H)	152.3	2 × CH	7.92 (s, 2H)	152.2	2 × CH
17	7.53 (dd, 1H)	132.1	CH	17	5.83 (m, 1H)	51.5	CH	4.46 (m, 1H)	34.8	CH
18, 18′	7.48 (m, 2H)	128.9	2 × CH	18	1.25 and 1.84 (2m, 4H)	32.3	CH_2	2.12 (m, 2H)	32.1	CH_2
19, 19′	7.95 (d, 2H)	130.0	2 × CH	19		24.0	CH_2	1.34 (m, 2H)	26.3	CH_2
20	–	127.9	–	20	2.08 (m, 2H)	59.1	CH_2	2.12 (m, 2H)	59.3	CH_2
21	–	169.9	–	21, 21′	2.05 (s, 6H)	45.8	2 × CH_3	2.05 (s, 6H)	46.0	2 × CH_3

[a] Refer structures (Fig. 2) for numbering. s, singlet; d, doublet; m, multiplet; dd, double doublet; brs, broad singlet.

impurities is given in Fig. 1. The optimized method is validated as per ICH guidelines [13] and validation data for identified impurities is tabulated in Table 1.

3.2. Detection of impurities

Sample solution equivalent to 0.5 mg/ml of rizatriptan benzoate prepared in diluent (buffer:acetonitrile, 98:2; buffer 0.1% orthophosphoric acid in water) was injected into the analytical LC using the solvent system as described in Section 2.2. Three impurities (about 0.05–0.1%) were detected at relative retention times (RRT) of 0.84, 0.89 and 1.51, respectively with respect to rizatriptan (retention time is about 14.7 min). The same samples were subjected to LC–MS analysis using conditions as described in Section 2.3 to identify the mass of the impurities. The masses of the impurities recorded in positive ion mode were 635, 635, and 469 for rizatriptan-2,2-dimer (RRT-0.84), rizatriptan-1,2-dimer (RRT-0.89) and Imp-3 (RRT-1.51), respectively.

3.3. Isolation of impurities by preparative HPLC

Crude sample was subjected to preparative HPLC as per the conditions described in Section 2.4. Fractions collected were analyzed by analytical LC conditions mentioned in Section 2.2. Fractions of >95% were pooled together; concentrated on rotavopour to remove acetonitrile. The pH of the fractions was adjusted to 9.0 with liquid ammonia. The impurities were extracted into dichloromethane and concentrated on rotavopour to remove the solvent. Rizatriptan-1,2-dimer was obtained as an off-white gummy mass with chromatographic purity of 94.0%, and rizatriptan-2,2-dimer was obtained as an off-white powder with chromatographic purity of 95.0%.

3.4. Structural elucidation

3.4.1. Rizatriptan-1,2-dimer

ESI mass spectrum of rizatriptan-1,2-dimer in positive ion mode showed a molecular ion peak at *m*/*z* 636 [$(MH)^+$] indicating the molecular weight of the compound as 635. In the ^{1}H NMR spectrum of this impurity, all signals corresponding to rizatriptan protons were observed with double integration, but signals corresponding to indole CH proton (7.40 ppm as singlet) and NH proton (11.18 ppm as exchangeable singlet) were integrated to one proton each. Additional signals were observed at 1.25 ppm (–CH_2), 1.84 ppm (–CH_2), 2.05 ppm [$N(CH_3)_2$], 2.08 ppm (–CH_2) and 5.83 ppm (triplet of –CH signal). In the ^{13}C NMR spectrum, additional signals were observed at 24.0 (CH_2), 24.8 (CH_2), 32.3 (CH_2), 46.0 (CH_3), 51.5 (CH) and 60.8 (CH_2). Signal corresponding to indole CH is present at 124.5 ppm. It is concluded from the ^{1}H NMR and ^{13}C NMR values that *N*,*N*-dimethyl butanal diethyl acetal which is used as reagent in the synthesis of rizatriptan is reacting at indole 2-position of one rizatriptan molecule and at NH position of another rizatriptan molecule, which is forming a dimer with loss of ethanol. The above spectral data confirms the impurity as 4-(5-((1H-1,2,4-triazol-1-yl)methyl)-3-(2-(dimethylamino)ethyl)-1H-indol-1-yl)-4-(5-((1H-1,2,4-triazol-1-yl)methyl)-3-(2-(dimethylamino)ethyl)-1H-indol-2-yl)-*N*,*N*-dimethylbutan-1-amine (rizatriptan-1,2-dimer) with molecular formula $C_{36}H_{49}N_{11}$ and molecular weight 635.

3.4.2. Rizatriptan-2,2-dimer

ESI mass spectrum of rizatriptan-2,2-dimer in positive ion mode showed a molecular ion peak at *m*/*z* 636 [$(MH)^+$] indicating the molecular weight of the compound as 635. In the ^{1}H NMR spectrum of this impurity, all signals corresponding

Table 3
FT-IR spectral data for Rizatriptan benzoate and impurities.

S. no.	Compounds	IR (KBr) absorption bands (Cm^{-1})
1	Rizatriptan benzoate	3430 (m) NH stretch; 2938, 2888 (w) CH_3, CH_2 stretch; 1608, 1505 (s) C=C and C=N stretch; 1569 (s) NH bend; 1446, 1377 (s) CH_2, CH_3 bend; 1271, 1140, 1016 (m) C–N stretch; 888, 853, 836, 794, 772 (m) CH & CN out of plane bend.
2	Rizatriptan–1,2-dimer	3384 (m) NH stretch; 2945, 2863, 2826, (m) CH_3, CH_2 stretch; 1650, 1508 (m) C=C and C=N stretch; 1559 (w) NH bend; 1459, 1364 (s) CH_2, CH_3 bend; 1273, 1137, 1017 (s) C–N stretch; 876, 856, 803, 766 (m) CH & CN out of plane bend.
3	Rizatriptan-2,2-dimer	3257 (m) NH stretch; 2945, 2863, 3826 (m) CH_3, CH_2 stretch; 1647, 1508 (m) C=C and C=N stretch; 1563 (m) NH bend; 1462, 1372 (s) CH_2, CH_3 bend; 1274, 1138, 1017 (s) C–N stretch; 858, 810, 766 (m) CH & CN out of plane bend.

w: weak; s: strong; m: medium.

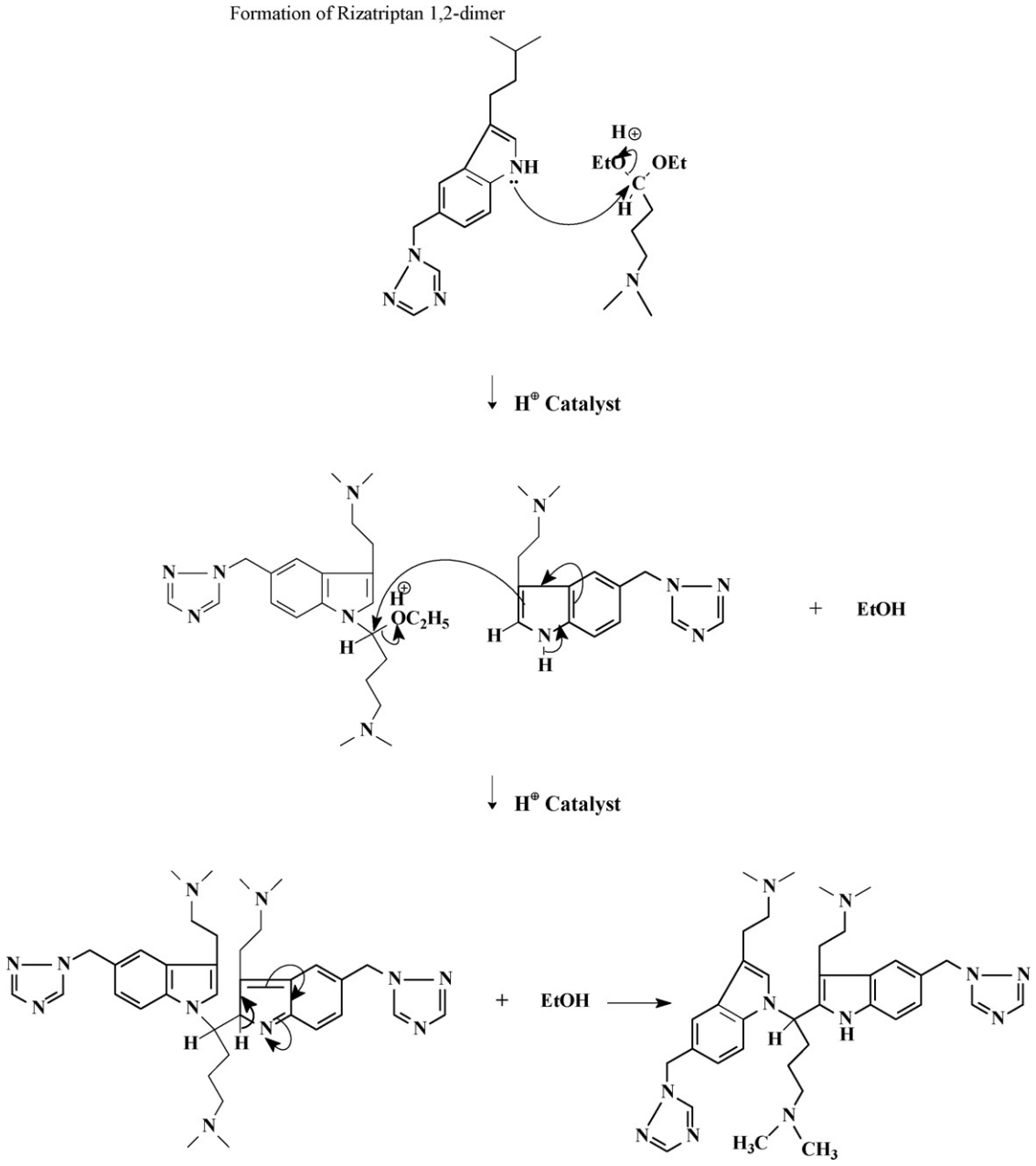

Fig. 3. Mechanism for formation of impurities.

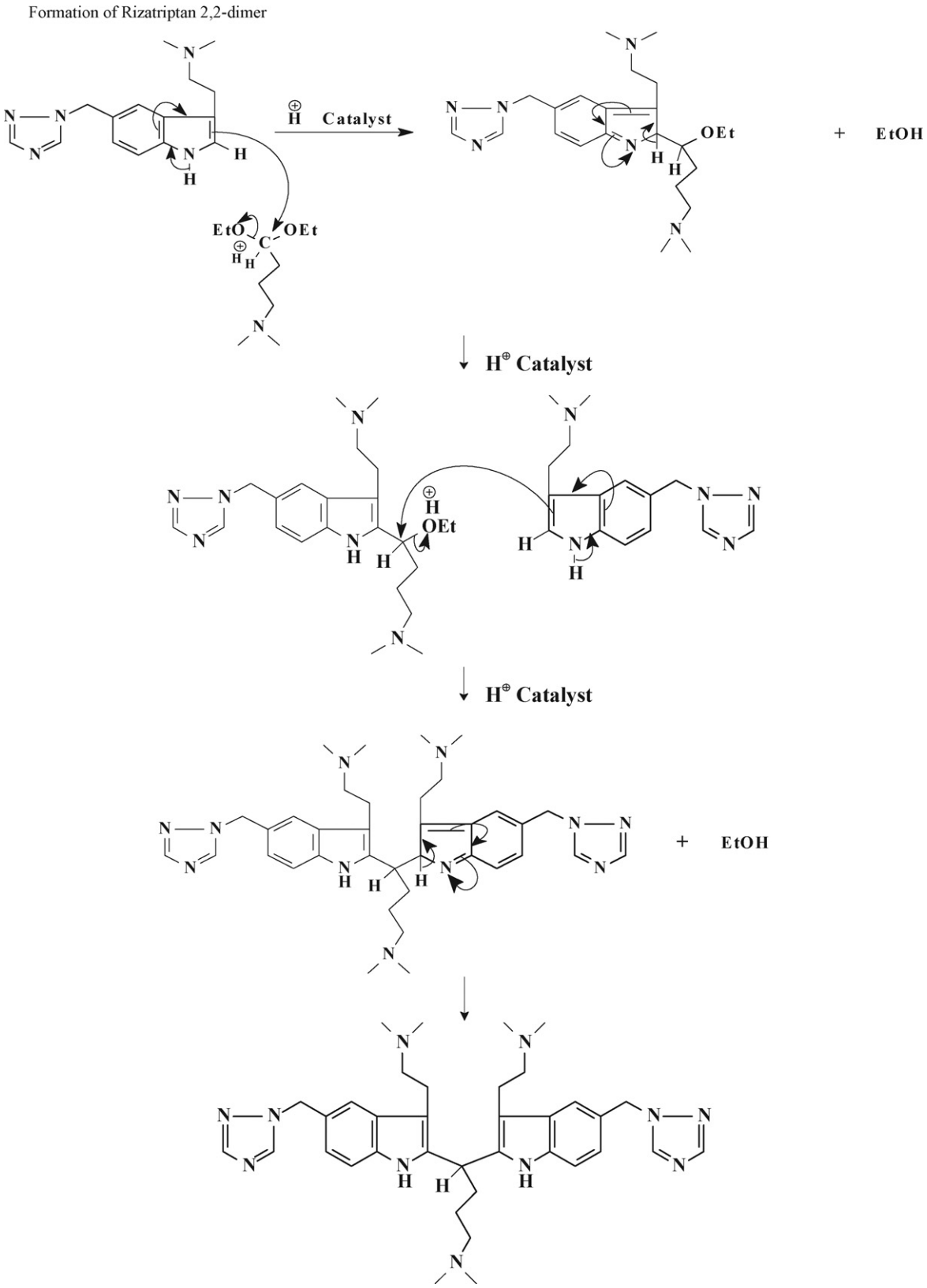

Fig. 3. (*Continued*).

to rizatriptan protons were observed with double integration, but signals corresponding to indole CH proton at 7.50 ppm is disappearing, and additional signals were observed at 1.34 ppm (two protons of CH_2), 2.05 ppm [six protons of $N(CH_3)_2$], 2.12 ppm (four protons of two CH_2 groups) and 4.46 ppm (triplet of CH signal). Indole NH signals at 10.74 ppm with double integration are also present. In the ^{13}C NMR spectrum, signal corresponding to indole CH proton at 124.5 ppm disappeared, and additional

signals were observed at 26.3 (CH_2), 32.1 (CH_2), 34.8 (CH), 46.0 (CH_3) and 61.1 (CH_2). It is concluded from the ^1H NMR and ^{13}C NMR values that *N*,*N*-dimethyl butanal diethyl acetal which is used as reagent in the synthesis of rizatriptan is reacting at indole position of two rizatriptan molecules forming a dimer with loss of ethanol. The above spectral data confirms the impurity as 4,4-bis-(5-((1H-1,2,4-triazol-1-yl)methyl)-3-(2-(dimethylamino)-ethyl)-1H-indol-2-yl)-*N*,*N*-dimethylbutan-1-amine (rizatriptan-2, 2-dimer) with molecular formula $C_{36}H_{49}N_{11}$ and molecular weight 635.

The chemical structures of rizatriptan benzoate and impurities are given in Fig. 2. ^1H and ^{13}C NMR spectral assignments for rizatriptan benzoate and for impurities are given in Table 2. FT-IR spectral assignments are given in Table 3.

3.5. Formation of impurities

Rizatriptan is synthesized by carrying out Fischer indole reaction using 4-(dimethylamino)butanal diethyl acetal as a reagent in the presence of aqueous sulphuric acid. During the Fischer indole reaction [14], the product rizatriptan further undergoes dimerization reaction through electrophilic substitution either at 2-position or 1-position of rizatriptan with 4-(dimethylamino)butanal diethyl acetal to form rizatriptan 1,2-dimer and rizatriptan 2,2-dimer. The mechanism for formation of impurities is given in Fig. 3.

4. Conclusion

Three process impurities, observed during regular monitoring by HPLC in rizatriptan benzoate bulk drug samples were identified by LC–MS. Imp-3 [rizatriptan 2,5-dimer] is cited in literature, other two unknown impurities were isolated and characterised by spectroscopic techniques viz., IR, NMR, and MS. The structures for these impurities were confirmed based on the spectral data.

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