Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
32 (2003) 29–39
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Impurity profile study of loratadine☆

K.V.S.R. Krishna Reddy [a], J. Moses Babu [a], Y. Ravindra Kumar [a], S. Vishnu Vardhan Reddy [a], M. Kishore Kumar [b], S. Eswaraiah [b], K. Rama Subba Reddy [b], M. Sayanarayana Reddy [b], B. Vijaya Bhaskar [b], P.K. Dubey [c], K. Vyas [a,*]

[a] *Department of Analytical Research, Discovery Research Division, Dr Reddy's Laboratories Ltd., Bollaram Road, Miyapur, Hyderabad 500 050, India*
[b] *Dr Reddy's Laboratories Ltd. Bulk Actives-III, Bollaram, Hyderabad 500 050, India*
[c] *Department of Chemistry, J.N.T. University, Kukatpally, Hyderabad 500 872, India*

Received 18 November 2002; received in revised form 24 January 2003; accepted 24 January 2003

Abstract

Three unknown impurities in loratadine bulk drug at levels below 0.1% (ranging from 0.05 to 0.1%) were detected by a simple isocratic reversed-phase high performance liquid chromatography (HPLC). These impurities were isolated from mother liquor sample of loratadine using reversed-phase preparative HPLC. Based on the spectral data (IR, NMR and MS) the structures of these impurities were characterized as 11-(*N*-carboethoxy-4-piperidylidene)-6,11-dihydro-5H-benzo(5,6) cyclopenta(1,2-b)-pyridine (I), 8-bromo-11-(*N*-carboethoxy-4-piperidylidene)-6,11-dihydro-5H-benzo(5,6) cyclopenta (1,2-b)-pyridine (II) and 8-chloro-11-(*N*-carboethoxy-4-piperidylidene)-5H-benzo(5,6) cyclopenta (1,2-b)-pyridine (III). The synthesis of these impurities was discussed.

Keywords: Loratadine; Impurities; Spectroscopy; Identification; Characterization and synthesis

1. Introduction

Loratadine, 4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-1-piperdine carboxylic acid ethylester, is a long acting non-sedating histaminic agent that was developed for the treatment of seasonal allergic rhinitis [1] whose anti-histaminic action is more effective than the other anti-histaminic drugs available commercially. A few chromatographic methods where the authors reported validated LC methods for the quantitative determination of loratadine and its related substances [2–5] and chromatographic-spectroscopic methods [6–8] have been described for the determination of loratadine and its metabolites.

During the analysis of different laboratory batches of loratadine, three unknown impurities were detected consistently in almost all the batches, whose area percentage ranged from 0.05

☆ DRL publication no. 272.

* Corresponding author. Fax: +91-40-304-5438.
E-mail address: vyask@drreddys.com (K. Vyas).

0731-7085/03/$ - see front matter
doi:10.1016/S0731-7085(03)00056-6

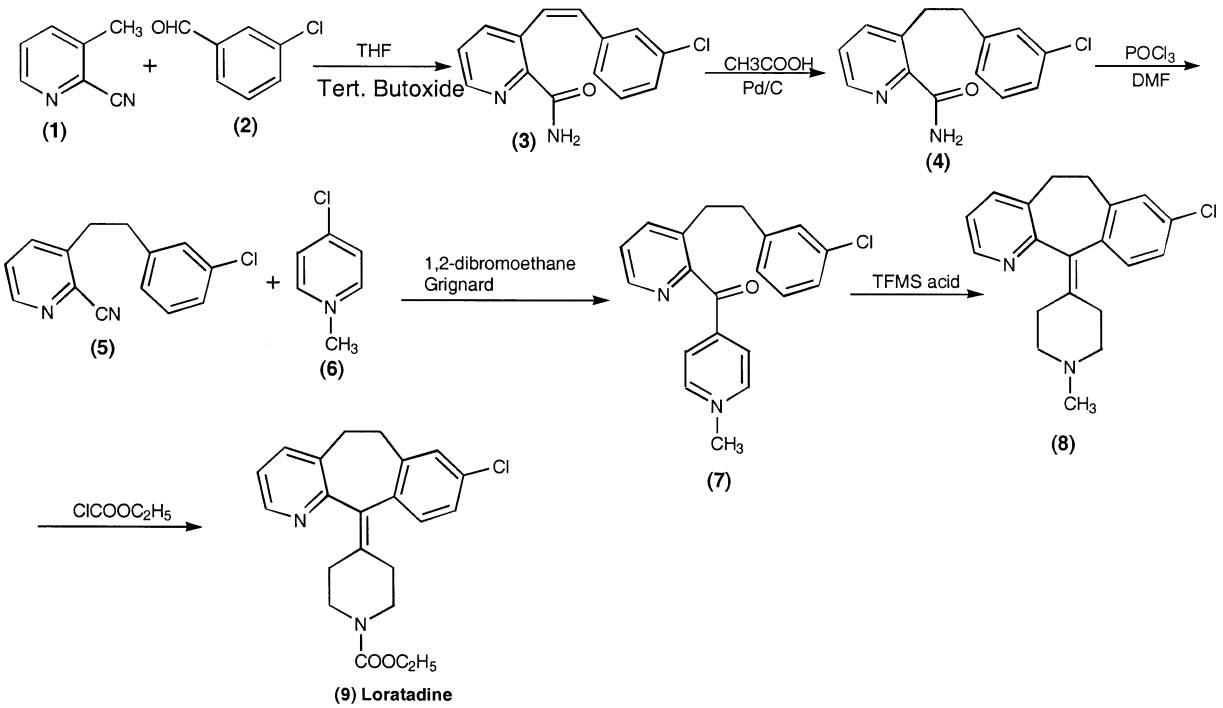


Fig. 1. Scheme for the synthesis of loratadine.

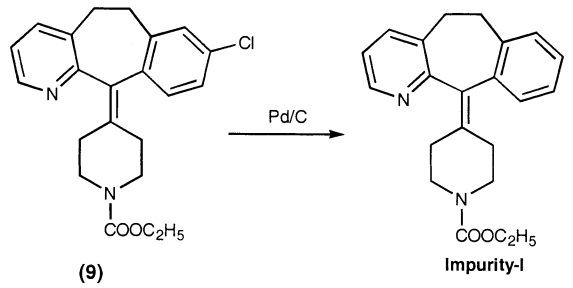


Fig. 2. Scheme for the synthesis of impurity I.

to 0.1%, by a simple isocratic reversed-phase LC method. A comprehensive study has been undertaken to isolate and characterize these impurities by spectroscopic techniques. The impurity profile study has to be carried out for any final product to identify and characterize all the unknown impurities that are present at a level of even below 0.05% [9]. The requirement of identifying and characterizing the impurities in the final product is extremely necessary in the wake of stringent purity requirements from the regulatory authorities. This paper not only describes the isolation and characterization of three impurities that are present in the range of 0.05–0.1% in the bulk drug of loratadine but also explains the formation of these impurities.

2. Experimental

2.1. Samples

The investigated samples of loratadine bulk material (B. No. Loratadine-Pharma Grade) and mother liquor samples (Lora-MLs) were obtained from Dr Reddy's Laboratories Ltd. Bulk Actives-III, Hyderabad, India.

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. An in-house LC

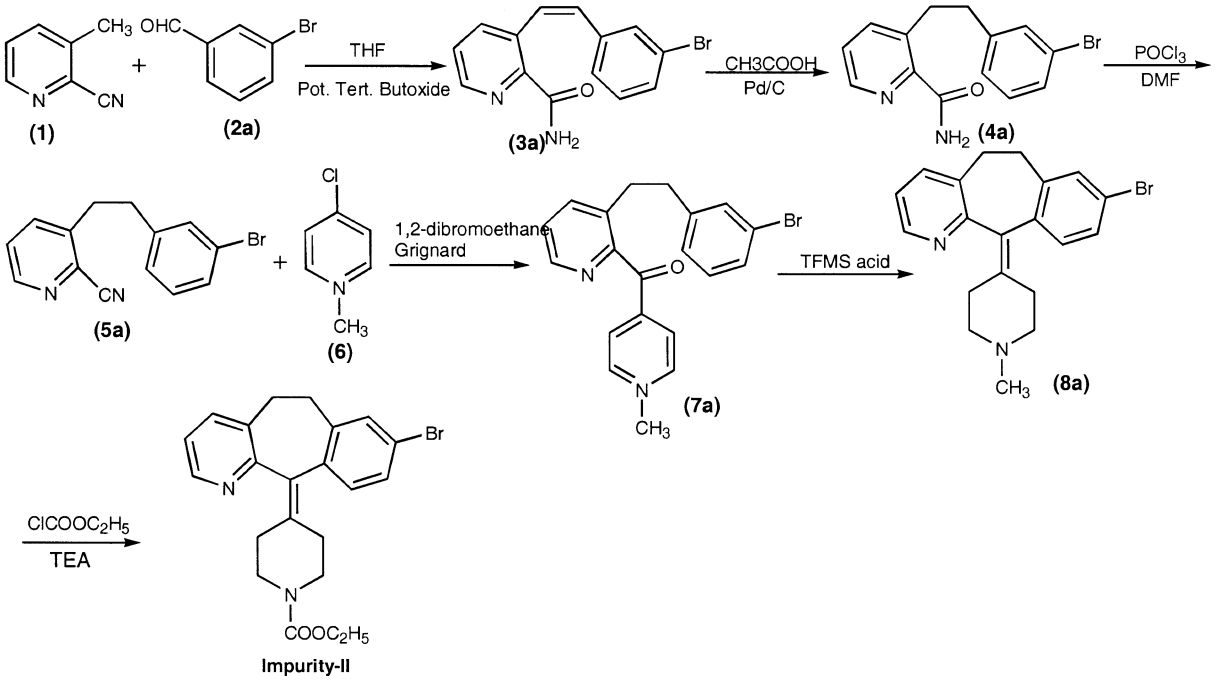


Fig. 3. Scheme for the synthesis of impurity II.

method was developed for the analysis of loratadine and its intermediates, where a C18 column (Hichrom-RPB, 250 × 4.6 mm i.d., 5 μ particle size, Hichrom Ltd., UK) with a mobile phase consisting of a mixture of 0.01 M KH_2PO_4 and acetonitrile in the ratio of 40:60 (v/v; pH 3.5) was used with UV-detection at 240 nm at a flow rate of 1.0 ml/min for the resolution of all impurities. The data was recorded using WATERS MILLENNIUM 32 software. This LC method was able to detect these impurities which ranged from 0.05 to 0.1% in the presence of parent compound.

2.3. High performance liquid chromatography (preparative)

A Shimadzu preparative HPLC equipped with LC-8A pump, SCL-8A System controller, SPD-6AV UV–Vis detector, FCV-100B Fraction collector and Rheodyne Injector Model 7725i with 2.0 ml loop. The data was collected and processed using Shimadzu CR7A chromatopak. A 250 × 10 mm i.d. column packed with 5 μ Hichrom-C18 (Hichrom Ltd., UK) was employed for separation. The mobile phase consisted of 0.01 M KH_2PO_4 and acetonitrile in the ratio of 50:50 (v/v; pH 3.5). The flow rate was set at 3.0 ml/min. Detection was carried out at 240 nm.

2.4. Mass spectrometry

Mass spectra were obtained using HP5989 with an electron energy set to 70 eV. The samples were introduced via the particle beam inlet using a LC pump (HP 1050 series) and a manual injector (Rheodyne model 7125i). The source manifold and quadrupole temperatures were maintained at 250 and 100 °C, respectively.

2.5. NMR spectroscopy

The ^{13}C and DEPT NMR experiments were performed on a 200 MHz instrument Varian Gemini model 2000 and the ^{1}H and 2D experiments (COSY, gHSQC and gHMBC) were performed on Mercury plus 400 MHz at 25 °C in $CDCl_3$.

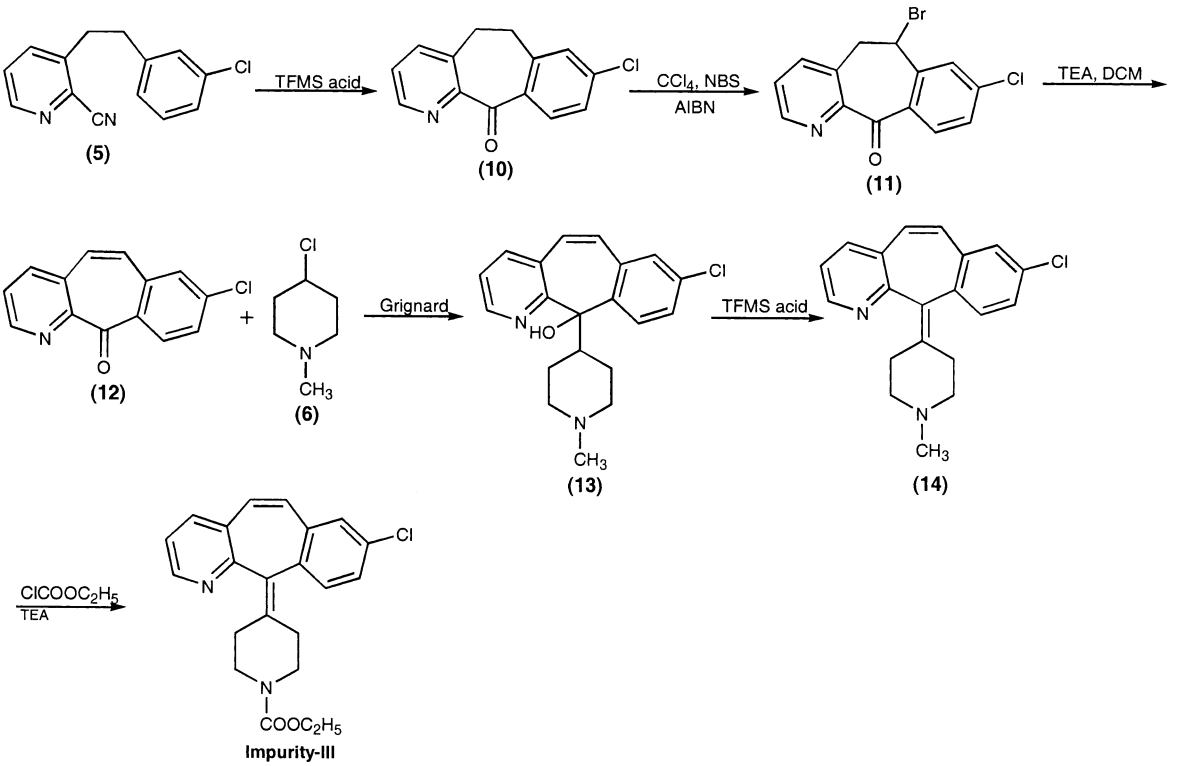


Fig. 4. Scheme for the synthesis of impurity III.

The ^{1}H chemical shift values were reported on the δ-scale in ppm, relative to TMS ($\delta = 0.00$) and the ^{13}C chemical shift values were reported relative to $CDCl_3$ ($\delta = 77.0$ ppm) as internal standards, respectively.

2.6. FT-IR spectroscopy

The IR spectra for loratadine, impurity I, II and III were recorded in the solid state as KBr dispersion using Perkin–Elmer 1650 FT-IR spectrophotometer.

2.7. Melting point determination

Melting points of all the impurities were determined on a Buchi digital melting point instrument Model 535 in open capillary tubes and were uncorrected.

2.8. Synthesis of impurities

2.8.1. Synthesis of loratadine

The scheme for the synthesis of loratadine is shown in Fig. 1.

2.8.2. Synthesis of impurity I

The scheme for the synthesis of impurity I is shown in Fig. 2.

Loratadine (**9**) was subjected to hydrogenation using Pd/C in the presence of methanol as solvent. During this reaction loratadine undergoes dehalogenation and gets converted to impurity I.

2.8.3. Synthesis of impurity II

The scheme for the synthesis of impurity II is shown in Fig. 3.

One of the starting materials used in the synthesis of loratadine (**9**) was 2-cyano-3-methyl pyridine (**1**). This (**1**) was allowed to react with 3-bromo benzaldehyde (**2a**) in the presence of

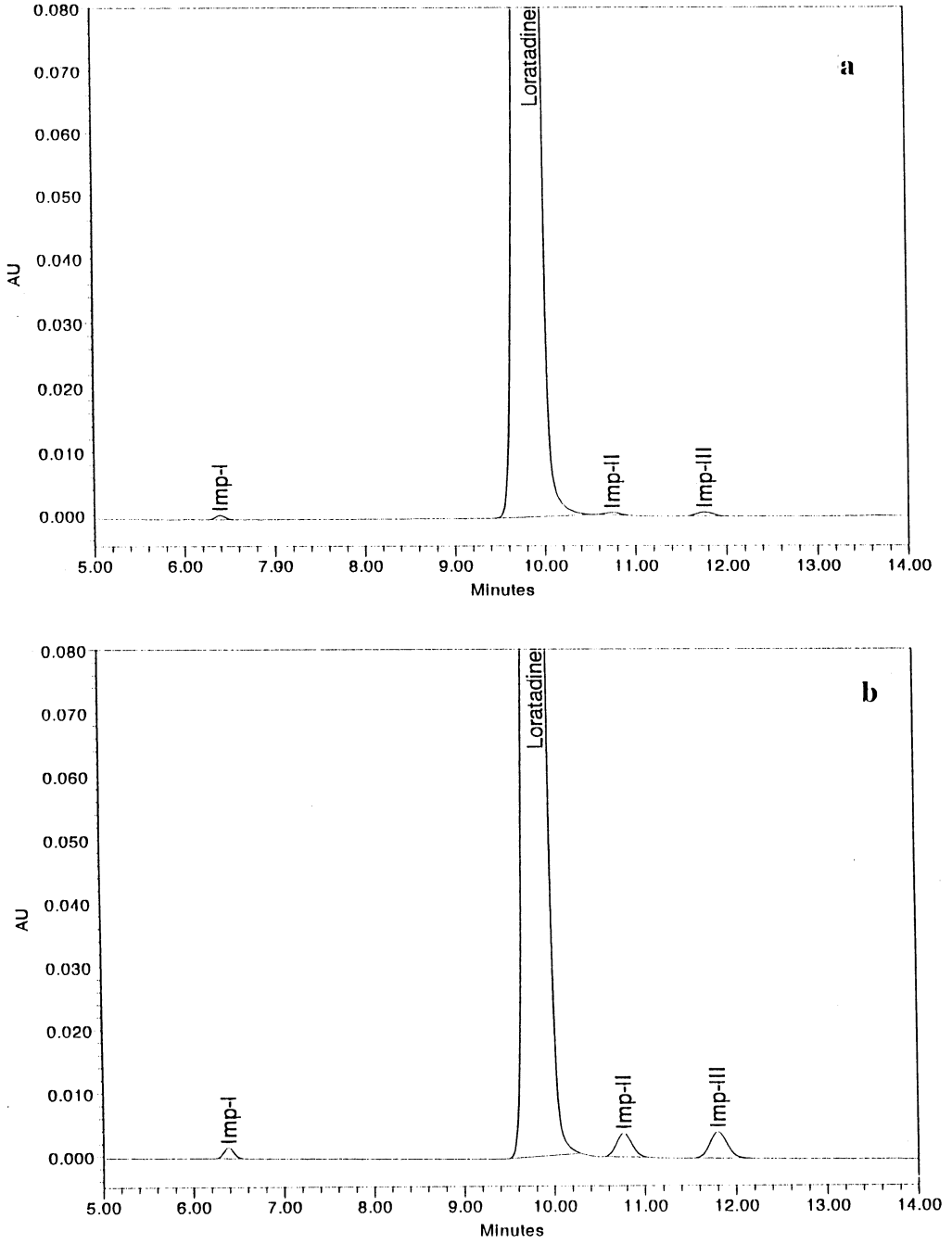
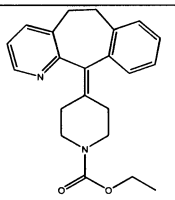
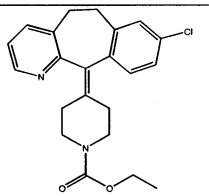
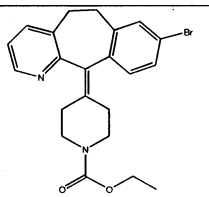
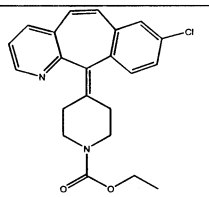


Fig. 5. (a) HPLC chromatogram of loratadine bulk drug. (b) HPLC chromatogram of loratadine bulk drug spiked with impurities.

tetrahydrofuran and potassium tertiary butoxide to yield its corresponding product (**3a**). This (**3a**) was subjected to hydrogenation in Pd/C in the presence of acetic acid to get (**4a**). This (**4a**) was allowed to react with phosphorous oxy chloride in the presence of dimethylformamide to yield (**5a**).

Table 1

S.No.	~Ret. Time (min.)		Compound	Structure	LC/MS Analysis		Nature
	Anal*	Prep**			~RT	M+	
01	6.0	16 – 17	Impurity I		16	348	Process-related
02	10.0	27 - 28	Loratadine		22	382	-
03	11.0	24 - 25	Impurity II		25@	426	Process-related
04	12.0	32 - 33	Impurity III		23	380	Process-related

* Analytical LC
** Preparative LC
@ Impurity II eluted after impurity III in LC/MS conditions (refer sec. 3.2).

This (**5a**) was allowed to couple with 4-chloro-*N*-methyl pyridine (**6**) to get its corresponding product (**7a**). This (**7a**) undergoes cyclyzation in the presence of trifluoromethane sulfonic acid to yield (**8a**). This (**8a**) upon reaction with ethylchloroformate in the presence of triethyl amine yielded impurity II.

2.8.4. Synthesis of impurity III

The scheme for the synthesis of impurity III is shown in Fig. 4.

One of the intermediates (**5**) was allowed to react with trifluoro methane sulfonic acid to covert into its subsequent derivative (**10**). This (**10**) was allowed to react with *N*-bromosuccinamide and *Azo iso* butyryl nitrile in the presence of CCl_4 to yield (**11**). This (**11**) in the presence of TEA and DCM yielded (**12**). This (**12**) on reaction with *N*-methyl-4-chloro piperdine (**6**) in the presence of Grignard gave (**13**). This (**13**) in the presence of trifluoro methane sulfonic acid yielded (**14**). This (**14**) was allowed to react with of chloroehyl

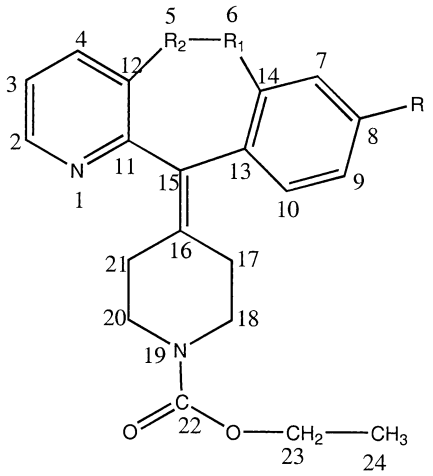


Fig. 6. Chemical structures of loratadine and impurities.

formate in the presence of triethyl amine yielded impurity III.

3. Results and discussions

3.1. Detection of impurities I, II and III

A typical analytical LC chromatogram (Fig. 5a) of a laboratory batch of loratadine bulk drug recorded using the LC method as described in Section 2.2. The target impurities under study are marked as Imp-I, Imp-II and Imp-III. Retention times and structures of these impurities and loratadine are shown in Table 1. These impurities were not only isolated from the mother liquor sample of loratadine on preparative LC but also synthesized for spectroscopic studies.

Table 2
FT-IR, mass spectral and melting range data of loratadine, impurities I, II and III

Serial number	Compound	IR (KBr)	MS data	Melting point (°C)
1	Impurity I	2977 (aliphatic C–H stretching), 1690 (C=O stretching), 1603 (C=C stretching), 1526 and 1379 (aliphatic C–H bending), 1432 (C–N stretching), 1227 and 1117 (C–O stretching), 789 and 757 (aromatic C–H bending)	*m/z* (EI; rel. int., %) 258(60), 246(84), 232(100), 217(46), M^+ 348(82)	139–177
2	Impurity-II	2902 (aliphatic C–H stretching), 1702 (C=O stretching), 1583 (C=C stretching), 1473 and 1385 (aliphatic C–H bending), 1433 (C=N stretching), 1228 (C–O stretching), 1118 (C–Br stretching), 879, 832 (aromatic C–H bending)	*m/z* (EI; rel. int., %) 338(40), 324(52), 310(67), 245(100), 230(63), 216(40), M^+ 426(97), M^{2+} 428(100)	149–152
3	Impurity-III	2924 (aliphatic C–H stretching), 1702 (C=O stretching), 1582 (C=C stretching), 1474 and 1386 (aliphatic C–H bending), 1435 (C=N stretching), 1230 (C–O stretching), 1117 (C–Cl stretching), 824, 766 (aromatic C–H bending)	*m/z* (EI; rel. int., %) 290(41), 278(52), 264(56), 243(60), 229(45), M^+ 380(100)	151–154
4	Loratadine	2983 (aromatic C–H stretching), 2883 (aliphatic C–H stretching), 1703 (C=O stretching), 1580 and 1560 (C=C stretching), 1474, 1323 (aliphatic C–H bending), 1435 (C=N stretching), 1225 (C–O stretching), 1116 (C–Cl stretching), 830, 764 (aromatic C–H bending)	*m/z* (EI; rel. int., %) 292(23), 280(20), 266(28), 245(20), M^+ 382(100), M^{2+} 384(35)	133-136

Table 3
Comparative ^{1}H NMR assignments for loratadine and its impurities

Position[a]	^{1}H	Loratadine		Impurity-I		Impurity-II		Impurity-III	
		δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
2	1H	8.4	d, 5.0	8.6	d, 5.0	8.38	d, 5.2	8.5	d, 5.2
3	1H	7.1	m	7.6	t, 6.8	7.1	dd, 8.0,4.8	7.20	dd, 8.0,4.4
4	1H	7.4	d, 7.8	7.9	d, 7.9	7.4	dd, 8.0,2.0	7.6	dd, 7.6,1.6
5	Ha	3.2–3.5	m	3.0	d, 11.6	2.8	m	6.9	d, 11.6
	Hb	2.7–2.9	m	3.5	m	3.34	m	–	–
6	Ha	3.2–3.5	m	2.8	d, 11.6	2.8	m	6.8	d, 1.6
	Hb	2.7–2.9	m	3.5	m	3.4	m	–	–
7	1H	7.0–7.3	m	7.1–7.3	br,m	7.3	d,2.4	7.3	d, 1.6
8	–	–	–	7.1–7.3	br,m	–	–	–	–
9	1H	7.0–7.3	m	7.1–7.3	br,m	7.23	dd, 8.0,2.4	7.3	dd, 8.0,2.4
10	1H	7.0–7.3	m	7.4	d,6.4	7.1	d, 8.0	7.2	d, 7.6
11	–	–	–	–	–	–	–	–	–
12	–	–	–	–	–	–	–	–	–
13	–	–	–	–	–	–	–	–	–
14	–	–	–	–	–	–	–	–	–
15	–	–	–	–	–	–	–	–	–
16	–	–	–	–	–	–	–	–	–
17	Ha	2.1	m	2.1	br, m	2.3	m	2.1	dt, 4.4,14.8
	Hb	2.6	m	2.8	br,m	2.8	m	2.3	m
18	Ha	3.8	m	3.2	m	3.2	m	3.7	m
	Hb	3.1	m	4.1	br,m	3.9	br,m	3.0	m
20	Ha	3.8	m	3.2	br,m	3.2	m	3.7	m
	Hb	3.1	m	3.9	br,m	3.8	m	3.0	m
21	2H	2.2–2.6	m	3.9	br,m	2.34	m	2.29	m
22	–	–	–	–	–	–	–	–	–
23	2H	4.1	q, 7.3	4.1	m	4.1	q, 7.2	4.1	q, 7.2
24	3H	1.2	t, 7.2	1.2	t, 7.2	1.3	t, 7.2	1.2	t, 7.6

d, doublet; q, quartet; t, triplet; m, multiplet; br,m, broad multiplet; dd, doublet of a doublet; dt, doublet of a triplet; J, coupling constant.

[a] Refer structural formula for numbering (Fig. 6).

3.2. LC/MS analysis

LC/MS analysis of mother liquor sample of loratadine was carried out on HP5989 with ionization electron beam energy of 70 eV. The sample was introduced into the source through LC with the help of a particle beam interface. An Hichrom C18 column (250 × 4.6 mm) at a flow rate of 0.4 ml/min. with a mobile phase consisting of 0.025 M CH_3COONH_4 (pH 4.0) and acetonitrile in a ratio of 30:70 v/v was used for the LC/MS analysis. The source manifold and quadrupole temperatures were maintained at 250 and 100 °C, respectively. The retention times of loratadine and the target impurities are given in Table 1. Based on the mass spectral information, tentative structures of all the three impurities were proposed. To confirm the proposed structures, mother liquor sample of loratadine containing all the target impurities was subjected preparative LC to isolate the impurities in adequate quantities in pure form and to carry out further spectroscopic experiments.

3.3. Isolation of the impurities by preparative HPLC

A simple reversed-phase solvent system discussed under Section 2.3 was used for isolating these impurities. The retention times of loratadine

Table 4
Comparative ^{13}C and DEPT NMR assignments for loratadine and its impurities

Position[a]	Loratadine		Impurity-I		Impurity-II		Impurity-III	
	^{13}C	DEPT	^{13}C	DEPT	^{13}C	DEPT	^{13}C	DEPT
2	146.4	CH	145.6	CH	146.6	CH	149.1	CH
3	121.9	CH	124.2	CH	122.2	CH	121.7	CH
4	137.1	CH	139.1	CH	137.4	CH	135.9	CH
5	31.4	CH_2	31.5	CH_2	31.4	CH_2	129.2	CH
6	31.1	CH_2	30.3	CH_2	31.6	CH_2	131.0	CH
7	125.8	CH	128.8	CH	131.8	CH	128.0	CH
8	133.9	–	127.0	CH	121.0	–	133.1	–
9	130.2	CH	131.0	CH	129.0	CH	130.3	CH
10	128.7	CH	128.7	CH	130.7	CH	128.4	CH
11	155.1	–	150.9	–	156.8	–	154.2	–
12	137.2	–	138.0	–	139.8	–	132.4	–
13	132.5	–	136.4	–	139.8	–	132.4	–
14	133.0	–	129.6	–	138.1	–	129.7	–
15	137.4	–	137.2	–	134.1	–	135.8	–
16	139.2	–	144.6	–	137.4	–	138.3	–
17	30.4	CH_2	31.2	CH_2	30.5	CH_2	29.7	CH_2
18	44.5	CH_2	43.8	CH_2	44.7	CH_2	44.8	CH_2
20	44.5	CH_2	43.8	CH_2	44.7	CH_2	44.7	CH_2
21	30.2	CH_2	30.6	CH_2	30.7	CH_2	29.8	CH_2
22	156.7	–	155.2	–	155.4	–	155.2	–
23	60.9	CH_2	61.3	CH_2	61.3	CH_2	61.2	CH_2
24	14.4	CH_3	14.5	CH_3	14.7	CH_3	14.6	CH_3

[a] Refer structural formula for numbering Fig. 6.

and impurities are shown in Table 1. Collected fractions of these impurities were pooled together and kept in the refrigerator. All the fractions of impurities isolated were concentrated under high vacuum on a Buchi Rotavapor Model R124 to strip off the organic solvent. The remaining aqueous layer was subjected to solvent–solvent extraction with chloroform to extract the compounds into organic layer. The chloroform fractions were pooled together and concentrated on Rotavapor under vacuum. The chromatographic purity of these impurities I, II and III was tested by analytical LC separately before and after concentration and found to be 94.5, 97.2 and 98.3%, respectively, indicating that these impurity fractions are quite stable during and after isolation. The isolated solids obtained from concentrated fractions of impurities were used to generate spectral data.

The details of the elucidation of structures of these impurities is presented in the following sections.

3.4. Structural elucidation of impurities

3.4.1. Structural elucidation of impurity I

The EI mass spectrum of impurity I exhibited molecular ion at *m/z* 348 atomic mass units (amu) which was less by 34 amu than that of loratadine. Interestingly, the characteristic M+2 molecular ion which was due to the presence of one chlorine atom in loratadine was absent in the mass spectrum of impurity I. In addition to this observation, the characteristic absorption band at around 1100 per cm due to C–Cl stretching in the FT-IR spectrum of loratadine was absent in the spectrum of impurity I. Further, an additional signal which was due to one proton was observed

in the ^{1}H NMR spectrum of impurity I and this was absent in the ^{1}H NMR spectrum of loratadine. This observation was supported by the appearance of one additional methine signal in the DEPT spectrum of impurity I which was not observed in the DEPT spectrum of loratadine.

Based on the above spectral data the molecular formula of impurity I was confirmed as $C_{22}H_{23}N_2O_2$ and the corresponding structure was characterized as 11-(*N*-carboethoxy-4-piperidylidene)-6,11-dihydro-5H-benzo(5,6) cyclopenta (1,2-b)-pyridine.

3.4.2. Structural elucidation of impurity II

The EI mass spectrum of impurity II exhibited molecular ion at *m*/*z* 426 amu with a characteristic M+2 molecular ion at 428 with an equal intensity. The molecular ion of impurity II was more by 44 amu than that of loratadine. This observation indicates that the chlorine atom in loratadine is replaced by bromine as observed form the mass spectral data.

In the ^{13}C NMR spectrum of loratadine, the quaternary carbon signal at δ 133.89 ppm which was assigned for carbon attached to chlorine, shifted to 120.95 ppm in impurity II. This shift can be rationalized in terms of higher inductive effect (-I) of chlorine.

Based on this data, the molecular formula of impurity II was confirmed as $C_{22}H_{23}BrN_2O_2$ and the corresponding structure was characterized as 8-bromo-11-(*N*-carboethoxy-4-piperidylidene)-6,11-dihydro-5H-benzo(5,6) cyclopenta (1,2-b)-pyridine.

3.4.3. Structural elucidation of impurity III

The EI mass spectrum of impurity III exhibited molecular ion at *m*/*z* 380 amu which was 2 amu less than that of loratadine with a characteristic isotopic abundance of chlorine.

The ^{1}H NMR spectrum of impurity III showed two signals in the aromatic region at δ 6.91 and 6.84 ppm which were integrated for one proton each. The signals at δ 31.35 and 31.11 in the ^{13}C NMR spectrum of spectrum of loratadine were absent in the ^{13}C NMR spectrum of impurity III. These two signals were shifted to the aromatic region in impurity III and appeared at 129.23 and 130.95 ppm. The DEPT spectrum of impurity III exhibited two additional signals due to methine groups (positive signals) which were not present in the DEPT spectrum of loratadine where two methylene signals (negative) were observed. This observation clearly indicates the formation of a double bond in impurity III. The possibility of double bond formation was only at position numbers 5 and 6 (refer Fig. 6). Based on the above spectral data the molecular formula of impurity III was confirmed as $C_{23}H_{21}ClN_2O_2$ and the corresponding structure was characterized as 8-chloro-11-(*N*-carboethoxy-4-piperidylidene)-5H-benzo(5,6) cyclopenta (1,2-b)-pyridine.

The spectral data for the synthesized and isolated impurities were found to be identical. The synthetic standards of impurities I, II and III were co-eluted on LC with loratadine and the area percentage at retention times 6, 10 and 12 min were enhanced and the LC chromatogram is shown in Fig. 5b.

The FT-IR, mass spectral and melting range data for loratadine, impurities I, II and III are shown in Table 2. The ^{1}H chemical shift values for loratadine, impurities I, II and III are given in Table 3. The ^{13}C NMR and DEPT assignments for loratadine, impurities I, II and III are given in Table 4. The chemical structures of loratadine, impurities I, II and III are shown in Fig. 6.

3.4.4. Formation of impurities

Impurity I may be formed if trace amounts of benzaldehyde is present in the starting material i.e. 3-chlorobenzaldehyde of loratadine synthesis. Impurity II may be formed during to the reaction of (**5**) with (**6**) (refer Fig. 1) in the presence of Grignard of 1,2-dibromoethane where the chlorine atom may be replaced by bromine leading to the formation of impurity II. Impurity III may be formed during cyclysation of (**7**) to form (**8**) (refer Fig. 1).

Acknowledgements

The authors wish to thank the management of Dr Reddy's Laboratories Ltd., Discovery Re-

search Division (Formerly Dr Reddy's Research Foundation) for supporting this work. Cooperation form colleagues of Dr Reddy's Laboratories Ltd., Bulk Actives III Division and Analytical Research Department of Discovery Research Division is appreciated.

References

[1] G.G. Kay, A.G. Harris, Clin. Expert Allergy 29 (3) (1999) 147–150.
[2] D. Zhong, H. Blume, Pharmazie 49 (10) (1994) 736–739.
[3] R. Johnson, J. Christensen, C.C. Lin, J. Chromatogr. B Biomed. Appl. 657 (1) (1994) 125–131.
[4] T. Radhakrishna, J. Satyanarayana, A. Satyanarayana, Indian Drugs 39 (6) (2002) 342–347.
[5] F.J. Ruperez, H. Fernandez, C. Barbas, J. Pharm. Biomed. Anal. 29 (2002) 35–41.
[6] F.C. Suthreland, A.D.J. Jager, D. Badenhorst, T. Scanes, H.K. Hundt, K.J. Swart, A.F. Hundt, J. Chromatogr. A 914 (1–2) (2001) 37–43.
[7] J. Martens, J. Chromatogr. B. Biomed. Appl. 673 (2) (1995) 183–188.
[8] N.E. Koury, L.I. Bebawy, J. Pharm. Biomed. Anal. 20 (4) (1999) 671–679.
[9] ICH Guideline Q3A (R), Impurities in New Drug Substances, 7 February, 2002.