Journal of Pharmaceutical and Biomedical Analysis 50 (2009) 405–412

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Identification and characterization of potential impurities of valsartan, AT1 receptor antagonist☆

A. Sampath[a], A. Raghupathi Reddy[a], B. Yakambaram[a], A. Thirupathi[a], M. Prabhakar[a], P. Pratap Reddy[a,*], V. Prabhakar Reddy[b]

[a] Department of Research and Development, Integrated Product Development, Innovation Plaza, Dr. Reddy's Laboratories Ltd., Survey Nos. 42, 45, 46 & 54, Bachupally, Qutubullapur, R.R. Dist-500072, A.P., India
[b] Department of Chemistry, P.G. College of Science, Saifabad, Hyderabad, A.P., India

ARTICLE INFO

Article history:
Received 14 February 2009
Received in revised form 21 May 2009
Accepted 22 May 2009
Available online 30 May 2009

Keywords:
Valsartan impurities
Desulfurization
Spectroscopy
Identification
Characterization

ABSTRACT

Five impurities (related substances) were detected during the impurity profile study of an antihypertensive drug substance, valsartan. A simple gradient high performance liquid chromatographic method (HPLC) and liquid chromatography–mass spectrometry (LC–MS) were used for the detection. Based on the spectral data (IR, NMR and MS) followed by synthesis, these impurities were characterized as (*S*)-*N*-(1-carboxy-2-methylprop-1-yl)-*N*-[2′-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]amine (impurity I); (*S*)-*N*-(1-carboxy-2-methylprop-1-yl)-*N*-(5-phenylthio)pentanoyl-*N*-[2′-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]amine (impurity II); (*S*)-*N*-(1-carboxy-2-methylprop-1-yl)-*N*-(5-phenyl)pentanoyl-*N*-[2′-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]amine (impurity III); (*S*)-*N*-(1-carboxy-2-methylprop-1-yl)-*N*-4-pentenoyl-*N*-[2′-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]amine (impurity IV); (*S*)-*N*-(1-carboxy-2-methylprop-1-yl)-*N*-(5-hydroxy)pentanoyl-*N*-[2′-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]amine (impurity V).

1. Introduction

Hypertension is one of the most prevalent diseases with an estimated one billion cases world wide [1]. The therapeutic standard was significantly improved in the 1980s by the introduction of losartan [2–3] as the first nonpeptidic angiotensin II type-1 (AT1) receptor antagonist. An entire therapeutic class, the sartans, has since been developed, among which valsartan, developed by Novartis and sold under the brand name DIOVAN currently holds the largest market share [4–9].

Valsartan is a US pharmacopeia listed drug substance and in this pharmacopeia, three impurities (*R*)-*N*-valeryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl]-methyl}valine, (*S*)-*N*-butyryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl]-methyl}valine and (*S*)-*N*-valeryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl]-methyl}valine benzyl ester were reported. Earlier, Nie et al. [10] have studied the impurity profile of valsartan, isolated three unknown impurities and one of these impurities was identified as (*S*)-*N*-valeryl-*N*-{[2′-(1-methyl-tetrazol-5-yl)biphenyl-4-yl]-methyl}-valine. The impurity profile mentioned in the present work is different from the reported impurities, as the synthetic scheme [11] used for the preparation of valsartan sample in our current study is different from the earlier reported synthetic schemes of valsartan.

During the analysis of the laboratory batches of valsartan crude, five impurities (related substances) with area percentage ranging from 0.1 to 0.2% were detected by a simple gradient HPLC method. To commercialize an active pharmaceutical ingredient (API), it is mandatory for the manufacturer to identify and characterize all the unknown impurities that are present in it at a level as low as 0.05% [12]. In this context, a comprehensive study has been undertaken to characterize all the five impurities present in the laboratory batches of valsartan using spectroscopic and spectrometric techniques. In this article, we report identification, characterization and synthesis of impurities obtained during the process development of valsartan (Fig. 1) [11].

2. Experimental

2.1. Samples

Samples of valsartan bulk materials were obtained from research and development department, Integrated Product Development, Innovation Plaza, Dr. Reddy's Laboratories Ltd., A.P., India. HPLC grade acetonitrile, potassium dihydrogen phosphate (KH_2PO_4),

☆ Dr. Reddy's Communication number: IPDO IPM-00163.

* Corresponding author. Tel.: +91 9989997176; fax: +91 4044346285.
E-mail address: prataprp@drreddys.com (P.P. Reddy).

0731-7085/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2009.05.024

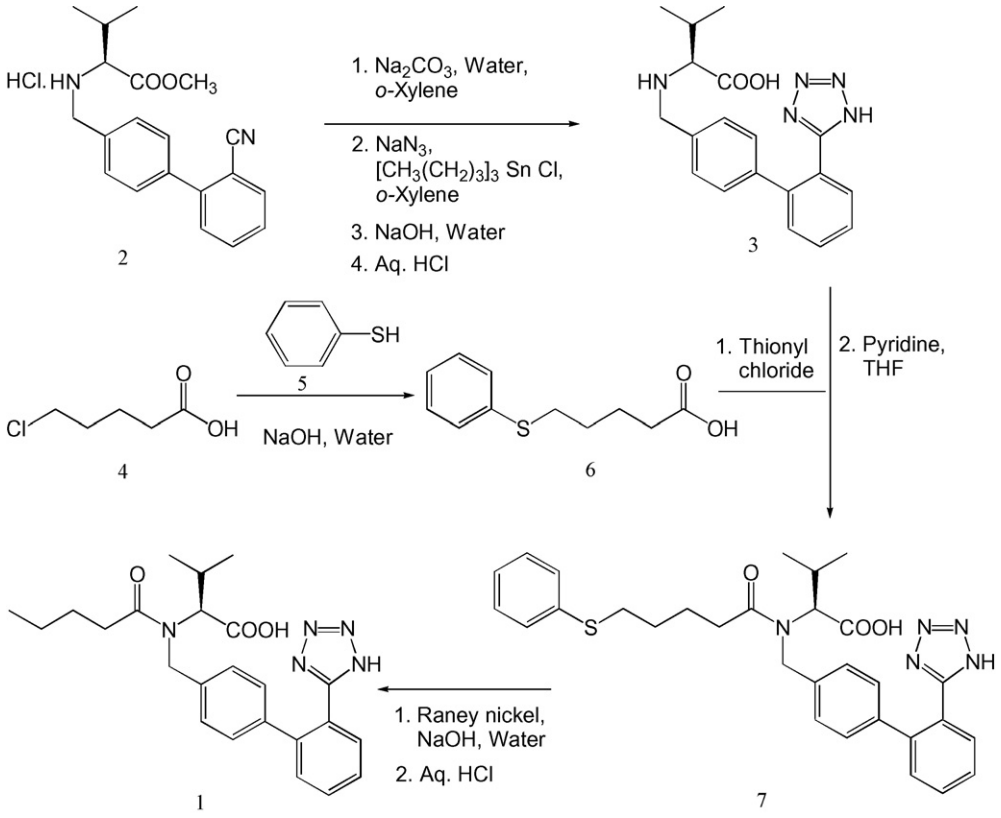

Fig. 1. Synthetic scheme for valsartan.

dipotassium hydrogen phosphate (K_2HPO_4), phosphoric acid, ammonium acetate and trifluoroacetic acid used in the analysis were procured from Merck, Mumbai, India. Water used for preparing mobile phase was purified using millipore milli-Q plus (Milford, MA, USA) purification system. NMR solvents $CDCl_3$ and DMSO-d_6 were procured from Cambridge Isotope Laboratories Inc., Andover, MA, USA. Pyridine, tetrahydrofuran, 5-phenylvaleric acid, thionyl chloride, 4-pentenoic acid, 5-chlorovaleric acid and sodium hydroxide used for the synthesis of impurities were purchased from Sigma–Aldrich, Hyderabad, India

2.2. High performance liquid chromatography (HPLC)

An in-house HPLC method was developed for the analysis of valsartan and its impurities (Agilent with empower software, 1100 series, G1312A Binary pump, G1314A variable wavelength detector, Waldbronn, Germany) where a column symmetry shield-RP18, 250 mm × 4.6 mm, 5 μm (Waters, Milford, MA, USA) with a mobile phase consisting of A: 0.01 M KH_2PO_4 and 0.005 M K_2HPO_4 (pH 3.0, adjusted with diluted phosphoric acid), B: water and acetonitrile (1:4, v/v), with a timed gradient program of T/%B: 0/50, 20/70, 30/70, 40/80, 50/50, 60/50 with a flow rate of 0.8 ml/min and UV detection at 210 nm was used. This HPLC method was able to detect all the impurities.

2.3. Liquid chromatography–mass spectrometry (LC–MS)

A LC–MS compatible method was developed for the analysis of valsartan and its impurities, where a column symmetry shield-RP18, 250 mm × 4.6 mm, 5 μm (Waters, Milford, MA, USA) with a mobile phase consisting of A: 0.01 M ammonium acetate (pH 3.0, adjusted with trifluoroacetic acid), B: water and acetonitrile (1:4, v/v), with a timed gradient program of T/%B: 0/50, 20/70, 30/70, 40/80, 50/50, 60/50 with a flow rate of 0.8 ml/min and UV detection at 210 nm was used. This LC–MS method was able to detect all the impurities.

2.4. Mass spectrometry

The LC–MS analysis has been performed on AB-4000 Q-trap LC–MS/MS mass spectrometer (Make: MDS SCIEX, vendor name: Applied Biosystems, Address: 850, incon Centre Drive, Foster City, CA, USA). The analysis was performed in positive ionization mode with turbo ion spray interface with the following conditions. Ion source voltage 5500 V, declustering potential 80 V, entrance potential 10 V, with the nebuliser gas as nitrogen at 30 psi. Negative ionization was performed by switching the polarity of the ion source voltage to −4500 V. The scanning range of MS and MS/MS experiments is 50–1000 (m/z).

2.5. NMR spectroscopy

The ^{1}H NMR were recorded on Varian Mercury plus 400 MHz FT-NMR spectrometer using $CDCl_3$ for valsartan and impurity II, using DMSO-d_6 for impurities I, III and IV and using mixture of DMSO-d_6 and $CDCl_3$ for impurity V. ^{13}C and DEPT (45, 90 and 135) were recorded on Varian Mercury plus 400 MHz FT-NMR (Varian, Germany) spectrometer using $CDCl_3$ for valsartan and using DMSO-d_6 for impurities I and V. ^{13}C NMR and DEPT (45, 90 and 135) for impurities II, III and IV were recorded on Gemini-2000 (400 MHz) FT-NMR (Varian, Germany) spectrometer using DMSO-d_6 as a solvent. The ^{1}H NMR chemical shift values were reported on the δ scale in ppm relative to TMS (δ = 0.00 ppm) and the ^{13}C NMR chemical shift values were reported relative to $CDCl_3$ (δ = 77.00 ppm) and DMSO-d_6 (δ = 39.50 ppm) as internal

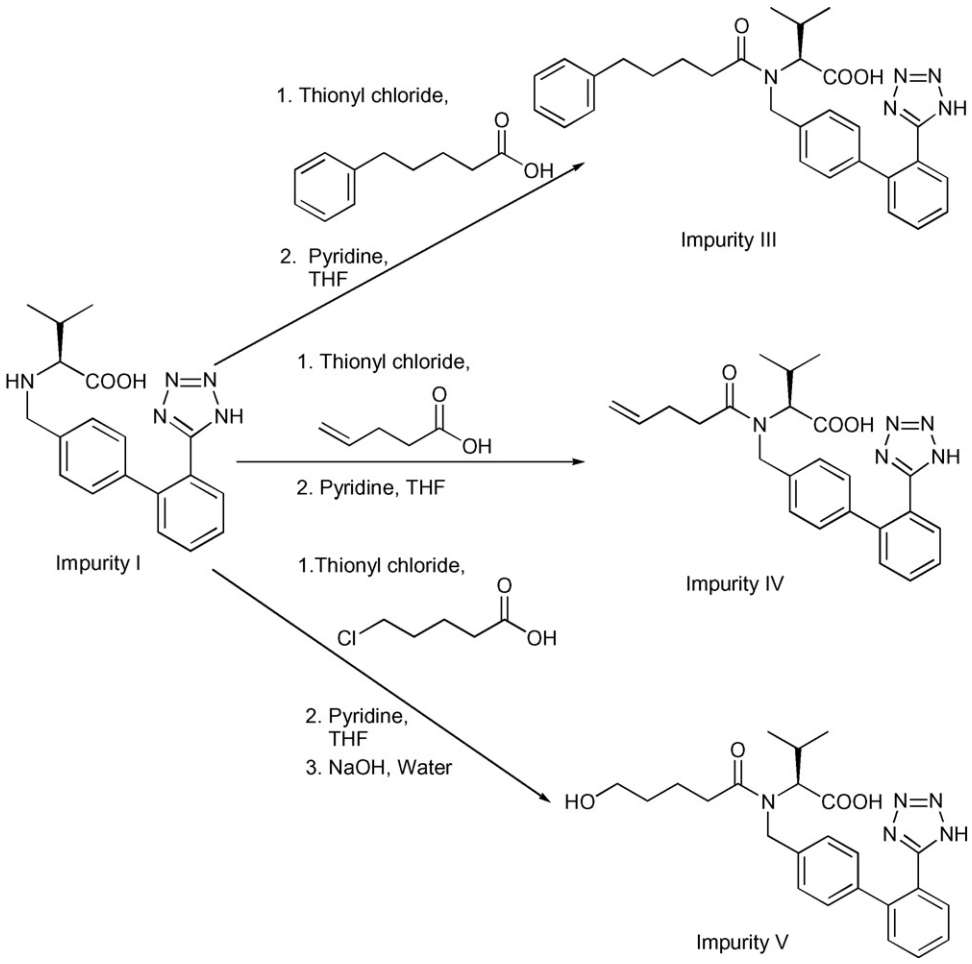

Fig. 2. Synthesis of valsartan impurities III, IV and V.

standards, respectively. DEPT spectra showed methyl and methine carbons as positive peaks and methylene carbons as negative peaks.

2.6. Melting point determination

Melting points of all impurities were determined in a Polmon digital melting point apparatus model no. MP96 (Polmon, Hyderabad, India).

2.7. FT-IR spectroscopy

The IR spectra were recorded in the solid state as KBr (Make: Merck and grade: IR) dispersion medium using PerkinElmer Spectrum One FT-IR spectrophotometer (PerkinElmer, Boston, MA, USA).

2.8. Synthesis of impurities

Impurity I (3) and impurity II (7) are the intermediates of valsartan, these compounds (Fig. 1) were prepared as per process mentioned in the patent [11]. The presence of these impurities was confirmed by co-injection with valsartan in the HPLC. Impurity III was prepared from the condensation of impurity I with in situ generated 5-phenylvaleroyl chloride (1.6 equiv) in the presence of pyridine (1.6 equiv) in tetrahydrofuran at −5 to 0 °C. 5-Phenylvaleroyl chloride was prepared from 5-phenylvaleric acid by reacting with thionyl chloride (1.2 equiv) at room temperature. Impurity IV and impurity V were prepared in the similar manner by using 4-pentenoic acid and 5-chlorovaleric acid, respectively. Preparation of impurity V involved the alkaline hydrolysis of condensation product using sodium hydroxide (1.2 equiv) in water medium (Fig. 2).

Impurities I, II, III, IV and V were prepared with HPLC purity 98.4%, 98.3%, 98.8%, 99.3% and 98.2%, respectively in the laboratory.

3. Results and discussion

3.1.1. Detection of impurities I, II, III, IV and V

A typical HPLC chromatogram of a laboratory batch of valsartan bulk drug was recorded as described in Section 2.2 (Fig. 3). The target impurities under study were marked as impurity I (retention time (RT): 3.0), impurity II (RT: 29.2), impurity III (RT: 27.8), impurity IV (RT: 15.8) and impurity V (RT: 6.8). The LC–MS compatible method described in Section 2.3 was used to detect all the impurities, whose structures were shown in Fig. 4.

3.2. Structure elucidation of impurities (related substances) of valsartan

3.2.1. Fragmentation pattern of valsartan

The fragmentation pattern of valsartan was generated as a reference to examine the behavior of impurities I, II, III, IV and V in the mass spectral analysis. The ES-MS–MS spectrum displayed daughter ions *m*/*z* at 207, 235, 306 and 352 (Fig. 5).

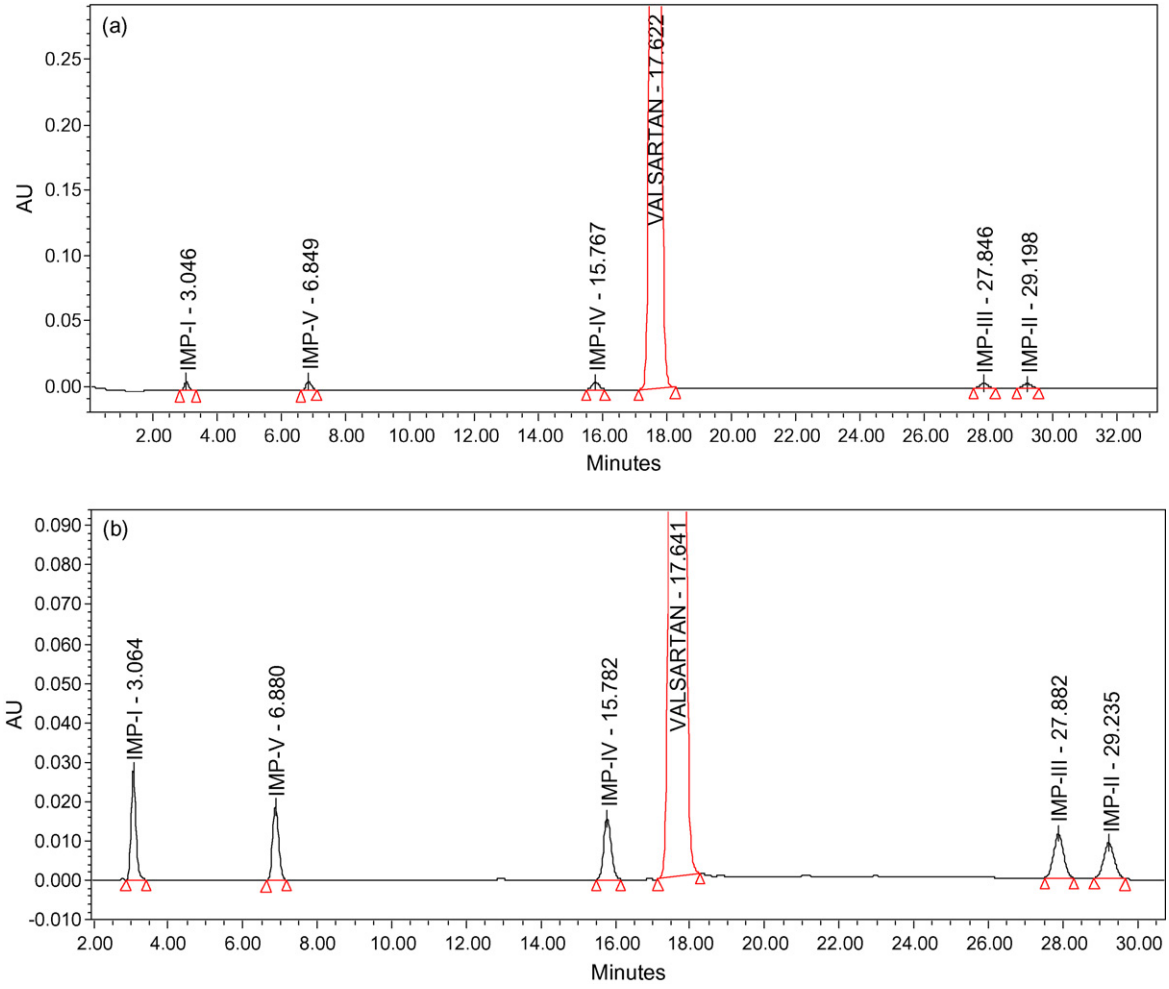

Fig. 3. (a) HPLC chromatogram of valsartan laboratory sample. (b) HPLC chromatogram of valsartan laboratory sample spiked with five impurities.

3.2.2. Structure elucidation of impurity I

The +ve ESI-MS spectrum of the impurity I showed peaks at *m*/*z* 352, 374 and 390 corresponding to the adduct ions $(M+H)^+$, $(M+Na)^+$ and $(M+K)^+$, respectively. The ES-MS–MS spectrum displayed daughter ions *m*/*z* at 207, 235 and 306. The fragmentation pattern is similar to the fragmentation pattern of valsartan (Fig. 5), which is indicating that the structure of impurity I is similar to the structure of valsartan. From the mass spectral data, the adduct ions confirmed the molecular ion of impurity I to be of *m*/*z* 351. The DEPT spectra displayed one negative signal due to one methylene group and 12 positive peaks corresponding to two methyl groups and ten methine groups (two in aliphatic and rest in aromatic region). IR spectrum displayed characteristic absorptions at 3419 cm^{-1} (NH and OH stretching) and 1622 cm^{-1} (C=O stretching), the latter being supported by the appearance of quaternary carbon signal characteristic of a carbonyl carbon in ^{13}C NMR spectrum. Based on the above spectral data the molecular formula of impurity I could be deduced as $C_{19}H_{21}N_5O_2$ and the corresponding structure was characterized as (*S*)-*N*-(1-carboxy-2-methylprop-1-yl)-*N*-[2′-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]amine.

3.2.3. Structure elucidation of impurity II

The +ve ESI-MS spectrum of the impurity II showed peaks at *m*/*z* 544, 566 and 582 corresponding to the adduct ions $(M+H)^+$, $(M+Na)^+$ and $(M+K)^+$, respectively. The ES-MS–MS spectrum displayed daughter ions *m*/*z* at 207, 235, 306, 352 and 399. The fragmentation pattern is similar to the fragmentation pattern of valsartan (Fig. 5), which is indicating that the structure of impurity II is similar to the structure of valsartan. From the mass spectral data, the adduct ions confirmed the molecular ion of impurity II to be of *m*/*z* 543. The DEPT spectra displayed 5 negative signals due to five methylene groups and 17 positive peaks corresponding to two methyl groups and fifteen methine groups (two in aliphatic and rest in aromatic region). IR spectrum displayed characteristic absorptions at 3444 cm^{-1} (NH and OH stretching), 1731 cm^{-1} (acid C=O stretching) and 1603 cm^{-1} (amide C=O stretching), the C=O stretching being supported by the appearance of quaternary carbon signals characteristic of a carbonyl carbon in ^{13}C NMR spectrum. Based on the above spectral data the molecular formula of impurity II could be deduced as $C_{30}H_{33}N_5O_3S$ and the corresponding structure was characterized as (*S*)-*N*-(1-carboxy-2-methylprop-1-yl)-*N*-(5-phenylthio)pentanoyl-*N*-[2′-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]amine.

3.2.4. Structure elucidation of impurity III

The +ve ESI-MS spectrum of the impurity III showed peaks at *m*/*z* 534 and 550 corresponding to the adduct ions $(M+Na)^+$ and $(M+K)^+$, respectively. The ES-MS–MS spectrum displayed daughter ions *m*/*z* at 207, 235, 306, 352 and 367. The fragmentation pattern is similar to the fragmentation pattern of valsartan (Fig. 5), which is indicating that the structure of impurity III is similar to the structure of valsartan. From the mass spectral data, the adduct ions confirmed the molecular ion of impurity III to be of *m*/*z* 511. The DEPT spectra displayed 5 negative signals

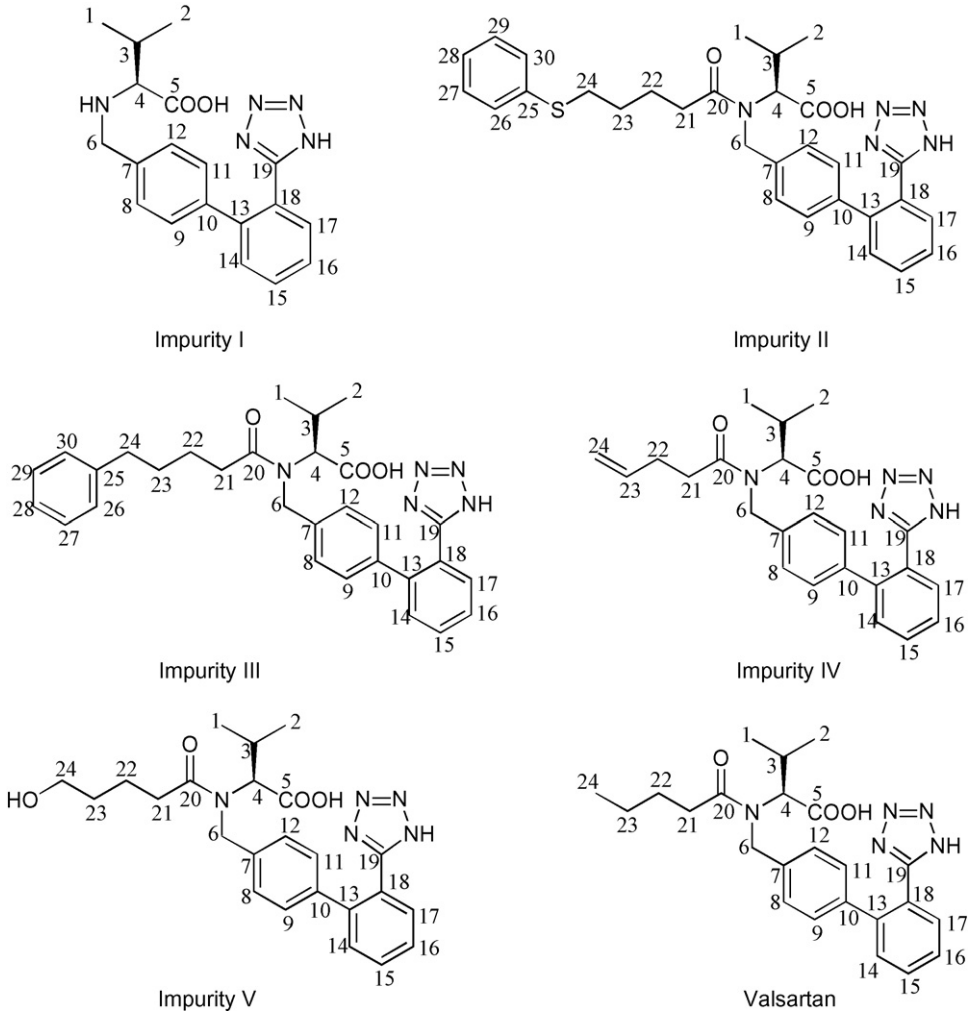

Fig. 4. Atom numbering used for NMR assignments.

due to five methylene groups and 17 positive peaks corresponding to two methyl groups and fifteen methine groups (two in aliphatic and rest in aromatic region). IR spectrum displayed characteristic absorptions at 1731 cm^{-1} (acid C=O stretching) and 1603 cm^{-1} (amide C=O stretching), being supported by the appearance of quaternary carbon signals characteristic of a carbonyl carbon in ^{13}C NMR spectrum. Based on the above spectral data the molecular formula of impurity III could be deduced as $C_{30}H_{33}N_5O_3$ and the corresponding structure was characterized as (*S*)-*N*-(1-carboxy-2-methylprop-1-yl)-*N*-(5-phenyl)pentanoyl-*N*-[2′-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]amine.

3.2.5. Structure elucidation of impurity IV

The +ve ESI-MS spectrum of the impurity IV showed peak at *m*/*z* 456 corresponding to the adduct ion $(M+Na)^+$. The ES-MS–MS spectrum displayed daughter ions *m*/*z* at 207, 235, 306 and 352. The fragmentation pattern is similar to the fragmentation pattern of valsartan (Fig. 5), which is indicating that the

Table 1
Melting point, FT-IR and mass spectral data of valsartan and impurities I, II, III, IV and V.

S. No.	Compound	M P (°C)	IR	MS
1	Impurity I	165–167	3419 (N–H and O–H stretching), 3033 (Aromatic C–H stretching), 2968 (Aliphatic C–H stretching), 1622 (C=O stretching), 1567 (Aromatic C=C, C=N stretching)	+ve ESI-MS: 352 $(M+H)^+$, 374 $(M+Na)^+$, 390 $(M+K)^+$
2	Impurity II	62–64	3444 (N–H and O–H stretching), 2965 (Aromatic C–H stretching), 2931 (Aliphatic C–H stretching), 1731 (Acid C=O stretching), 1603 (Amide C=O stretching)	+ve ESI-MS: 544 $(M+H)^+$, 566 $(M+Na)^+$, 582 $(M+K)^+$
3	Impurity III	58–60	2929 (Aliphatic C–H stretching), 1731 (Acid C=O stretching), 1603 (Amide C=O stretching)	+ve ESI-MS: 534 $(M+Na)^+$, 550 $(M+K)^+$
4	Impurity IV	87–89	3435 (N–H and O–H stretching), 2968 (Aromatic C–H stretching), 2927 (Aliphatic C–H stretching), 1732 (Acid C=O stretching), 1606 (Amide C=O stretching)	+ve ESI-MS: 456 $M+Na)^+$
5	Impurity V	71–75	3421 (N–H and O–H stretching), 3031 (Aromatic C–H stretching), 2935 (Aliphatic C–H stretching), 1731 (Acid C=O stretching), 1614 (Amide C=O stretching)	+ve ESI-MS: 452 $(M+H)^+$, 474 $(M+Na)^+$
6	Valsaratan	105–108	2923 (Aliphatic C–H stretching), 1732 (Acid C=O stretching), 1602 (Amide C=O stretching)	+ve ESI-MS: 436 $(M+H)^+$, 458 $(M+Na)^+$

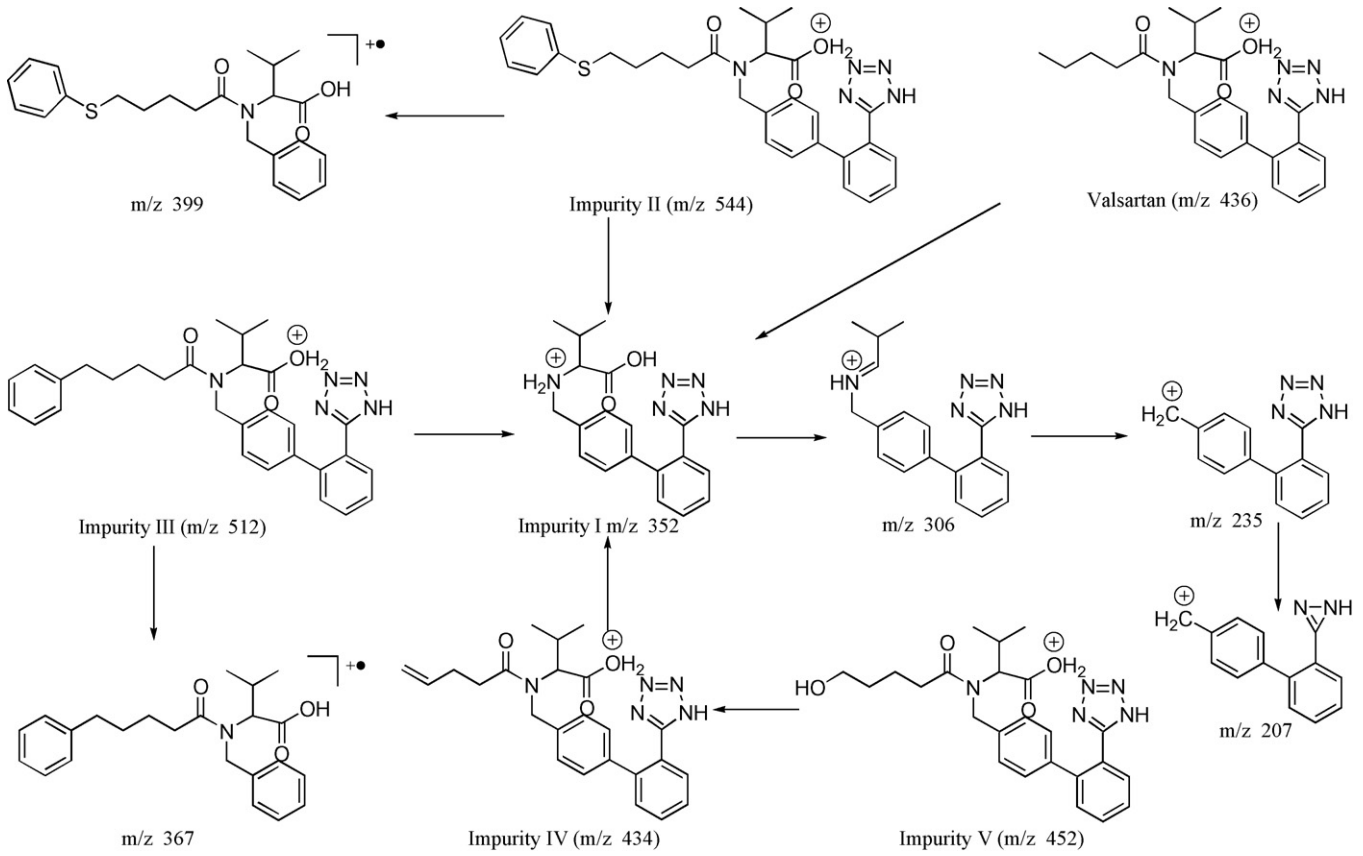

Fig. 5. Fragmentation pattern of valsartan, impurities I, II, III, IV and V.

Table 2
^{1}H and ^{13}C NMR assignments for valsartan and impurities I and II.

Position[a]	Valsartan				Impurity I				Impurity II			
	^{1}H	ppm/m[b]	^{13}C	DEPT	^{1}H	ppm/m[b]	^{13}C	DEPT	^{1}H	ppm/m[b]	^{13}C	DEPT
1	3H	0.97/d	18.5	CH_3	3H	0.90/d	18.4	CH_3	3H	0.95/d	18.5	CH_3
2	3H	0.99/d	18.5	CH_3	3H	0.93/d	18.4	CH_3	3H	1.00/d	18.5	CH_3
3	1H	2.75/m	26.7	CH	1H	2.0/m	29.5	CH	1H	2.65/m	28.2	CH
4	1H	3.4/d	65.4	CH	1H	3.0/d	65.8	CH	1H	3.4/d	65.7	CH
5	–	–	177	–	–	–	171.9	–	–	–	173.1	–
6	2H	4.23/d	53.9	CH_2	2H	3.72/d	50.3	CH_2	2H	4.21/d	48.8	CH_2
		4.99/d				3.92/d				4.97/d		
7	–	–	141.2	–	–	–	156.0	–	–	–	155.0	–
8 and 12	2H	7.22/d	128.7	CH	2H	7.1/d	128.8	CH	2H	7.17/d	128.8	CH
9 and 11	2H	7.26/d	128	CH	2H	7.3/d	125.0	CH	2H	7.34/d	126.9	CH
10	–	–	137.3	–	–	–	134.6	–	–	–	137.8	–
13	–	–	139.2	–	–	–	139.4	–	–	–	138.1	–
14	1H	7.48/d	128.3	CH	1H	7.64/d	127.6	CH	1H	7.48/d	127.8	CH
15	1H	7.55/t	129.8	CH	1H	7.51/t	130.6	CH	1H	7.58/t	130.9	CH
16	1H	7.62/t	128.3	CH	1H	7.55/t	127.6	CH	1H	7.62/t	127.8	CH
17	1H	8.08/d	128.2	CH	1H	7.66/d	128.9	CH	1H	8.11/d	127.6	CH
18	–	–	140.3	–	–	–	140.9	–	–	–	141.1	–
19	–	–	154.5	–	–	–	156.0	–	–	–	155.0	–
20	–	–	172.5	–	–	–	–	–	–	–	171.8	–
21	2H	2.6/t	33.3	CH_2	–	–	–	–	2H	2.6/t	31.7	CH_2
22	2H	1.75/m	27.2	CH_2	–	–	–	–	2H	1.7/m	24.0	CH_2
23	2H	1.4/m	22.4	CH_2	–	–	–	–	2H	1.85/m	30.6	CH_2
24	3H	1.0/t	13.7	CH_3	–	–	–	–	2H	2.95/t	32.3	CH_2
25	–	–	–	–	–	–	–	–	–	–	136.4	–
26 and 30	–	–	–	–	–	–	–	–	2H	7.34/d	126.3	CH
27 and 29	–	–	–	–	–	–	–	–	2H	7.25/t	128.9	CH
28	–	–	–	–	–	–	–	–	1H	7.45/t	125.3	CH

[a] Refer structural formula (Fig. 4) for numbering.
[b] Multiplicity; s, singlet; d, doublet; t, triplet; m, multiplet.

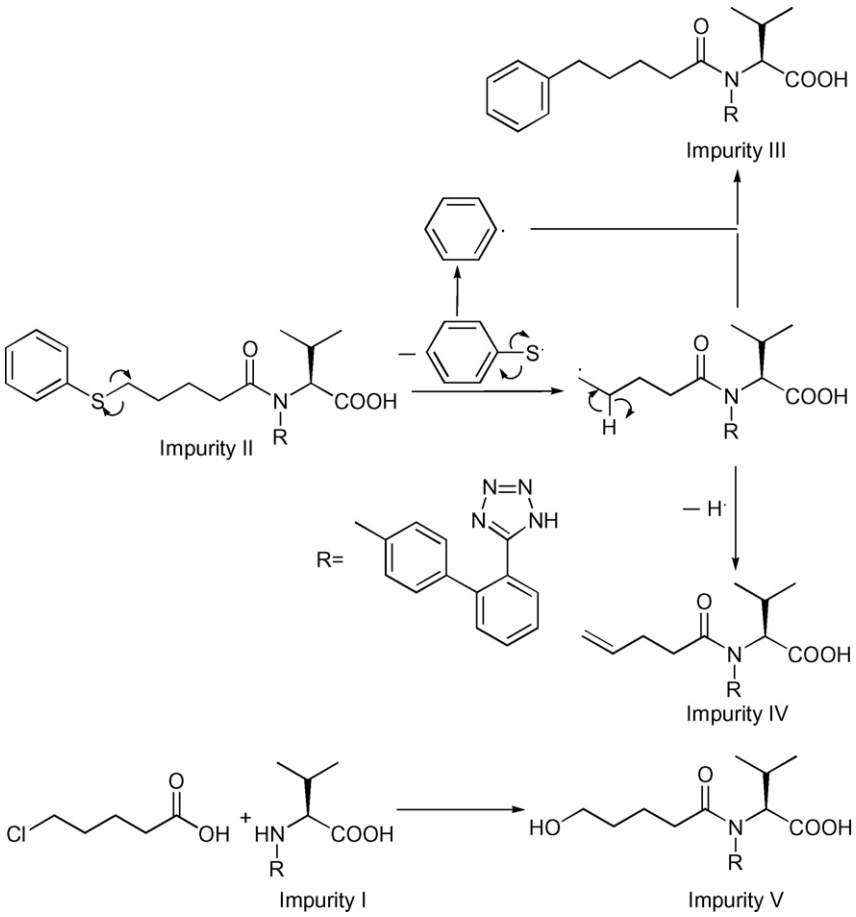

Fig. 6. Pathway of formation of valsartan impurities.

Table 3
^{1}H and ^{13}C NMR assignments for impurities III, IV and V.

Position[a]	Impurity III				Impurity IV				Impurity V			
	^{1}H	ppm/m[b]	^{13}C	DEPT	^{1}H	ppm/m[b]	^{13}C	DEPT	^{1}H	ppm/m[b]	^{13}C	DEPT
1	3H	0.75/d	18.6	CH_3	3H	0.77/d	18.5	CH_3	3H	0.72/d	18.5	CH_3
2	3H	0.93/d	18.6	CH_3	3H	0.94/d	18.5	CH_3	3H	0.77/d	18.5	CH_3
3	1H	2.6/m	24.6	CH	1H	2.70/m	26.4	CH	1H	2.2/m	21.5	CH
4	1H	4.08/d	65.9	CH	1H	4.1/d	65.8	CH	1H	4.08/d	65.8	CH
5	–	–	174.5	–	–	–	172.8	–	–	–	173.6	–
6	2H	4.50/d 4.61/d	48.9	CH_2	2H	4.47/d 4.63/d	48.7	CH_2	2H	4.52/d 4.62/d	48.8	CH_2
7	–	–	141.2	–	–	–	141.3	–	–	–	141.4	–
8 and 12	2H	7.13/d	128.9	CH	2H	6.98/d	128.3	CH	2H	7.08/d	128.3	CH
9 and 11	2H	7.2/d	126.4	CH	2H	7.11/d	126.3	CH	2H	7.2/d	127.0	CH
10	–	–	137.7	–	–	–	137.8	–	–	–	137.8	–
13	–	–	138.2	–	–	–	138.0	–	–	–	138.2	–
14	1H	7.6/d	127.7	CH	1H	7.65/d	127.7	CH	1H	7.63/d	127.7	CH
15	1H	7.50/t	130.6	CH	1H	7.52/t	128.8	CH	1H	7.53/t	128.8	CH
16	1H	7.56/t	127.7	CH	1H	7.57/t	127.7	CH	1H	7.55/t	127.7	CH
17	1H	7.69/d	127.0	CH	1H	7.68/d	127.0	CH	1H	7.65/d	127.6	CH
18	–	–	137.2	–	–	–	141.2	–	–	–	141.2	–
19	–	–	155.2	–	–	–	155.1	–	–	–	–	–
20	–	–	173.4	–	–	–	171.8	–	–	–	171.9	–
21	2H	2.2/t	32.7	CH_2	2H	2.2/t	32.3	CH_2	2H	2.15/t	32.0	CH_2
22	2H	1.5/m	26.4	CH_2	2H	2.3/m	30.6	CH_2	2H	1.6/m	21.5	CH_2
23	2H	1.6/m	30.7	CH_2	1H	5.8/m	137.2	CH	2H	1.45/m	32.7	CH_2
24	2H	2.5/t	35.0	CH_2	2H	5.08/d	115.1	CH_2	2H	3.4/t	60.5	CH_2
25	–	–	142.0	–	–	–	–	–	–	–	–	–
26 and 30	2H	7.16/d	128.2	CH	–	–	–	–	–	–	–	–
27 and 29	2H	7.28/t	128.2	CH	–	–	–	–	–	–	–	–
28	1H	7.0/t	125.6	CH	–	–	–	–	–	–	–	–

[a] Refer structural formula (Fig. 4) for numbering.
[b] Multiplicity; s, singlet; d, doublet; t, triplet; m, multiplet.

structure of impurity IV is similar to the structure of valsartan. From the mass spectral data, the adduct ion confirmed the molecular ion of impurity IV to be of *m*/*z* 433. The DEPT spectra displayed 4 negative signals due to four methylene groups and 13 positive peaks corresponding to two methyl groups and eleven methine groups (three in aliphatic and rest in aromatic region). IR spectrum displayed characteristic absorptions at 3435 cm^{-1} (NH and OH stretching), 1732 cm^{-1} (acid C=O stretching) and 1606 cm^{-1} (amide C=O stretching), the C=O stretching being supported by the appearance of quaternary carbon signals characteristic of a carbonyl carbon in ^{13}C NMR spectrum. Based on the above spectral data the molecular formula of impurity IV could be deduced as $C_{24}H_{27}N_5O_3$ and the corresponding structure was characterized as (*S*)-*N*-(1-carboxy-2-methylprop-1-yl)-*N*-4-pentenoyl-*N*-[2′-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]amine.

3.2.6. Structure elucidation of impurity V

The +ve ESI-MS spectrum of the impurity V showed peaks at *m*/*z* 452 and 474 corresponding to the adduct ions $(M+H)^+$ and $(M+Na)^+$, respectively. The ES-MS–MS spectrum displayed daughter ions *m*/*z* at 207, 235, 306, 352 and 434. The fragmentation pattern is similar to the fragmentation pattern of valsartan (Fig. 5), which is indicating that the structure of impurity V is similar to the structure of valsartan. From the mass spectral data, the adduct ions confirmed the molecular ion of impurity V to be of *m*/*z* 451. The DEPT spectra displayed 5 negative signals due to five methylene groups and 12 positive peaks corresponding to two methyl groups and ten methine groups (two in aliphatic and rest in aromatic region). IR spectrum displayed characteristic absorptions at 3421 cm^{-1} (NH and OH stretching), 1731 cm^{-1} (acid C=O stretching) and 1614 cm^{-1} (amide C=O stretching), the C=O stretching being supported by the appearance of quaternary carbon signals characteristic of a carbonyl carbon in ^{13}C NMR spectrum. Based on the above spectral data the molecular formula of impurity V could be deduced as $C_{24}H_{29}N_5O_4$ and the corresponding structure was characterized as (*S*)-*N*-(1-carboxy-2-methylprop-1-yl)-*N*-(5-hydroxy)pentanoyl-*N*-[2′-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]amine.

Structure elucidation of the impurities I, II, III, IV and V was done with the help of mass, IR and NMR spectral data (Tables 1–3).

3.3. Pathway of formation of impurities

Impurities I and II are the intermediates in the synthetic sequence of valsartan. (*S*)-*N*-(1-carboxy-2-methylprop-1-yl)-*N*-(5-phenylthio)pentanoyl-*N*-[2′-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]amine (impurity II) undergoes desulfurization by reductive cleavage of phenyl sulfide in the presence of Raney nickel in aqueous sodium hydroxide solution to provide valsartan. Impurities III and IV may be resulted during the course of desulfurization as this reaction proceeds through the free radical mechanism. 5-Chlorovaleric acid is a precursor of 5-phenylthiovaleric acid, which is one of the starting materials for impurity II. The unreacted 5-chlorovaleric acid in 5-phenylthiovaleric acid reacts with impurity I and subsequently undergoes alkaline hydrolysis resulting in impurity V (Fig. 6).

Acknowledgements

The authors wish to thank the management of Dr. Reddy's Laboratories Ltd. for permitting to carryout the present work. The authors also wish to thank the colleagues of Analytical Research & Development Department and Process Research & Development Department of Active Pharmaceutical Ingredients, IPDO, Dr. Reddy's Laboratories Ltd.

References

[1] U.S. Department of Health and Human Services, National Heart, Lung, and Blood Institute, National High Blood Pressure Education Program, NIH Publication No. 03-5233, December, 2003.
[2] J.V. Duncia, A.T. Chiu, D.J. Carini, G.B. Gregory, A.L. Johnson, W.A. Price, G.J. Wells, P.C. Wong, J.C. Calabrese, P.B.M.W.M. Timmermans, J. Med. Chem. 33 (1990) 1312–1329.
[3] D.J. Carini, J.V. Duncia, P.E. Aldrich, A.T. Chiu, A.L. Johnson, M.E. Pierce, W.A. Price, J.B. Santella III, G.J. Wells, R.R. Wexler, P.C. Wong, S.E. Yoo, P.B.M.W.M. Timmermans, J. Med. Chem. 34 (1991) 2525–2547.
[4] Novartis Annual Report, 2006, Novartis International AG, Basel, Switzerland, 2007.
[5] P. Bühlmayer, F. Ostermayer, T. Schmidlin, EP 0443983 B1, February 12 (1991).
[6] P. Bühlmayer, F. Ostermayer, T. Schmidlin, US 5399578, March 21 (1995).
[7] P. Bühlmayer, P. Furet, L. Criscione, M. De Gasparo, S. Whitebread, T. Schmidlin, R. Lattmann, J. Wood, Bioorg. Med. Chem. Lett. 4 (1994) 29–34.
[8] Novartis Pharma Schweiz, vol. 2, AG. Health & Science, 2005, pp. 8–9.
[9] J. McMurray, S. Solomon, K. Pieper, S. Reed, J. Rouleau, E. Velazquez, H. White, J. Howlett, K. Swedberg, A. Maggioni, L. Køber, F. Van de Werf, R. Califf, M. Pfeffer, J. Am. Coll. Cardiol. 47 (2006) 726–733.
[10] J. Nie, B. Xiang, Y. Feng, D. Wang, J. Liq. Chromatogr. Related Technol. 29 (2006) 553–568.
[11] J. Iqbal, A.R. Reddy, A. Sampath, B. Yakambram, P. Rajagopal, A. Thirupathi, Indian patent application 1049/CHE/2006, November 28 (2008).
[12] ICH harmonized tripartite guideline, Impurities in New Drug Substances Q_3A (R2), current step 4 version, October 25, 2006.