APL Research Centre, (a division of Aurobindo Pharma Limited), Hyderabad, India

Identification and characterization of new impurities in rivastigmine

M. Srinivasa Rao, Sumathi V. Rao, D. V. N. Srinivasa Rao, Ch. Bharathi, Pradeep Rajput, Hemant Kumar Sharma

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V. Sumathi Rao, Department of Analytical Research, Aurobindo Pharma Research Centre, 313 Bachupally, Qutubullapur Mandal, Hyderabad, 500072, India
sumathirao@aurobindo.com

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Rivastigmine is a drug against Alzheimer's disease, and is a non-pharmacopoeial compound. During the preparation of rivastigmine in our laboratory, two impurities were detected and identified with a simple and sensitive reversed-phase liquid chromatography coupled with electrospray-mass spectrometry. The same impurities were also observed in commercial batches. These impurities were isolated by preparative HPLC and co-injected with rivastigmine sample to confirm the retention times in HPLC. These impurities were characterized as *N,N*-dimethyl-3-[1-dimethylaminoethyl]phenylcarboxylate (dimethyl-rivastigmine) and *N,N*-diethyl-3-[1-dimethylaminoethyl]phenylcarboxamide (diethyl-rivastigmine). Structural elucidation of these impurities by spectral data (^{1}H NMR, ^{13}C NMR and MS) is discussed.

1. Introduction

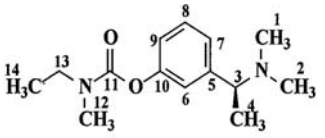
Rivastigmine (Exelon®), (*S*)-*N*-ethyl-3-[(1-dimethylamino)ethyl]-*N*-methyl-phenylcarbamate hydrogentartrate, is an acetylcholineesterase inhibitor (Martindale 2002) of the carbamate type approved for the treatment of Alzheimer's disease (AD). People with AD suffer mainly from impaired memory and orientation, personality changes and later also perceptual, speech and walking disorders. Neuropathologically, AD is characterized by the presence of neurofibrillary tangles (Melchiorre et al. 2004) and seniles plaques, impaired synaptic function and cell loss.

During the preparation of rivastigmine in APL Research Centre two impurities were detected consistently in HPLC up to a level of 0.5% (ICH Guideline 2002). Extensive literature survey revealed no information about impurities of rivastigmine. Since it is mandatory to identify and characterize all unknown impurities in the final product as per regulatory requirement, an attempt was made to isolate and characterize these two impurities of rivastigmine.

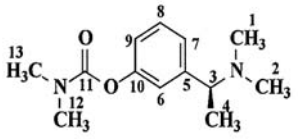
2. Investigations, results and discussion

2.1. Detection of impurities

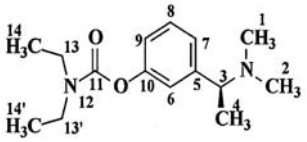
Laboratory batches of rivastigmine were analyzed using the HPLC method described in Section 3.2. These samples were subjected LC-MS/MS analysis (Pommier and Frigola 2003) using the method as described in section 3.4. The unknown impurities, which were isolated by preparative HPLC were co-injected with rivastigmine to confirm the retention times. The two impurities were well resolved from the rivastigmine peak and the representative resolution mixture chromatogram is shown in the Figure.



Rivastigmine



Dimethyl rivastigmine



Diethyl rivastigmine

Rivastigmine and its impurities

2.2. Structural elucidation of impurity dimethyl rivastigmine

The molecular ion peak at m/z; 237.1 [$(MH)^+$] in positive ion mode by LC-MS analysis indicated a molecular weight of 236 amu, which is 14 amu less than that of rivastigmine. The major fragmentation peak at m/z 192.2 corresponds to *N,N*-dimethyl-3-[1-dimethylaminoethyl]phenyl carboxylate

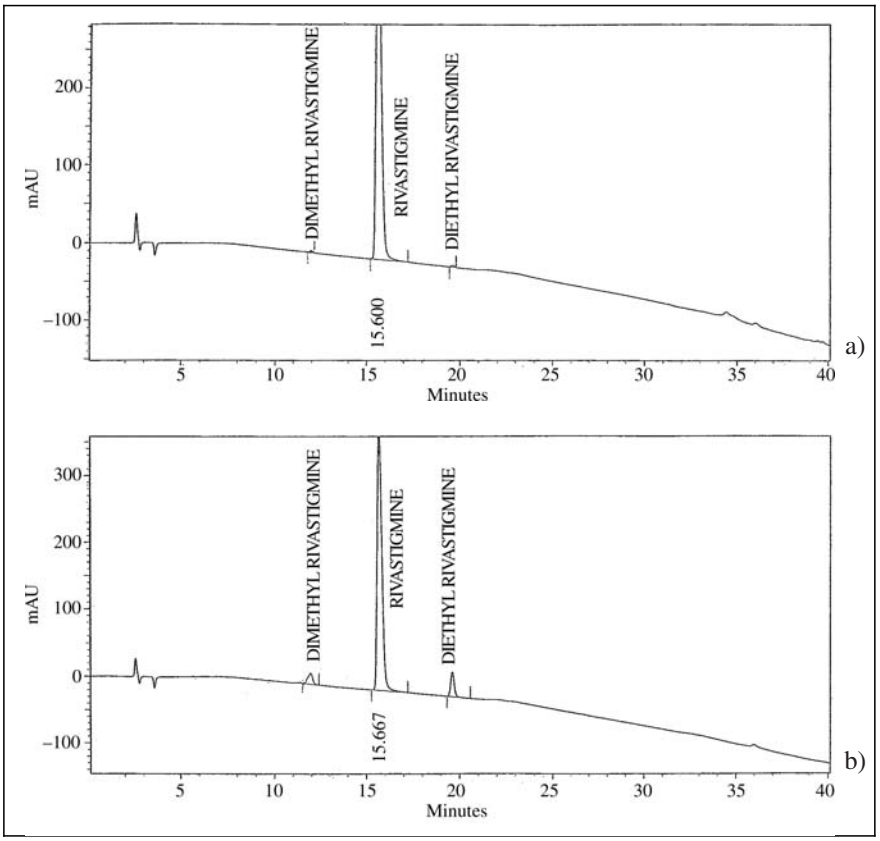


Fig.:
LC-Chromatogram of (a) rivastigmine sample; b: LC-Chromatogram of (b) rivastigmine sample spiked with dimethyl rivastigmine and diethyl rivastigmine

ion. To confirm the retention time, pure dimethyl rivastigmine was co injected with rivastigmine sample in HPLC. It was observed that the retention time was matching the retention time of dimethyl rivastigmine. The structure of this impurity was confirmed by ^{1}H NMR and ^{13}C NMR spectrum. In ^{1}H NMR spectrum a multiplet at 3.44 ppm is observed in rivastigmine sample and the absence of this peak indicated dimethyl rivastigmine. In ^{13}C NMR spectrum 44.40 ppm corresponding to CH_2 carbon disappeared in dimethyl rivastigmine, confirmed by DEPT experiment. Anal.calcd. For $C_{13}H_{20}N_2O_2$ (236). ^{1}H NMR, ^{13}C NMR and DEPT data for rivastigmine and its dimethyl impurity are

Table: Comparative ^{1}H, ^{13}C (proton decoupled) and DEPT NMR assignments for rivastigmine and its dimethyl and diethyl impurities

S.No	Rivastigmine			Dimethyl impurity			Diethyl impurity		
	^{1}H NMR (ppm)	^{13}C NMR (ppm)	DEPT	^{1}H NMR (ppm)	^{13}C NMR (ppm)	DEPT	^{1}H NMR (ppm)	^{13}C NMR (ppm)	DEPT
1	2.20 (s, 3 H)	43.59	CH_3	2.61, 2.62 (2 s, 3 H)	36.90	CH_3	2.62, 2.63 (2 s, 3 H)	39.18	CH_3
2	2.20 (s, 3 H)	43.59	CH_3	2.77, 2.78 (2 s, 3 H)	36.90	CH_3	2.77, 2.78 (2 s, 3 H)	42.50	CH_3
3	3.24 (q, 1 H)	66.01	CH	4.29 (q, 1 H)	66.15	CH	4.28 (q, 1 H)	66.15	CH
4	1.36 (d, 3 H)	20.48	CH_3	1.87 (d, 3 H)	17.60	CH_3	1.88 (d, 3 H)	17.65	CH_3
5	–	146.0, 146.1	C	–	135.89	C	–	135.81	C
6	7.06 (s, 1 H)	121.09	CH	7.26 (s, 1 H)	123.92	CH	7.27 (s, 1 H)	123.83	CH
7	7.01 (d, 1 H)	124.60	CH	7.44 (d, 1 H)	126.19	CH	7.44 (d, 1 H)	126.00	CH
8	7.27 (dd, 1 H)	129.26	CH	7.46 (dd, 1 H)	130.70	CH	7.45 (dd, 1 H)	130.66	CH
9	7.11 (d, 1 H)	120.64	CH	7.21 (d, 1 H)	123.06	CH	7.21 (d, 1 H)	123.06	CH
10	–	151.8	C	–	151.6	C	–	151.7	C
11	–	154.8	C	–	156.8	C	–	156.2	C
12	2.99, 3.06 (2 s, 3 H)	34.15, 34.57	CH_3	3.02 (s, 1 H)	37.13	CH_3	–	–	–
13	3.44 (m, 2 H)	44.40	CH_2	–	–	–	3.39, 3.46 (2 q, 4 H)	42.65, 42.83	CH_2
14	1.19, 1.24 (2 t, 3 H)	12.86, 13.61	CH_3	3.12 (s, 3 H)	37.13	CH_3	1.21, 1.27 (2 t, 6 H)	13.68, 14.62	CH_3

s, singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quartet; m, multiplet
DEPT: Distortionless enhancement by polarisation transfer
[a]Refer chemical structures Figure for numbering of rivastigmine and its dimethyl, diethyl impurities

given in the Table. This impurity may be arising due to contamination of *N*-dimethyl carbonyl chloride during the preparation of rivastigmine.

2.3. Structural elucidation of impurity diethyl rivastigmine

The molecular ion peak at m/z; 265.3 [$(MH)^+$] in positive ion mode by LC-MS analysis indicated a molecular weight of 264 amu, which is 14 amu more than that of rivastigmine. The major fragmentation peak at m/z 220.2, corresponds *N*,*N*-diethyl-3-[(1-dimethylaminoethyl]phenylcarboxamide ion. To confirm the retention time of pure diethyl rivastigmine, was co injected with rivastigmine sample in HPLC. It was observed that the retention time was matching the retention time of diethyl rivastigmine. The structure of this impurity was confirmed by ^{1}H NMR and ^{13}C NMR spectrum. In ^{1}H NMR spectrum a multiplet at 2.99 and 3.06 ppm, is observed in rivastigmine sample and the absence of these peaks indicated diethyl rivastigmine. In ^{13}C NMR spectrum 34.15 and 34.57 ppm corresponding to CH_3 carbon was disappeared in diethyl rivastigmine, confirmed by DEPT experiment. Anal.calcd. For $C_{15}H_{24}N_2O_2$ (236). ^{1}H NMR, ^{13}C NMR and DEPT data for rivastigmine and its diethyl impurity are given in the Table. This impurity may arise due to contamination of *N*-diethyl carbonyl chloride during the preparation of rivastigmine.

3. Experimental

3.1. Samples

The investigated samples of rivastigmine and its unknown impurities were prepared in APL Research Centre (a unit of Aurobindo Pharma Limited, Hyderabad, India). Reagents used for analysis i.e., ammonium acetate (BDH grade, England), acetonitrile (HPLC grade) procured from Merck (India) Limited and milliQ water.

3.2. High Performance Liquid Chromatography (Analytical)

Chromatographic separation was performed on a HPLC system with. Shimadzu binary gradient system with SCL-10At Vp pumps, SIL-10AD Vp auto injector and Class-Vp Software for instrument control and data acquisition [Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan]. Analysis was carried out on Xterra RP18 250 mm long 4.6 mm i.d., and 5 μm particle diameter column. Mobile phase A was 0.01 M ammonium acetate (Tse and Laplanche 1998; Kavalìrova et al. 2004; Kenndler et al. 2005; Wang et al. 2006) as such [acetate buffer prepared by dissolving 0.778 g of ammonium acetate in 1000 ml of water]. Mobile phase B was acetonitrile. UV detection was at 215 nm and flow rate was kept at 1.5 ml/min. Data acquisition time was 40 min. Pump mode was gradient and the program was as follows, 0–15 min: 90% A–10% B; 15–25 min. 70% A–30% B; 25–35 min. 50% A–75% B; 35–40 min. 25% A–75% B; 40–42 min. 25% A–10% B; 42–50 min 90% A–10% B.

3.3. High Performance Liquid Chromatography (preparative)

A Shimadzu LC-8A Preparative Liquid Chromatograph equipped with SPD-10A VP, UV-Vis detector [Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan] was used. Hypersil BDS C18 (250 mm long × 21.2 mm i.d.) preparative column packed with 8 μm particle size [Thermo Electron Corporation, UK] was employed for isolation of impurity. The mobile phase consisted of (A) 0.1 M ammonium acetate solution and (B) acetonitrile. Flow rate was kept at 25 ml/min and detection was carried out at 215 nm. The gradient program was as follows, 0–0.01 min.: 100% A; 0.01–50 min.: 95% A–5% B; 50–80 min.: 90% A–10% B; 80–100 min.: 50% A–50% B; 100–101 min.: 100% A; 101–110 min.: 100% A.

3.4. LC-MS/MS analysis

LC-MS/MS analysis was carried out using Perkin Elmer triple quadrupole mass spectrometer (API 2000, PE SCIEX) coupled with Shimadzu HPLC equipped with SPD 10 A VP US-VIS detector and LC 10 AT VP pumps [Foster City, CA]. 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. High pure nitrogen gas was used as auxiliary gas and curtain gas. Zero air was used as nebulizer gas. LC-MS spectra were acquired from m/z 100–1000 in 0.1 amu steps with 2.0 s dwell time. Rivastigmine sample was subjected to LC-MS/MS analysis (Enz et al. 2003). The analysis was carried out using Xterra RP18, 250 × 4.6 mm column with 5 μm particle diameter with mobile phase consisting of a mixture of 0.01 M ammonium acetate solution as such and acetonitrile. Flow rate was 1.0 ml/min. Two unknown impurities were detected in laboratory batch sample. The masses of detected impurities corresponding to mass of m/z 236 and m/z 264 were observed in sample of rivastigmine.

3.5. NMR Spectroscopy

The ^{1}H, ^{13}C NMR (proton decoupled) spectra were recorded on Bruker 300 MHz spectrometer using D_2O as solvent and tetramethylsilane (TMS) as internal standard.

3.6. Mass Spectrometry

Mass spectra were recorded on a Perkin Elmer PE SCIEX-API 2000 mass spectrometer equipped with a Turbo ionspray interface at 375 °C.

3.7. Isolation of the impurities

Rivastigmine was prepared by the reaction of *N*-ethyl-*N*-methyl carbamoyl chloride with (*S*)-3-(1-dimethylaminoethyl)phenol. Commercially available samples of *N*-ethyl-*N*-methyl carbamoyl chloride are contaminated with *N*-dimethyl carbamoyl chloride and *N*-diethyl carbamoyl chloride. Because of the presence of these impurities in *N*-ethyl-*N*-methyl carbamoyl chloride, structurally related corresponding impurities i.e., dimethyl rivastigmine and diethyl rivastigmine will be present in rivastigmine.

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