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Spectral Characterization of Degradation Impurities of Paroxetine Hydrochloride Hemihydrate

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Abstract

Two unknown impurities were detected in stressed samples (by hydrochloric acid and hydrogen peroxide) of paroxetine hydrochloride hemihydrate (an active pharmaceutical ingredient – API) using a gradient reversed-phase high performance liquid chromatography (HPLC). These impurities were enriched and were present up to 30% in the degraded sample. The impurities were isolated from the degraded sample by column purification.

Spectral data of the isolated impurities were collected. Based on the spectral data of two dimensional nuclear magnetic spectroscopy (2D-NMR) and mass spectrometry (MS) Impurity-1 and Impurity-2 were characterized as (3*S*,4*R*)-3-{[(6-chloro-1,3-benzodioxol-5-yl)oxy]methyl}-4-(4-fluorophenyl)piperidine and [(3*S*,4*R*)-4-(4-fluorophenyl)piperidin-3-yl]methanol respectively.

Keywords

Paroxetine ••Degradation•• NMR•• HPLC ••LC-MS.

Introduction

Paroxetine belongs to the class of antidepressants, as it is a selective serotonin reuptake inhibitor (SSRI) [1]. It is sold as Paxil in United States and is also approved for the

treatment of post-traumatic stress disorder. Like some other antidepressants, it is also prescribed in the treatment of obsessive-compulsive disorder (OCD). Degradation studies form an integral part of stability testing of active pharmaceutical ingredients. To our knowledge the degradation impurities of paroxetine presented here, have not been reported. The aim of the present study was to isolate and characterize the two major degradation impurities [2], as it is necessary to identify impurities above 0.1% [3]. The impurities were generated during the stability testing of the Active Pharmaceutical Ingredient (API) in acid and peroxide [4]. The impurities were enriched by degradation of paroxetine hydrochloride hemihydrate, and then subjected to column purification; identified using LC-MS [5] and NMR.

Results and Discussion

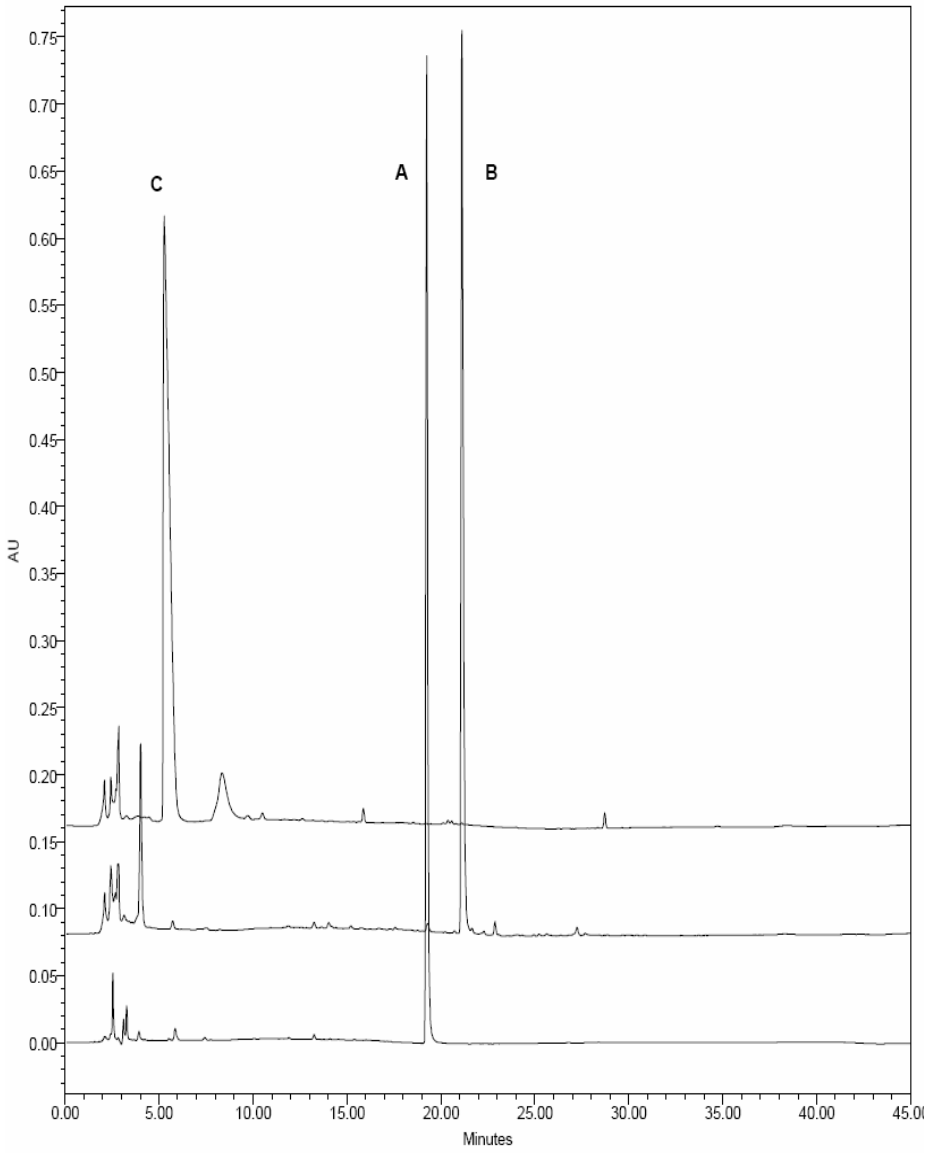


Fig. 1. Overlay of HPLC chromatograms of Paroxetine hydrochloride hemihydrate API (A), impurity-1(B) and impurity-2 (C).

Degradation of paroxetine hydrochloride hemihydrate (henceforth referred to as API) under acidic condition and with hydrogen peroxide was found to give two unknown impurities: impurity-1 and impurity-2, respectively whereas using a base and heat stress for 48 hrs did not give rise to unknown impurities.

The analytical HPLC chromatograms of API, impurity-1 and impurity-2 after isolation are shown in Fig. 1 and their structures are shown in Table.1 respectively. API (Fig. 1A) eluted at a retention time of 19.25 min while impurity-1 and impurity-2 after isolation eluted at retention times of 21.12 min (Fig. 1B) and 5.28 min (Fig. 1C) respectively.

The NMR data of the isolated impurities were collected and the structures of impurities were assigned with the help of gradient double quantum correlation spectroscopy (gDQCOSY), gradient heteronuclear single quantum correlation spectroscopy (gHSQC) and gradient heteronuclear multiple bond correlation spectroscopy (gHMBC) data.

Structure elucidation of impurity-1

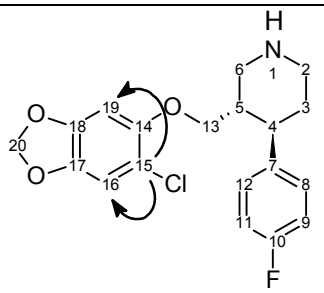
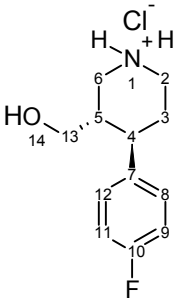
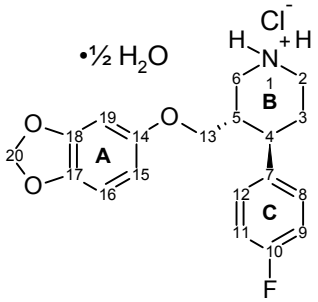
The mass spectral (MS) data indicates that the diagnostic molecular ion peak (M+1) of API at m/z 330 whereas the MS data of impurity-1 displayed a diagnostic protonated molecular ion at m/z 364 with the characteristic isotopic abundance of a chlorine atom, an additional chlorine atom may be present in impurity-1. The high resolution mass spectral (HR MS) data of impurity-1 also showed the exact mass of the protonated molecular ion at m/z 364.1130 (Cal. 364.1116 for $C_{19}H_{20}ClFNO_3$), which corresponds, to the molecular formula $C_{19}H_{19}ClFNO_3$ with characteristic isotopic abundance of chlorine. This confirms the addition of chlorine atom to API.

To arrive at the position of the substituted chlorine atom in the structure 1D-NMR and 2D-NMR spectra were recorded. The ^{1}H, ^{13}C NMR signal assignments of API (known) and impurity-1 (unknown) are tabulated in Table 2.

The ^{1}H NMR data of impurity-1 (Fig. 2B) were compared with those of API (Fig. 2A). The spectral data was similar except for the absence of signal at δ 6.12 in ^{1}H NMR of impurity-1 (Fig. 2B) indicating the absence of a proton in impurity-1, the corresponding gHSQC spectrum also indicates the absence of signal due to carbon at δ 106. A new signal at δ 114 was observed in gHSQC spectrum of impurity-1 indicating that there may be a different substituent on this carbon. It is also interesting to note that the aromatic doublet at δ 6.33 in ^{1}H NMR of API due to H at position 16 was found to be a singlet at δ 6.77 in Impurity-1. Further confirmation was obtained from long-range proton carbon experiment (gHMBC). The cross peaks of quaternary carbon at C-15 with those of C-16 and C-17 positions confirm the point of attachment of chlorine at position 15. These interactions are shown in Table.1.

Based on the above results, impurity-1 structure can be rationalized in terms of the addition of a chlorine atom to the benzodioxol ring (ring A-refer API structure in Table.1) at position 15. The structure of the impurity-1 was characterized as (3*S*,4*R*)-3-{[(6-chloro-1,3-benzodioxol-5-yl)oxy]methyl}-4-(4-fluorophenyl)piperidine.

Tab. 1. LC-MS data and HPLC data of Paroxetine hydrochloride hemihydrate and impurities

S.No.	HPLC RT(min)	LC-MS RT (min)	Structure/Molecular weight	Remarks
1	21.12 min	39.68	 Molecular Formula = $C_{19}H_{19}ClFNO_3$ Formula Weight = 363.81 Monoisotopic Mass = 363.10	impurity-1 (Acid stressed-Degradation related)
2	5.28 min	4.18	 Molecular Formula = $C_{12}H_{17}FNO^+$ Monoisotopic Mass = 210.13	impurity-2 (Peroxide stressed-Degradation related)
3	19.25min	11.26	 Molecular Formula = $C_{19}H_{21}ClFNO_3 \bullet ½H_2O$ Monoisotopic Mass = 374.83	API

Structure elucidation of impurity-2

The mass spectral data of API and impurity-2 were compared. The data indicates that the diagnostic protonated molecular ion of API at m/z 330 and that of impurity-2 displayed a diagnostic protonated molecular ion at m/z 210 with the absence of characteristic isotopic abundance for chlorine atoms. The HR MS data of impurity-2 showed exact mass of the protonated molecular ion at m/z 210.13 (Cal. 210.12 for $C_{12}H_{16}NOF$), which corresponds, to the molecular formula $C_{12}H_{16}N_1O_1F_1$. To arrive at the structure1D-NMR (1 Dimensional NMR) and 2D-NMR were recorded.

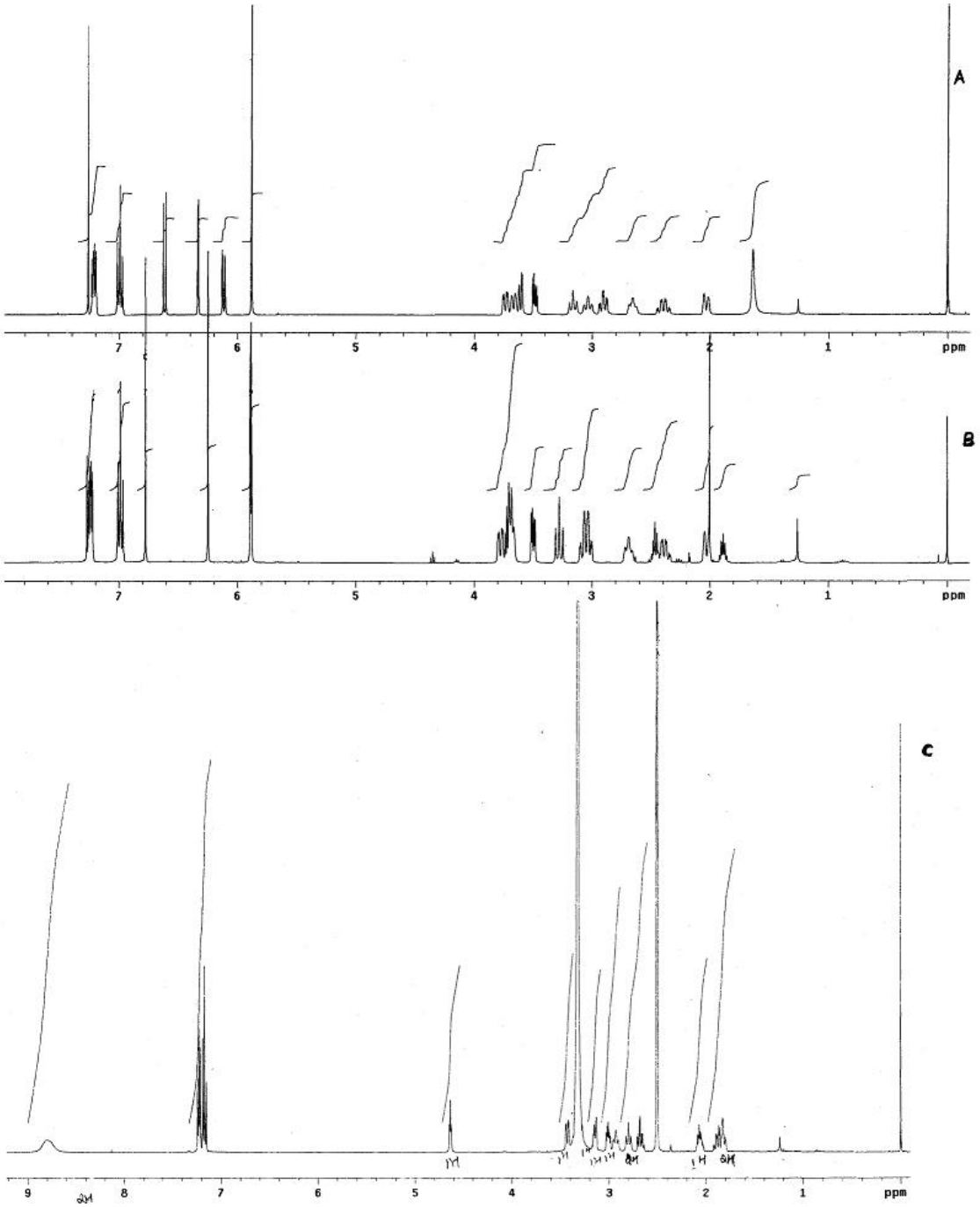


Fig. 2. Overlay of Proton NMR spectra of Paroxetine hydrochloride hemihydrate (A), impurity-1 (B) and impurity-2 (C).

The ^{1}H NMR data of impurity-2 (Fig. 2C) were compared with those of API (Fig. 2A). The spectral data were different, the resonances in API due to the benzodioxol ring (ring A - refer API structure in Table.1) attached were absent in impurity-2, whereas the pattern due to the rings B & C were found to be similar to that of API. The additional broad singlet at δ 8.80 is due to the hydroxy (-OH) group present in the degraded impurity indicates the disappearance of the benzodioxol ring in impurity-2. This was also confirmed by gHSQC and gHMBC spectra.

The MS/MS fragmental data and crystal structure of impurity-2 (crystals were obtained as hydrochloride by slow evaporation in chloroform and methanol) also lend support to the proposed structure of impurity-2.

The structure of impurity-2 was characterized as [(3*S*,4*R*)-4-(4-fluorophenyl)piperidin-3-yl]methanol and is shown in Table-1 along with numbering (refer impurity-2 structure in Table.1), the ^{1}H and ^{13}C NMR assignments of impurity-2 from gHSQC data are tabulated in Table. 2.

Tab. 2. ^{1}H and ^{13}C NMR assignments of chemical shifts for Paroxetine hydrochloride hemihydrate, impurity-1 and impurity-2

Atom No.*	Paroxetine hydrochloride hemihydrate (API)				Paroxetine HCl (0.1N HCl stressed) (Impurity-1)				Paroxetine HCl (3.0% H_2O_2 stressed) (Impurity-2)			
	^{1}H	ppm/J**	^{13}C	DEPT	^{1}H	ppm/J	^{13}C	DEPT	^{1}H	ppm/J	^{13}C	DEPT
1	1H	1.60/s	–	–	1H	1.24/s	–	–	1H	8.75/br	–	–
2	2H	H_a 3.10m, H_b 3.65/m	44	CH_2	2H	H_a 3.10/m, H_b 3.75/m	45	CH_2	2H	H_a 2.80/m, H_b 3.42/m	46	CH_2
3	2H	H_a 2.04m, H_b 2.42/m	30	CH_2	2H	H_a 2.00/m, H_b 2.40/m	30	CH_2	2H	H_a2.08/m, H_b2.75/m	41	CH_2
4	1H	2.90/m	42	CH	1H	3.02/m	42	CH	1H	2.75/m	42	CH_3
5	1H	2.65/m	40	CH	1H	2.69/m	40	CH	1H	1.85/m	31	CH
6	2H	H_a 3.20/m H_b 3.75/m	47	CH_2	2H	H_a 3.28m, H_b 3.76/m	47	CH_2	2H	H_a2.95/m, H_b3.42/m	47	–
7	–	–	138	–	–		138	–	–	–	126	–
8	1H	7.20/d8.8	129	CH	1H	7.25/d8.8	129	CH	1H	7.23/m	140	CH
9	1H	6.95/d8.2	116	CH	1H	6.98/d8.2	116	CH	1H	7.19/m	130	CH
10	–	–	162	–	–	–	164	–	–	–	162	–
11	1H	6.95/d8.2	116	CH	1H	6.98/d8.2	116	CH	1H	7.19/m	130	CH
12	1H	7.20/d8.8	129	CH	1H	7.25/d8.8	129	CH	1H	7.23/m	140	CH
13	2H	H_a 3.45/m, H_b 3.60/m	68	CH_2	2H	H_a 3.48/m, H_b 3.70/m	69	CH_2	2H	H_a3.02/m, H_b3.15/m	61	CH_2
14	–	–	152	–	–	–	148	–	1H	4.65/m	–	–
15	1H	6.12/d2.6	106	CH	–	–	114	–	–	–	–	–
16	1H	6.33/d2.6	98	CH	1H	6.77/s	110	–	–	–	–	–
17	–	–	143	–	–	–	170	–	–	–	–	–
18	–	–	149	CH	–	–	142	–	–	–	–	–
19	1H	7.03/d2.4	116	CH	1H	6.23/s	105	–	–	–	–	–
20	2H	5.88/s	102	CH_2	2H	5.88/s	106	–	–	–	–	–

*: Refer Table.1 for numbering; ** J: This column gives the ^{1}H–^{1}H coupling constant, (s) singlet, (d) doublet, (t) triplet, (m) multiplet.

Experimental

Materials

The investigated sample of paroxetine hydrochloride hemihydrate (API-B.No. CPAX 2A 00305) was supplied by Dr. Reddy's Laboratories Ltd. Bulk Actives – III, Hyderabad, India (US Process Patent 2006). The materials used for HPLC analysis were triethylamine (AR grade) and acetonitrile (gradient grade) and they were purchased from Fluka chemicals, Switzerland and Ranbaxy Laboratories, India respectively. Water used was purified using Milli-Q plus purification system (Make: Millipore, Milford, USA).

Acid, Base and Oxidative degradation of API

About 0.5g of API (B.No. CPAX 2A 00305) was weighed separately into three 100 mL volumetric flasks and into each flask 30mL of acetonitrile (gradient grade, purchased from Ranbaxy Laboratories, India) and tetrahydrofuran (HPLC grade, supplied by Qualigens Fine Chemicals, Mumbai, India) in 1:1 v/v ratio were added and each of the volumetric flasks were diluted to 100mL with 1 N hydrochloric acid solution (HCl AR grade, supplied by Qualigens Fine Chemicals, Mumbai, India), 0.5 N sodium hydroxide solution (sodium hydroxide pellets AR grade, supplied by Qualigens Fine Chemicals, Mumbai, India) and 3% hydrogen peroxide solution, (diluted from 30% hydrogen peroxide supplied by LOBA CHEMIE, Mumbai, India) respectively. All these solutions were kept for 48 hrs at 60 °C with stirring and the HPLC of all these degraded samples were recorded.

Column purification

Purification of degradation related impurities (impurity-1 and impurity-2) of API

Column purification was done with chloroform and methanol (LR grade, supplied by Ranbaxy Fine Chemicals Limited, New Delhi, India). Impurity-1 and impurity-2 of API were first extracted into chloroform and water, respectively. The extracted impurities about 50 mg each were loaded onto a column packed with 200 mg of 100-120 mesh silica; for isolation of impurity-1 elution was done initially with chloroform then followed by chloroform: methanol ($CHCl_3$: MeOH) 99.5: 0.5 %v/v; the column was eluted gradually with 0.5 % v/v increments of methanol up to 5% v/v of methanol, fractions exhibiting similar thin layer chromatography (TLC) profiles were combined and evaporated on a rota vapor. Purity of the required fraction was checked by HPLC; since the sample was only 85% pure, further purification was done by chloroform wash and the supernatant layer was extracted. The purity of the isolated impurity-1 was about 93% by HPLC and the spectral data of impurity-1 was collected and the structure was elucidated.

Purification of impurity-2 was done by gradually eluting the extracted impurity about 800mg with $CHCl_3$: MeOH (90:10 % v/v); 12 % v/v, 15% v/v and 30 % v/v increments of methanol and fractions exhibiting similar thin layer chromatography (TLC) profiles were combined and evaporated on a rota vapor. The quantity of the isolated impurity-2 was found to be 110 mg. The purity of this fraction was checked by HPLC and since the sample was only 30% pure by HPLC, further purification was done by crystallizing the sample in chloroform and adding minimum amount of methanol. Crystals of impurity-2 were obtained by slow evaporation. The crystals were found to be about 96% pure by HPLC, the spectral data was recorded and the structure was elucidated; the structure was also confirmed by Single Crystal as hydrochloride salt of [(3*S*,4*R*)-4-(4-fluorophenyl)piperidin-3-yl]methanol.

High performance liquid chromatography (analytical)

A Waters Model Alliance 2695 Separations module equipped with a Waters 2996 photo diode array UV detector (Supplied by Waters, USA) was used. A simple in-house gradient LC method was developed for the analysis of paroxetine hydrochloride hemihydrate drug substance and its degradation products. A C18 Column, X-Terra RP18, 250x4.6mm I.D., 5µ (Waters) with a mobile phase consisting of a mixture of A: triethyl amine (0.1%) pH adjusted to 10 with dilute orthophosphoric acid; B: acetonitrile; the gradient program used was T / %B: 0/30, 5/30, 25/80, 35/80, 40/30, 45/30. Detection was done at UV 285 nm for API and impurity-1 and UV 265 nm for impurity-2. Flow rate of 1.0ml/min was used. The data was recorded using Waters Empower software (Build 1154).

This HPLC method was found to be linear with respect to both impurity-1 and impurity-2. Calibration curves for impurities 1 and 2 were constructed in the concentration range of 250 µg/mL to 750 µg/mL. The regression equation obtained was Y= 4E+06 X-42049 and Y= 2E+06 X-59853 respectively for impurity-1 and impurity-2. The correlation coefficient was found to be greater than 0.99 for both the impurities, confirms the method is linear.

ESI-LC-MS

LC-MS was carried out on the degraded drug substance of API. The mobile phase used was A: 1mL formic acid in 1000 mL Milli Q water; B: 1mL Formic acid in 1000 mL acetonitrile using the following gradient elution (T/%B: 0/20, 30/20, 50/80, 60/80, 65/20, 70/20) with a flow rate of 1.0mL/min and monitored at 285 nm. An Inertsil C8-3 (250 x 4.6mm) 5 µ column was used. The concentrations of the injected samples were 0.5 mg/mL and injected volume was 20 µL.

The MS/MS experiments were performed on a PESCIEX API 3000. The sample was introduced through a turbo ion spray interface in positive ionization mode using infusion pump. The nebulizer and curtain gases used were zero air and nitrogen respectively and the ion spray voltage was maintained at 4500 V. Focusing potential, declustering potential and entrance potential were kept at 200 V, 50 V and 10 V respectively.

NMR

The ^{1}H, ^{13}C and 2D NMR experiments (gDQCOSY, gHSQC and gHMBC) for API, impurity-1 were performed in $CDCl_3$ solvent (procured from Cambridge Isotopic Labs, Inc., USA) and peroxide stressed impurity was performed in dimethyl sulphoxide-D_6 (procured from Cambridge Isotopic Labs, Inc., USA) using Varian Mercury plus 400 MHz FT NMR spectrometer. The ^{1}H, ^{13}C and 2D NMR experiments (gDQCOSY, gHSQC and gHMBC) using Varian Mercury plus 400 MHz FT NMR spectrometer (Varian, Germany) equipped with a 5-mm ID probe at 298 K. The data were collected and processed by Varian NMR 6.1C version software running on SUN ULTRA-10 PC with Microsoft windows xp. The ^{1}H-^{1}H bond correlations were confirmed by gDQCOSY experiments. The protonated carbon positions were confirmed by gHSQC experiments. The non-protonated carbons were confirmed by gHMBC experiments. The ^{1}H chemical shifts are reported in ppm with reference to tetramethylsilane (δ 0.0 ppm). The ^{13}C NMR experiments chemical shift values were reported relative to $CDCl_3$ (δ 77.00 ppm) and DMSO-d_6 (δ 39.50 ppm) as internal standards, respectively.

High resolution mass

All samples were analysed on the Micromass LCT Premier XE mass spectrometer equipped with an ESI Lock spray source for accurate mass values. Leucine enkephalin was used as an internal reference compound, which was introduced via the lock spray channel. The mass range was calibrated with the cluster ions of sodium formate using a fifth order polynomial fit. Data were acquired using the positive mode.

The mass spectrometer was equipped with a Waters Acquity system. API was dissolved in methanol at a concentration level of 1mg/ml, sonicated for 5 minutes and centrifuged for 6 minutes at 16000 rpm. This was diluted 1:100 with methanol and introduced to the mass spectrometer via infusion syringe.

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The authors' Intellectual property management department (IPM) has given this manuscript the DRL-Publication No. 628.

Authors' Statements

Competing Interests

The authors declare no conflict of interest.

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