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Identification of degradation products in stressed tablets of Rabeprazole sodium by HPLC-hyphenated techniques†

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Three unknown impurities of Rabeprazole, a proton pump inhibitor, were formed in the formulated drug under the stress conditions, [40 °C/75% relative humidity (RH) for 6 months] with relative retention times (RRTs) 0.17, 0.22 and 0.28. The Impurity-I (0.17 RRT) was isolated using preparative HPLC and characterized by NMR and MS. The other two impurities, Impurity-II (RRT 0.22) and Impurity-III (RRT 0.28) could not be isolated, hence they are characterized by HPLC-hyphenated techniques, LC–NMR and high-resolution LC–MS. On the basis of the spectral data, the Impurity-I, Impurity-II and Impurity-III were characterized as 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-methyl-4-oxo-1,4-dihydropyridine-2-carboxylic acid, 1*H*-benzo [*d*] imidazole-2-sulfonic acid and 4-(3-methoxy propoxy)-3-methyl-2-pyridine carboxylic acid, respectively.

Keywords: Rabeprazole sodium; degradation products; LC–NMR; LC–MS

Introduction

Rabeprazole, (±)-2-[4-(3-methoxypropoxy)-3-methylpyridin-2-yl] methylsulfinyl]-1*H*-benzimidazole sodium is a proton pump inhibitor that covalently binds and inactivates the gastric parietal cell proton pump (H^+/K^+ ATPase). It is an important alternative to H_2 antagonists and an additional treatment option to other proton pump inhibitors in the management of acid-related disorders.[1] It has also demonstrated efficacy in healing and giving symptomatic relief for gastric and duodenal ulcers, as well as a high eradication rate of the microorganism *Helicobacter pylori* when associated with antimicrobial therapy.[2] The molecular structure is shown in Fig. 1.

In general, solid active pharmaceutical ingredients (APIs) are formulated with excipients as tablets and/or capsules. Since the active ingredient is interacting with the excipients and the formulated product is stored at different conditions, the study of stability of APIs is critical in the drug development process. Many factors can affect the stability of a pharmaceutical product, some of them include the stability of the active ingredient, the manufacturing process, the environmental conditions (such as heat, light and moisture during storage), as well as some chemical reactions such as oxidation, reduction, hydrolysis and racemization that might occur.[3,4] Study of stability under stressed conditions is important since it can cause many degradation reactions.

The identification of process-related Rabeprazole impurities in bulk substance by LC–MS and spectral data (IR and NMR) has been reported.[5] Rabeprazole sodium photodegradation products have been published recently.[6] The instability of Rabeprazole under acidic conditions is known; hence, it is manufactured as enteric coated tablets.[7] However, the stability of Rabeprazole sodium tablets under stressed conditions is not yet reported. Hence, the present manuscript deals with the identification and characterization of three unknown impurities obtained by storage of the tablets at stressed conditions [40 °C/75% relative humidity (RH)] for 6 months. Of the three polar impurities, the most polar one was isolated by preparative HPLC, but the other two could not be isolated due to low resolution. Therefore, HPLC-hyphenated techniques were utilized for the structure elucidation.

HPLC-hyphenated techniques are now widely used for the structure elucidation of trace amounts of the degradation products without complicated isolation process. LC–MS has been one of the powerful techniques for the identification of small quantities of drug degradation products.[8] Recently LC–NMR has been increasingly utilized to obtain detailed structural information of degradation products.[9,10]

The isolated impurity was characterized by NMR and MS. The combined fraction of other two impurities was characterized by using hyphenated techniques such as LC–NMR and LC–MS.

Results and Discussion

The purpose of this work is to study the stability of Rabeprazole sodium tablets under stressed conditions. A few tablets were kept at 40 °C/75% RH in stability chambers for about 6 months.

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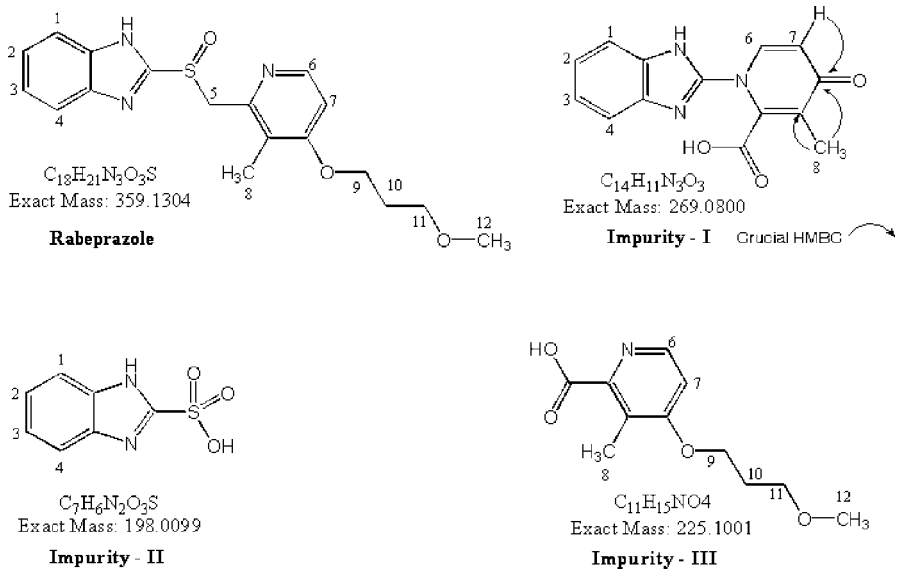

Figure 1. Structures of Rabeprazole, Impurity-I, Impurity-II and Impurity-III 150 × 108 mm (120 × 120 DPI).

The initial purity and that after stressed conditions was studied by HPLC. The chromatogram reveals three unknown impurities formed during the accelerated stress conditions (Fig. 2). The unknown impurities were eluted at relative retention times (RRTs) 0.17, 0.22 and 0.28 which are referred as Impurity-I, Impurity-II and Impurity-III, respectively. Impurity-I was isolated by preparative HPLC and the structure was identified by spectroscopic techniques (High Resolution (HR)-MS and NMR). Impurity-II and Impurity-III could not be isolated individually due to low resolution; hence, the fractions were collected together and characterized by LC–NMR and LC–MS. The spectral data of the impurities and the parent drug are compared for the identification (Table 1).

The structure elucidations of these impurities are discussed below. The NMR and MS data (Fig. 3) of the impurities were compared with the Rabeprazole sodium data. The elucidated structures are shown in Fig. 1.

Impurity-I

The NMR and high-resolution MS data (Fig. 3) of Impurity-I were compared with those of Rabeprazole data ($C_{18}H_{21}N_3O_3S$). The HR-MS data of Impurity-I exhibited protonated ion $[M + H]^+$ at *m/z* 270.1093 corresponding to the molecular formula $C_{14}H_{12}N_3O_3$. Interestingly, the sulfur atom is absent in Impurity-I. The ^1H NMR signals showed resonances corresponding to that of benzimidazole and methyl substituted pyridine moieties. Signals from the 3-methoxypropoxy group are not observed. The diagnostic quaternary carbon chemical shifts at $\delta = 183.96$ and 170.08 ppm were attributed to ketone and carboxylic acid functionality. The HMBC experiment shows long-range $^1H-^{13}C$ correlations across two to four bonds. The HMBC experiment (Fig. 4) showed long-range $^1H-^{13}C$ correlations of $\delta = 183.96$ ppm carbon signal with $\delta = 1.95$ (methyl–^1H) and 6.50 ppm (methine–^1H). This confirms the presence of the ketone group in 4-position in the pyridine moiety. The HMBC correlations are shown by arrows in the Fig. 1; NMR assignments are shown in Table 1. Thus, from the NMR and high-resolution MS data the structure of the Impurity-I was identified as 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-methyl-4-oxo-1,4-dihydropyridine-2-carboxylic acid.

Table 1. ^1H NMR data of Rabeprazoel sodium, Impurities I, II and III (Fig. 1)[a]

Position	^1H	Rabeprazole Na	Impurity-I[b]	Impurity-II[c]	Impurity-III[c]
1,4	2H	7.30 (m)	7.30(m)	7.3 (m)	–
		(117.39)	(126.47)	–	–
2,3	2H	7.65 (m)	7.55(m)	7.6 (m)	–
		(118.40)	(117.50)	–	–
5	Ha	4.80 (d, 13.5)	–	–	–
		(61.13)	–	–	–
	Hb	4.70 (d, 13.5)	–	–	–
		(61.13)	–	–	–
6	1H	8.21 (d, 6.0)	7.90(d, 6.0)	–	8.20 (d, 6.0)
		(148.17)	(142.58)	–	–
7	1H	6.95 (d, 6.0)	6.50(d, 6.0)	–	7.1 (d, 6.0)
		106.17	(117.47)	–	–
8	3H	2.14 (s)	1.95 (s)	–	2.10 (s)
		(10.76)	(14.11)	–	–
9	2H	4.10 (t, 6.0)	–	–	4.20 (t, 6.0)
		(65.09)	–	–	–
10	2H	1.97 (m)	–	–	1.95 (m)
		(28.74)	–	–	–
11	2H	3.48 (t, 6.0)	–	–	3.60 (t, 6.0)
		(68.39)	–	–	–
12	3H	3.21 (s)	–	–	3.20 (s)

[a] The ^1H NMR data of Rabeprazole were taken in DMSO-d_6, those of Impurity-I data in D_2O. Multiplicities and coupling constants (Hz) are given in parentheses; s, singlet; d, doublet; t, triplet; m, multiplet. ^{13}C chemical shifts are noted below the ^1H data in parentheses.
[b] Quaternary ^{13}C NMR signals were observed at $\delta = 124.25$, 139.00, 142.58, 150.12, 170.08 and 183.96 ppm.
[c] The data of the impurities II and III are from LC–NMR spectra which were referenced to CH_3CN at $\delta = 1.95$ ppm.

Impurity-II

The HR-MS data of Impurity-II exhibited a protonated molecular ion $[M + H]^+$ at *m/z* 199.0197 corresponding to the molecular

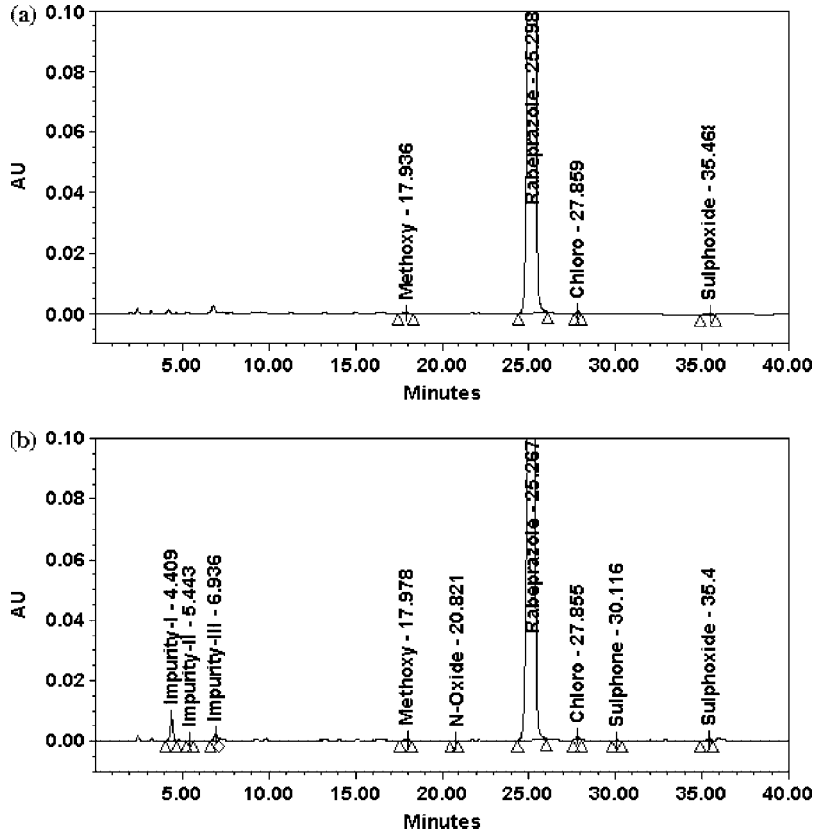

Figure 2. Typical HPLC chromatograms: (a) Rabeprazole sodium tablets (initial), and (b) stressed Rabeprazole sodium tablets (6 months at 40 °C/75% RH). 125 × 123 mm (120 × 120 DPI).

formula $C_7H_7N_2O_3S$. The one-dimensional (1D) ^{1}H NMR data were collected using LC–NMR by the stopped-flow method and are shown in Fig. 5 overlaid with those of Rabeprazole. It displays only two aromatic signals at $\delta = 7.3$ (m) and 7.6 ppm (m). The splitting pattern and the chemical shifts showed that these two signals correspond to the benzimidazole moiety with a molecular formula $C_7H_5N_2$. The remaining atoms in the molecular formula correspond to SO_3H substituted at the benzimidazole ring system. Thus, from the 1D LC–NMR and LC–MS data, the structure of the impurity-II was elucidated as 1*H*-benzo[*d*] imidazole-2-sulfonic acid.

Impurity-III

The high-resolution MS data of Impurity-III exhibited a protonated molecular ion $[M + H]^+$ at *m/z* 226.1092. Hence, the molecular formula is found to be $C_{11}H_{16}NO_4$. The 1D ^{1}H NMR data were collected using LC–NMR by the stopped-flow method. The ^{1}H NMR spectrum of Impurity-III overlaid with Rabeprazole for the comparison is shown in Fig. 5. It shows signals at $\delta = 2.00$ (m, 2H), 2.10 (s, 3H), 3.20 (s, 3H), 3.60 (t, $J = 6.0$ Hz, 2H), 4.20 (t, $J = 6.0$ Hz, 2H), 7.10 (d, $J = 6.0$ Hz, 1H) and 8.20 (d, $J = 6.0$ Hz, 1H) ppm. Comparison of the ^{1}H NMR spectra of the Impurity-III and Rabeprazole reveals that the impurity signals correspond to 4-(3-methoxypropoxy)-3-methyl-2-pyridine, whereas the methylsulfinyl benzimidazole moiety is absent. The molecular formula showed the presence of four oxygen atoms in the impurity. Two oxygen atoms were assigned to the methoxy and propoxy groups. The remaining two oxygen atoms were assigned to the free acid group on the pyridine at 4-position. Thus, from the 1D LC–NMR and the LC–MS data the structure of the impurity-III was identified as 4-(3-methoxy porpoxy)-3-methyl-2-pyridine carboxylic acid.

Formation of the impurities

The formation of Impurity-I may be due to a rearrangement of Rabeprazole. Detailed studies on omeprazole and similarly rearranged impurities have been reported by Arne Brändström *et al.*[11–15] Impurity-II and Impurity-III may be formed by the cleavage of the bond adjacent to sulfur followed by oxidation.

Experimental

Stress conditions

In order to study the stability of formulated Rabeprazole, the tablets were stressed (kept at 40 °C/75% RH for 6 months in 500 count high-density polyethylene bottles, 150 cm^3).

Isolation of the impurities

The HPLC analysis of the tablets has shown *ca* 0.03–0.3% of unknown degraded impurities I, II and III at RRT's *ca* 0.17, 0.22 and 0.28, respectively. In order to accelerate the formation of unknown degraded impurities, approximately 10 g of tablet blend was kept in an autoclave at 105 °C for 5 days. The purity of the blend before and after autoclave was determined using HPLC, and it was found that the Impurity-I has increased to *ca* 3.0% while Impurity-II and Impurity-III have increased to *ca* 0.3%. The sample enriched with

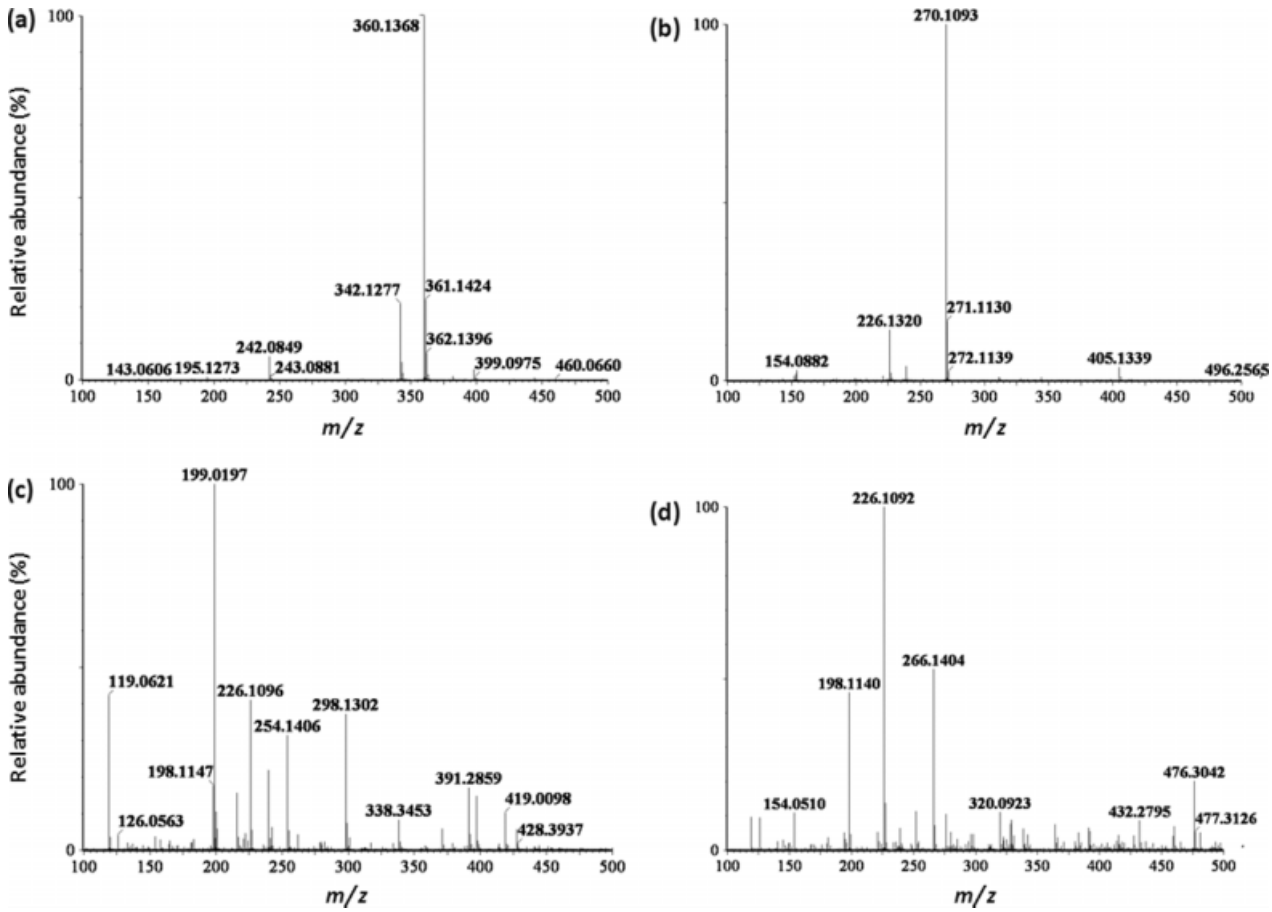

Figure 3. Protonated LC–MS spectra: (a) Rabeprazole, (b) Impurity-I, (c) Impurity-II and (d) Impurity-III. 200 × 153 mm (120 × 120 DPI).

impurities was subjected to preparative HPLC. The fractions were collected and analyzed by analytical HPLC. The Impurity-I fraction was isolated with *ca* 95% purity, whereas the Impurity-II and Impurity-III were collected together because of low resolution.

HPLC method for the separation of Rabeprazole sodium tablets

Analytical HPLC was carried out using a reversed-phase Inertsil ODS 3 V column (250 × 4.6 mm i.d., 5 µm, GL Sciences Inc., Japan) on a Waters HPLC solvent delivery system. Separations were achieved by an acetonitrile/0.01 M KH_2PO_4 isocratic elution method (35 : 65) at a flow-rate of 1.0 ml/min. The column temperature was maintained at ambient and the eluents were monitored at 280 nm.

Preparative HPLC conditions

Preparative HPLC was performed using a reversed-phase C18 column (250 × 20 mm i.d., 5 µm, (Zodiac Company) on Agilent Prep. HPLC system. Mobile phase-A was buffer (1.0 g of ammonium acetate in 1000 ml of water). Mobile phase-B was acetonitrile. Preparative HPLC was carried out at a flow-rate of 20 ml/min, gradient (T/%B, 0/5, 10/5, 11/95, 30/95, 31/5, 40/5) at ambient temperature. About 100 mg of the stressed tablet powder was dissolved in 5 ml of methanol : water (80 : 20) and injected into the preparative HPLC system using a rheodyne injector. The eluents were detected at 280 nm.

Ultra Performance Liquid Chromatography (UPLC)-TOF MS analysis

HPLC method converted into UPLC compatible method using Aquity column converter software (Water corporation, Manchester, UK), where a column Aquity UPLC BEH C18 50 × 2.1 mm 1.7 µm particle size (Water corporation, Manchester, UK) with mobile phase consisting of 0.01 M ammonium acetate filtered and acetonitrile using gradient elution T/% acetonirile 0/5, 0.23/5, 1.64/50, 2.42/85, 3.19/85, 3.51/5, 4/5, with a flow-rate of 0.5 ml/min, UV detection at 280 nm was used. This LC method was able to detect the impurities. Leucine enkephalin was used as lock spray for accurate mass measure. The mass spectra of impurities were recorded on LCT premier XE.

Stopped-flow LC–NMR analysis

LC–NMR was performed on a Varian LC–NMR instrument (Varian Associates, Inc., Palo Alto, CA) using a Pro Star pump system, a Pro Star UV detector, an INOVA 500 MHz NMR spectrometer and a microflow LC–NMR probe. The probe has $^{1}H\{^{13}C\}$ channels with pulsed-field gradient along *z*-axis. The active sample volume of the probe was approximately 60 µl and the transfer time from the UV cell to the active volume was calibrated to be 21 s at a flow-rate of 1.0 ml/min. LC–NMR compatible chromatographic method was developed for the analysis of Rabeprazole sodium and its degradation products where a column Zorbax SB CN

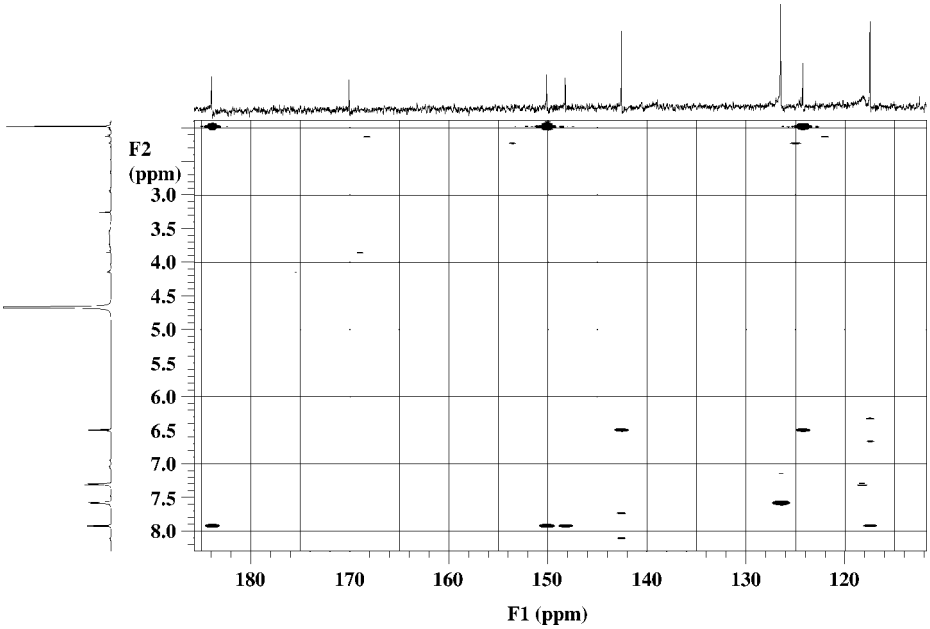

Figure 4. HMBC spectrum of Impurity-I 1397 × 1079 mm (120 × 120 DPI).

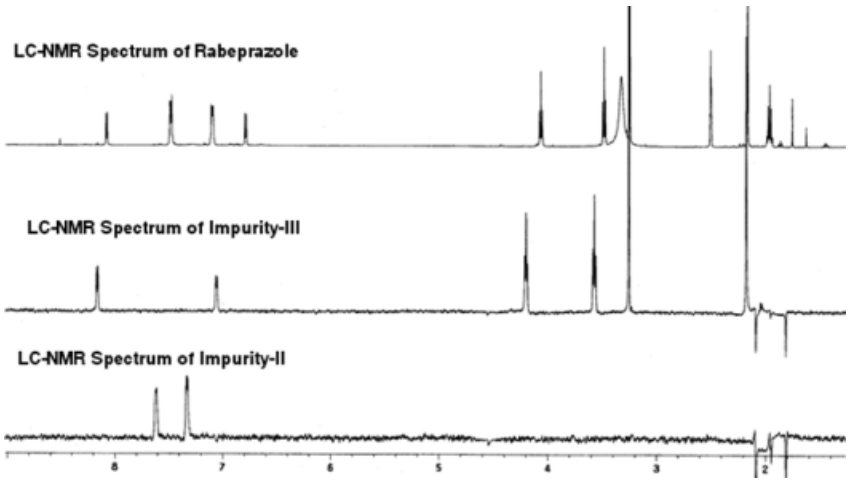

Figure 5. Overlaid 1D LC–NMR spectra ($CH_3CN + D_2O$) of Impurity-II, Impurity-III with Rabeprazole sodium pure in DMSO-d_6 136 × 108 mm (120 × 120 DPI).

250 mm × 4.6 mm, 5.0 µm particle size (Agilent Technologies, UK) with a mobile phase consisting of NMR Chromasolv® acetonitrile (Riedel-de Haen) and D_2O (99.9%, Cambridge Isotope Laboratory, MA) using gradient elution T/% acetonirile: 0/5, 1.0/5, 10/85, 15/85, 12/95, 20/95, 25/5, 35/5, with a flow-rate of 1.0 ml/min, and the UV detection at 280 nm was used. The column was kept at ambient temperature and the eluent was monitored via UV detection at 280 nm. ^{1}H NMR experiments were performed in 'stopped-flow' mode where the HPLC flow was halted after the sample elution fraction was transferred to the LC–NMR probe which was equilibrated at 25 °C. Pulse sequences '*lc1d*' from the Standard Varian Pulse Sequence Library were used. Double solvent suppression was applied on the ^{1}H NMR resonances of water and acetonitrile. 1D ^{1}H NMR spectra were recorded into 32.000 data points with a spectral width of 9.500 Hz and 1.7 s of acquisition time. A total of 2.000 transients were collected in approximately 1.5 h for each 1D ^{1}H NMR spectrum.

Conclusions

Three unknown impurities of Rabeprazole sodium tablets were generated during the accelerated stressed conditions. The most polar impurity-I was isolated by preparative HPLC and characterized by NMR and MS. The low-resolution impurities II and III were characterized by the HPLC-hyphenated techniques LC–NMR and LC–MS-TOF. The LC–MS spectra provided the molecular formulae of Impurity-II and Impurity-III, and the molecular structures were elucidated by LC–NMR analysis. Complementary use of these two hyphenated techniques facilitated in the unambiguous structure identification of the degradation products.

Online combination of these analytical tools (HPLC, NMR and MS) brings superior efficiency to their uncoupled use in the identification and structural determination of individual chemical components in the complex mixtures. Such advanced methods will be utilized for structure elucidation of impurities of other active ingredients.

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