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Short communication

Identification, isolation and characterization of new impurity in rabeprazole sodium

P. Sreenivasa Rao[a], Uttam Kumar Ray[a,*], P. Badarinadh Gupta[a], D.V.N. Srinivasa Rao[a], Aminul Islam[a], Pradeep Rajput[a], K. Mukkanti[b]

[a] Department of Chemical Research, Aurobindo Pharma Ltd. Research Centre, 313, Bachupally, Quthubullapur Mandal, Hyderabad 500090, India
[b] Department of Chemistry, J.N.T. University, Kukatpally, Hyderabad 500085, India

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ABSTRACT

Rabeprazole sodium [1] is a proton pump inhibitor, used as an antiulcerative. During the manufacturing of rabeprazole sodium, we observed an unknown impurity at levels 0.05–0.1% in HPLC analysis along with the known potential impurities. This new unknown impurity was isolated using preparative liquid chromatography. Based on the complete spectral analysis (^{1}H NMR, ^{13}C NMR, DEPT, Mass and IR), this new impurity was designated as 2-[[(3-methyl-4-(methylthio)-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole (methylthio impurity of rabeprazole). Impurity isolation, structure elucidation and probable formation mechanism was discussed.

1. Introduction

Rabeprazole sodium [1], chemically known as 2-[[(4-(3-methoxypropoxy)-3-methyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole, is a proton pump inhibitor and used as an antiulcerative. Several analytical methods have been reported in the literature for the determination of rabeprazole and its impurities [2–6]. During the preparation of rabeprazole sodium [7,8] (Fig. 1) one unknown impurity was detected consistently in HPLC analysis along with the potential known impurities (Fig. 2). The HPLC analysis of rabeprazole sample has been performed and described as in Section 2.2. As per regulatory requirement [9], new impurity present above 0.10% level in the drug substance need to be identified and characterized.

2. Experimental

2.1. Samples

The investigated samples of rabeprazole and known impurities were prepared in APL Research Centre (A unit of Aurobindo Pharma Limited, Hyderabad, India). Reagents used for analysis, i.e., ammonium acetate (AR grade), methanol (HPLC grade) and acetonitrile (HPLC grade) were obtained from Merck (India) Limited. Milli-Q grade water was used.

2.2. High performance liquid chromatography (analytical)

A Waters 2695 separation module equipped with 2996 photo diode array detector with Empower pro data handling system (Waters Corporation, Milford, MA, USA) was used. The analysis was carried out on YMC C8, 150 mm long, 4.6 mm i.d., and 5-μm particle size column. Mobile phase A consists a phosphate buffer (pH 7.6 ± 0.05) and acetonitrile in the ratio of 98:2 (v/v). The phosphate buffer was prepared by dissolving 3.2 g of dipotassium hydrogen phosphate and 0.85 g of potassium dihydrogen phosphate in 1000 mL of water and solution pH was adjusted to 7.6 ± 0.05 with 5N potassium hydroxide solution. Mobile phase B was acetonitrile. UV detection was at (set) 284 nm and flow rate 1.0 mL/min. Data acquisition time was 40 min. The gradient program as follows: time (min)/A (v/v):B (v/v); $T_{0.01}$/90:10, $T_{10.0}$/80:20, $T_{25.0}$/65:25, $T_{30.0}$/50:50, $T_{40.0}$/25:75, $T_{42.0}$/10:90, and $T_{50.0}$/10:90.

2.3. Isolation of impurity (methylthio impurity) by preparative HPLC

A Shimadzu LC-8A Preparative Liquid Chromatograph equipped with SPD-10A VP, UV–Vis detector (Shimadzu Corporation, Analytical Instruments Division, Japan), Symmetry C18 (250 mm long × 19 mm i.d.) preparative column packed with 7 μm parti-

* Corresponding author. Tel.: +91 40 23040261; fax: +91 40 23042932.
E-mail address: raykuttam@gmail.com (U.K. Ray).

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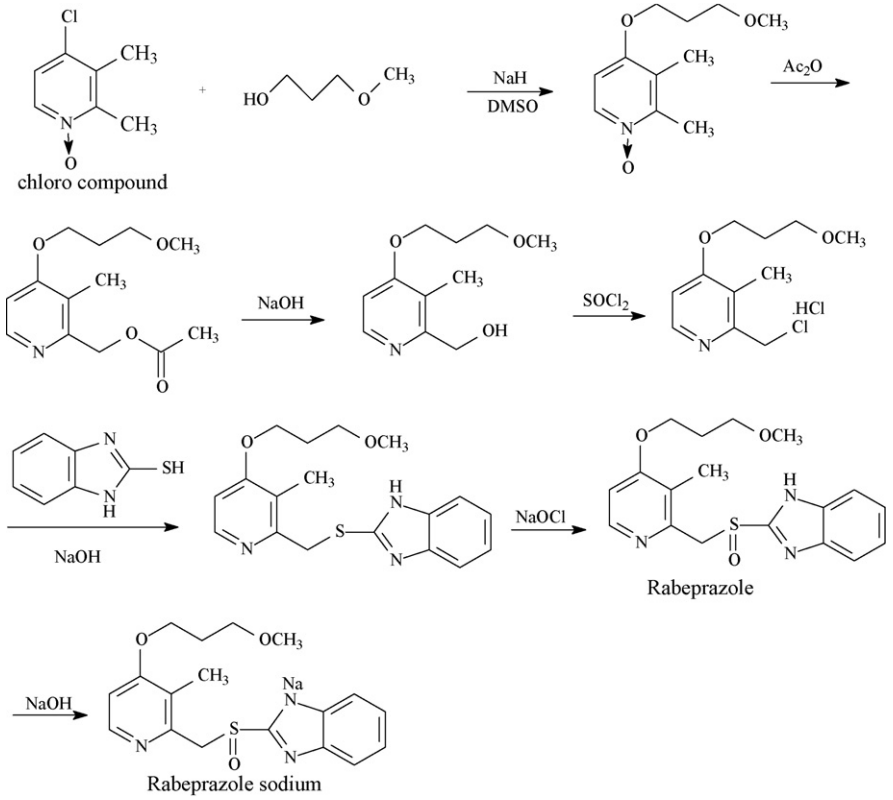

Fig. 1. Scheme for the synthesis of rabeprazole sodium.

cle size (Waters, USA) was used for isolation of new impurity. The mobile phase of (A) 0.1 M ammonium acetate solution and (B) methanol and acetonitrile are in the ratio of 70:30. Flow rate was kept at 20 mL/min and detection was carried out at 284 nm. The gradient program was as follows: time (min)/A (v/v):B (v/v); $T_{0-0.01}$/100% A; $T_{0.01-50}$/95% A: 5% of 75:25 B; T_{50-80}/90% A: 10% of 70:30 B; T_{80-100}/50% A: 50% of 30:70 B; $T_{100-110}$/100% A. Rabeprazole sodium samples containing the new impurity at ~0.1% level (determined by the method given in Section 2.2) were dissolved at 50 mg/mL in the mobile phase for the preparative HPLC. Injection volume is 10 mL. Peak cut criteria was set based on peak retention time. Fractions >95% purity were pooled together and concentrated by rotavapour to remove solvents. Concentrated fraction was passed through the preparative HPLC column using water as

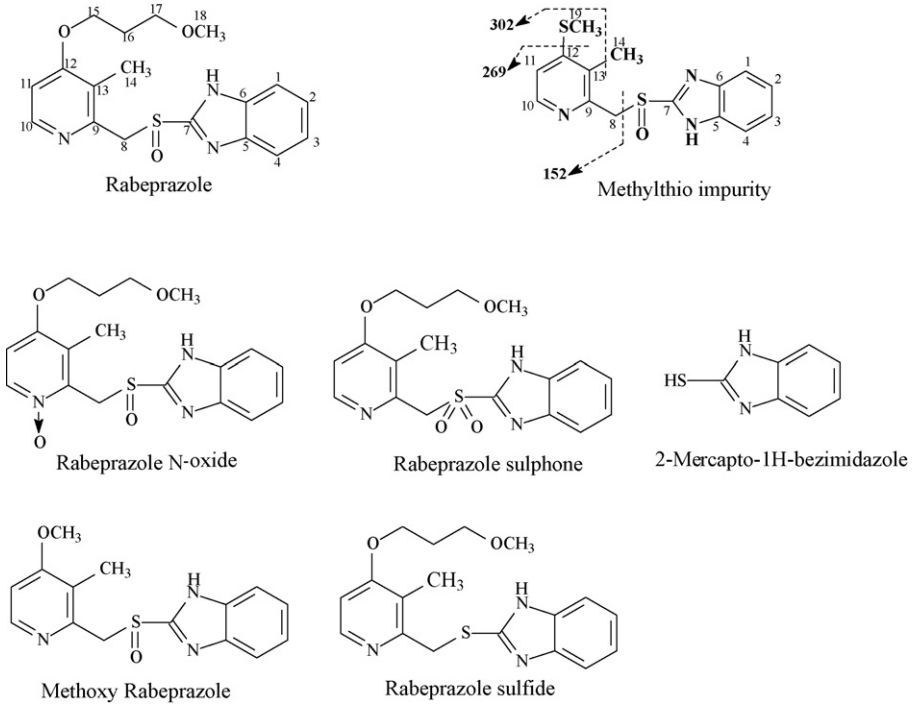

Fig. 2. Chemical structures of rabeprazole impurities.

mobile phase to remove buffer and then compound was eluted with a mixture of water and equal volumes of methanol and acetonitrile (50:50). Again the elute was concentrated using rotavapour to remove solvents then lyophilized using freeze dryer (Virtis advantage 2XL) to obtain a white powder with 96% purity.

2.4. LC–MS/MS analysis

LC–MS/MS analysis was carried out using Perkin Elmer triple quadrupole mass spectrometer (Perkin Elmer, API 2000, PE SCIEX) coupled with Shimadzu HPLC equipped with SPD 10A VP UV–Vis

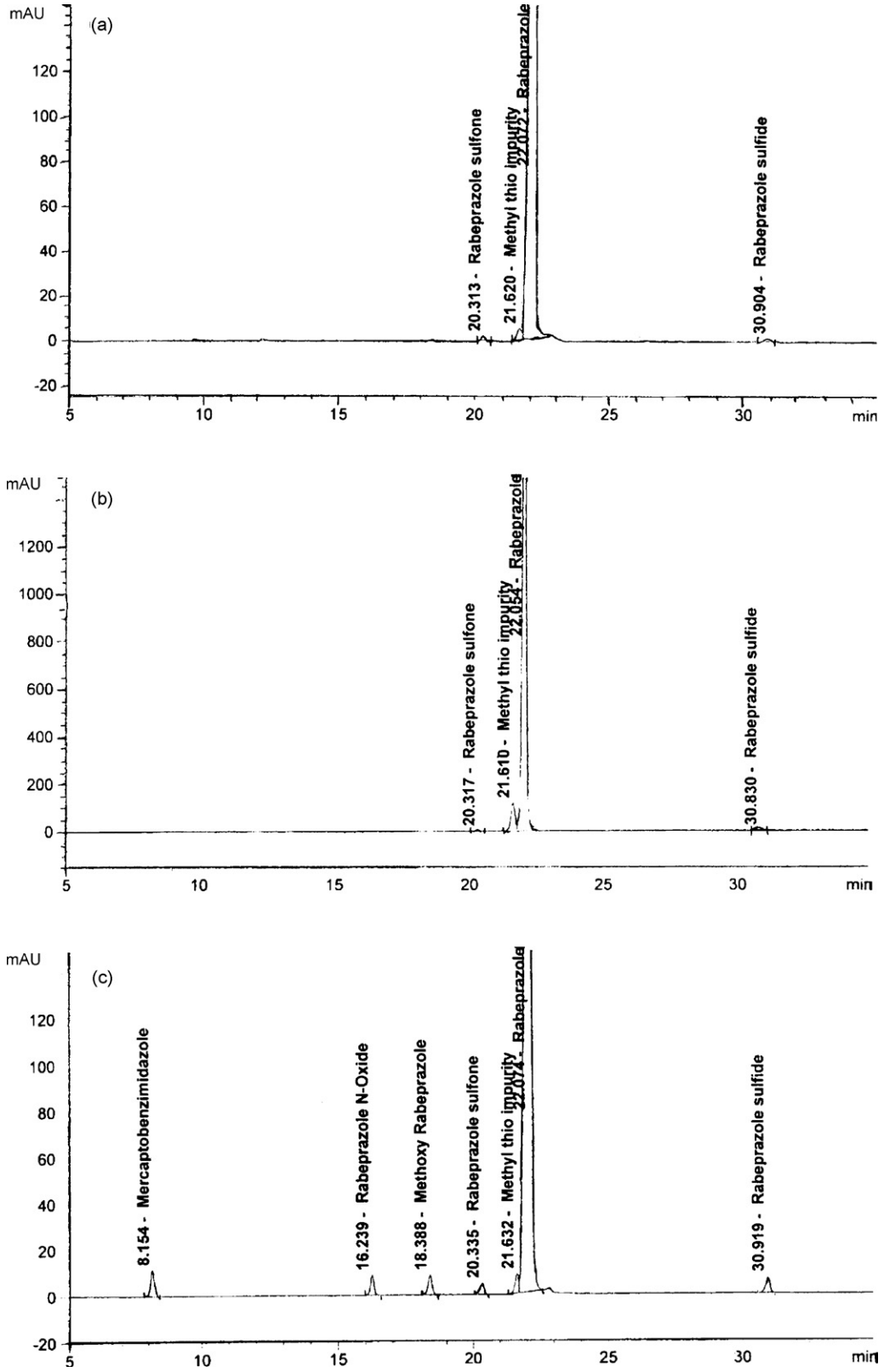

Fig. 3. LC-chromatogram of (a) rabeprazole sample, (b) rabeprazole sample spiked with methylthio impurity and (c) rabeprazole impurities mixture.

Table 1
Comparative ^{1}H, ^{13}C (proton decoupled) and DEPT NMR assignments for rabeprazole and methylthio impurity.

Position[a]	Rabeprazole			Methylthio impurity		
	^{1}H, δ (ppm) multiplicity	^{13}C, δ (ppm)	DEPT	^{1}H, δ (ppm) multiplicity	^{13}C, δ (ppm)	DEPT
1	7.58 (brs, 1H)	106.6	CH	7.36 (brs, 1H)	117.8	CH
2	7.28 (m, 1H)	124.0	CH	7.68 (m, 1H)	124.0	CH
3	7.28 (m, 1H)	124.0	CH	7.68 (m, 1H)	124.0	CH
4	7.58 (brs, 1H)	100.6	CH	7.36 (brs, 1H)	117.8	CH
5	–	149.7	C	–	147.8	C
6	–	149.7	C	–	147.8	C
7	–	153.6	C	–	151.6	C
8	4.78 and 4.83 (ABq, 2H)	69.2	CH_2	4.66 and 4.85 (ABq, 2H)	61.4	CH_2
9	–	164.0	C	–	153.4	C
10	8.29 (d, 1H)	148.7	CH	8.30 (d, 1H)	146.8	CH
11	6.70 (d, 1H)	148.7	CH	7.01 (d, 1H)	146.8	CH
12	–	153.6	C	–	151.6	C
13	–	123.0	C	–	130.2	C
14	2.12 (s, 3H)	11.4	CH_3	2.49 (s, 3H)	15.5	CH_3
15	4.07 (t, 2H)	61.2	CH_2	–	–	–
16	2.03 (m, 2H)	29.7	CH_2	–	–	–
17	3.51 (t, 2H)	65.5	CH_2	–	–	–
18	3.34 (s, 3H)	59.2	CH_3	–	–	–
19	–	–	–	2.29 (s, 3H)	14.7	CH_3

[a] Refer chemical structures in Fig. 2 for numbering of rabeprazole and methylthio impurity. s: singlet; d: doublet; dd: doublet of a doublet; m: multiplet.

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 to 1000 in 0.1 amu steps with 2.0 s dwell time. Rabeprazole sample was subjected to LC–MS/MS analysis. The analysis was carried out using YMC Pack C8, 150 mm × 4.6 mm column (YMC Corporation, USA) with 5 μm particle. Mobile phase A consists of 0.01 M ammonium acetate solution and acetonitrile in the ratio of 50:50 (v/v). Mobile phase B consists of acetonitrile. Flow rate was 1.0 mL/min and gradient program, time (min)/A (v/v):B (v/v); $T_{0.01}$/90:10, $T_{10.0}$/75:25, $T_{30.0}$/55:45, $T_{40.0}$/25:75, $T_{41.0}$/90:10, and $T_{50.0}$/90:10. Potential impurities were detected in rabeprazole sample. The detected peak masses were identical to the values of the known impurities. Additionally, 317 mass observed in rabeprazole.

2.5. NMR spectroscopy

The ^{1}H, ^{13}C NMR (proton decoupled) and DEPT spectra were recorded on Bruker 300 MHz spectrometer using DMSO-d_6 as solvent and tetramethylsilane as internal standard.

2.6. FT-IR spectroscopy

IR spectrum was recorded as KBr pellet on Perkin Elmer instrument model spectrum one.

3. Results and discussion

3.1. Detection of impurities

Rabeprazole sodium samples were analyzed by the HPLC method described in Section 2.2. These samples were subjected to LC–MS/MS analysis using the method described in Section 2.4. The chemical structures of these impurities are shown in Fig. 2. The known impurities and isolated methylthio impurity were co-injected with rabeprazole to confirm the retention times. All the impurities were well resolved from rabeprazole peak and the representative rabeprazole impurities mixture chromatogram was shown in Fig. 3.

3.2. Structural elucidation of methylthio impurity

The molecular ion peak m/z 317.9[$(MH)^+$] by LC–MS analysis indicated a mass of 317, which was 42 amu less than rabeprazole. The isotopic [M+2] peak is 9.5% of the intensity of M, suggesting the presence of two sulfur atoms. The major fragment ions m/z 302, 269, and 152 resemble the probable fragmentation pattern of methylthio impurity (Fig. 2).

In ^{1}H NMR and ^{13}C NMR spectra of new impurity show chemical shift similarity with rabeprazole, except signals corresponding to methoxypropoxy moiety. In addition, a singlet of three protons at δ 2.29 ppm in ^{1}H NMR and at δ 14.4 ppm in ^{13}C NMR are characteristic signal of $-CH_3$. Moreover, in comparison with rabeprazole ^{1}H NMR, a significant downfield chemical shift was observed for the positions C-11 and C-14 (Fig. 2) from δ 6.70 ppm and δ 2.12 ppm to 7.01 ppm and 2.49 ppm respectively. In ^{13}C NMR spectra, C-11 and C-14 were shifted to δ 146.8 ppm and 15.5 ppm from δ 148.7 ppm and 11.4 ppm respectively. Comparative ^{1}H NMR, ^{13}C NMR and DEPT spectral data for rabeprazole and new impurity is given in Table 1.

The IR spectra of new impurity show the following absorption bands 3196 (NH stretch), 2984, 2922 (aliphatic CH stretch), 1567, 1542, 1491 (C=C and C=N stretch), 1218 (C–N stretch), 1058 (S=O stretch) and 735 (C–S).

The elemental analysis data calculated for $C_{15}H_{15}N_3OS_2$ (317): C, 56.68; H, 4.73; N, 13.25; S, 20.20 and found: C, 56.71; H, 4.71; N, 13.24; S, 20.25. From the above spectral data, the impurity was confirmed as 2-[[(3-methyl-4-(methylthio)-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole.

3.3. Formation of methylthio impurity

This impurity is believed to be originated from the reaction of 4-chloro-2,3-lutidine-N-oxide (chloro compound) with methyl mercaptan, which was formed by decomposition of dimethyl sulfoxide [10] during the preparation of rabeprazole (Fig. 1).

4. Conclusion

The process related new impurity in rabeprazole sodium which was isolated, identified, and characterized using HPLC (analytical and preparative), MS, NMR and IR techniques as the methylthio impurity.

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