CHAPTER 4

Omeprazole

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Contents

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Profiles of Drug Substances, Excipients, and Related Methodology, Volume 35
ISSN 1871-5125, DOI: 10.1016/S1871-5125(10)35004-7

1. DESCRIPTION

1.1. Nomenclature

1.1.1. Systematic chemical names

- (*RS*)-5-Methoxy-2-(4-methoxy-3,5-dimethyl-2-pyridinyl-methyl-sulfinyl)-benzimidazole
- 5-Methoxy-2-[[(4-methoxy-3,5-dimetyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole
- 5-Methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole
- 2-[[(3,5-Dimethyl-4-methoxy-2-pyridyl)methyl]sulfinyl-5-methoxy]-1*H*-benzimidazole [1–4]

1.1.2. Nonproprietary names

Omeprazole, H-168/68, Omeprazol, Omeprazolas, Omeprazolum, Omepratosoli [1, 2].

1.1.3. Proprietary names

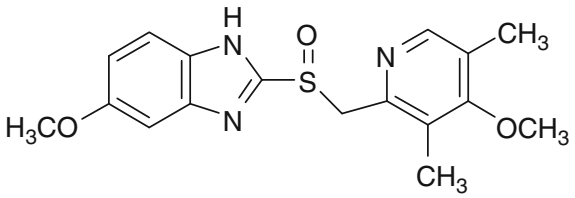
Antra, Gastroloc, Gastroguard, Logastric, Losec, Mepral, Mopral, Omapren, Omelich, Omelind, Omepral, Omeprazen, Omeprazole, Ompanyt, Osiren, Parizac, Pepticum, Prilosec, Prilosico, Zegerid, Zoltum [1, 3].

1.2. Formulae

1.2.1. Empirical, molecular weight, and CAS number

Omeprazole	$C_{17}H_{19}N_3O_3S$	345.42	[73590-58-6]
Omeprazole sodium	$C_{17}H_{18}N_3O_3S,Na$	367.42	[95510-70-6]
Omeprazole magnesium	$C_{34}H_{36}MgN_6O_6S_2$	713.1	[95382-33-5]

1.2.2. Structural formula



1.3. Elemental analysis

C 59.11%, H 5.54%, N 12.16%, O 13.90%, S 9.28%.

1.4. Appearance

Omeprazole: A white or almost white powder [3].
Omeprazole sodium: A white or almost white powder, hygroscopic [3].

1.5. Uses and applications

Omeprazole is a proton pump inhibitor which inhibits secretion of gastric acid by irreversibly blocking the enzyme system of hydrogen/potassium adenosine triphosphatase (H^+/K^+-ATPase), the ''proton pump'' of the gastric parietal cell. The drug is used in conditions where the inhibition of gastric acid secretion may be beneficial, including aspiration syndromes [5], dyspepsia [6], gastro-oesophageal reflux disease [7], peptic ulcer disease [8], and the Zollinger–Ellison syndrome [9]. Esomeprazole which is an isomer of omeprazole is also used [10]. The dose of omeprazole may need to be reduced in patients with hepatic impairment [1, 11].

Omeprazole may be given by mouth as the base or magnesium salt or intravenously as the sodium salt. Doses are expressed in terms of the base. Omeprazole magnesium 10.32 mg and omeprazole sodium 10.64 mg are each equivalent to about 10 mg of omeprazole. For the relief of the acid-related dyspepsia, the drug is given in usual doses of 10 or 20 mg daily by mouth for 2–4 weeks. The usual dose for the treatment of gastro-oesophageal reflux disease is 20 mg by mouth once daily for 4 weeks,

followed by a further 4–8 weeks if not fully healed. In refractory oesophagitis, a dose of 40 mg daily may be used. Maintenance therapy after healing of oesophagitis is 20 mg once daily, and for acid reflux is 10 mg daily. In children, over 1 year of age, licensed UK oral doses for treatment are 10 mg daily in those weighing 10–20 kg, and 20 mg daily in those weighing over 20 kg. These doses may be doubled if necessary. The British National Formulary for Children (BNFC) recommends a dose of 700 μg/kg daily in children 1 month to 2 years of age, increased if necessary up to 3 mg/kg daily, or 20 mg daily, whichever is less. Similar initial doses are suggested in neonates [1].

In the management of peptic ulcer a single daily dose of 20 mg by mouth, or 40 mg in severe cases, is given. Treatment is continued for 4 weeks for duodenal ulcer and 8 weeks for gastric ulcer. Where appropriate, a dose of 10–20 mg once daily may be given for maintenance [1].

For the eradication of *Helicobacter pylori* in peptic ulceration, omeprazole may be combined with antibacterials in dual or triple therapy. Effective triple therapy regimens include omeprazole 20 mg twice daily combined with: amoxycillin 500 mg and metronidazole 400 mg, both three times daily; clarithromycin 500 mg and metronidazole 40 mg (or tinidazole 500 mg) both twice daily; or with amoxycillin 1 g and clarithromycin 500 mg both twice daily. These regimens are given for 1 week. Dual therapy regimens, such as omeprazole 40 mg daily with either amoxycillin 750 mg to 1 g twice daily or clarithromycin 500 mg three times daily, are less effective and must be given for 2 weeks. Omeprazole alone may be continued for a further 4–8 weeks [1].

Doses of 20 mg are used in the treatment of Non-steroidal Anti-inflammatory Drugs (NSAID)-associated ulceration; a dose of 20 mg daily may also be used for prophylaxis in patients with a history of gastroduodenal lesions who require continued NSAID treatment [1].

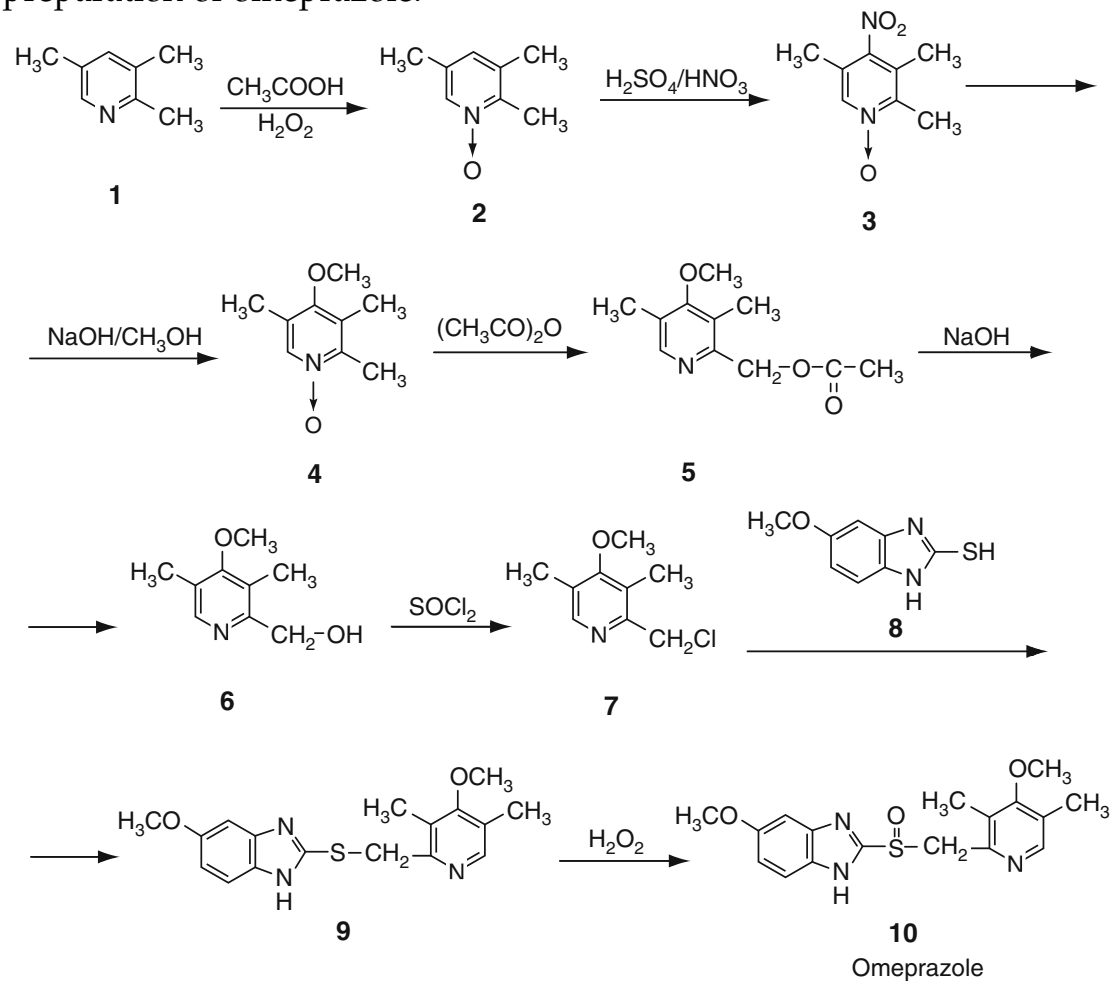
The initial recommended dosage for patients with the Zollinger–Ellison syndrome is 60 mg by mouth once daily, adjusted as required. The majority of patients are effectively controlled by doses in the range 20–120 mg daily, but doses up to 120 mg three times daily have been used. Daily doses above 80 mg should be given as divided doses (usually 2) [1].

Omeprazole is also used for the prophylaxis of acid aspiration during general anesthesia, in dose of 40 mg the evening before surgery and a further 40 mg twice to 6 h before the procedure [1].

Patients who are unsuited to receive oral therapy, omeprazole sodium may be given on a short-term basis by intravenous infusion, in a usual dose equivalent to 40 mg of the base over a period of 20–30 min in 100 ml of sodium chloride 0.9% or glucose 5%. It may also be given by slow intravenous injection. Higher intravenous doses have been given to patients with Zollinger–Ellison syndrome [1].

2. METHODS OF PREPARATION

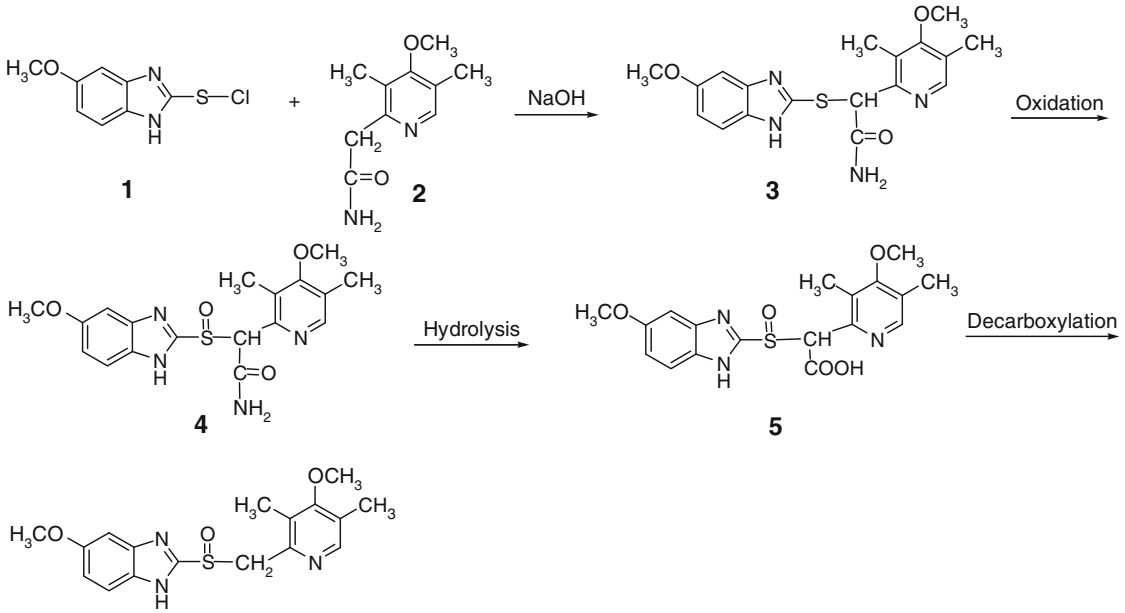
2.1. Brandstrom and Lamm [12] used the following method for the preparation of omeprazole:



2,3,5-Trimethyl pyridine **1** was oxidized by hydrogen peroxide in acetic acid to give the *N*-oxide **2** and the latter was nitrated using a mixture of sulfuric acid and nitric acid to give the 4-nitro derivative **3**. The nitro group in **3** was displaced by hydroxymethylation to yield **4**. Treatment of compound **4** with acetic acid anhydride reduces the ring and forms an ester derivative **5**. The corresponding alcohol **6** was formed by the treatment with base, followed by displacement of the hydroxyl group with a chloride using thionyl chloride to give 2-chloromethyl-4-methoxy-2,3,5-trimethyl pyridine **7**. The benzimidazole portion **8** displaces the chloride giving 5-methoxy-2-[((4-mrthoxy-3,5-dimethyl-2-pyridinyl)methyl)thio]-1*H*-benzimidzole **9**. Omeprazole **10** is formed in a final step where the thioether group is oxidized by hydrogen peroxide to the corresponding sulfoxide.

2.2. Slemon and Macel [13] used several intermediates for the preparation of omeprazole. The drug was produced from the corresponding acetamide–sulfide compounds by a process of oxidation to form the amide sulfinyl compound, followed by alkaline hydrolysis to the sulfinyl carboxylate or salt, and decarboxylation. The methods are as follows:

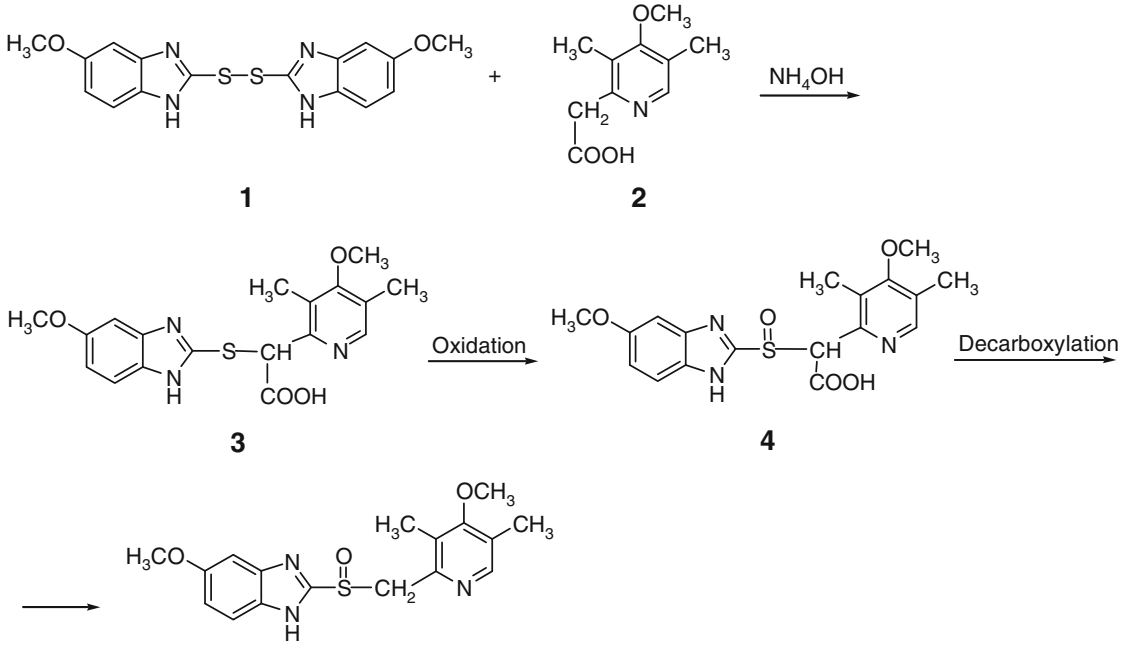
2.2.1. Reaction of 5-methoxy-2-chloromercaptobenzimidazole **1** with 3,5-dimethyl-4-methoxy-2-methylamidopyridine **2** yielded the acetamide thioether **3**. Compound **3** was oxidized to give the acetamide sulfoxide **4**. The acetamide **4** was hydrolyzed to give the carboxylate **5** which was decarboxylated to yield omeprazole **6**.



6

Omeprazole

2.2.2. Reaction of 2-*S*-*S*-bis(5-methoxybenzimidazole) **1** with 3,5-dimethyl-4-methoxy-2-pyridine methyl carboxylate **2** followed by reaction with ammonia to give the carboxylate thioether **3**. Compound **3** was oxidized to give the carboxylate sulfoxide **4** which was decarboxylated to give omeprazole **5**.



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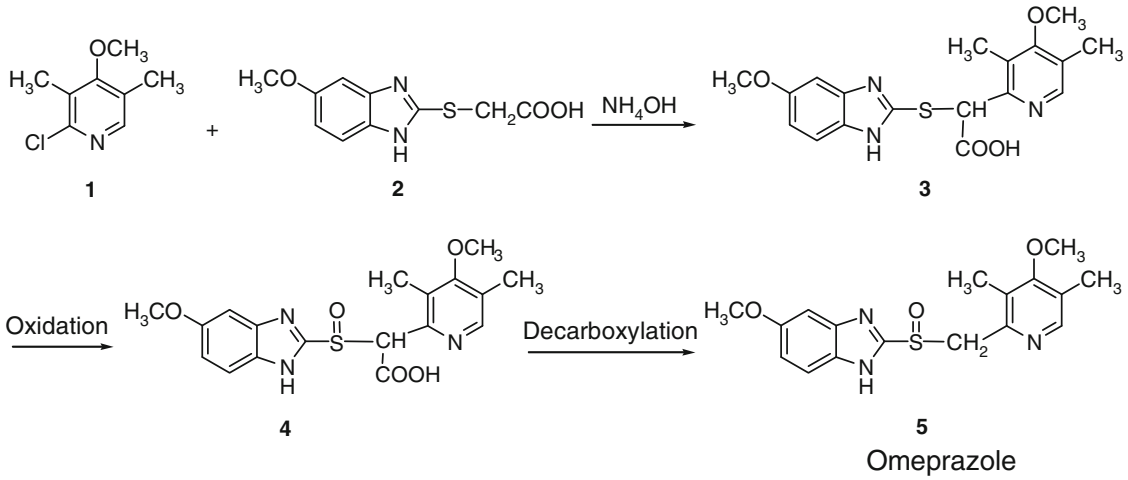
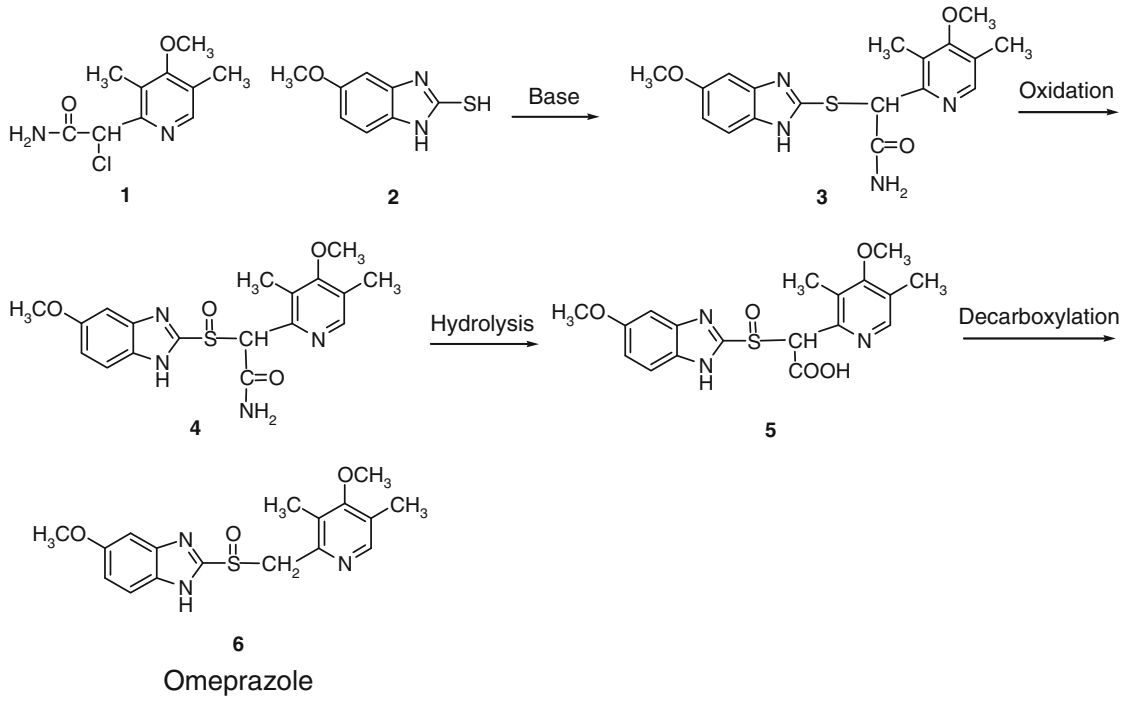
Omeprazole

2.2.3. Reaction of 3,5-dimethyl-4-methoxy-2-chloropyridine **1** with 5-methoxy-2-(methylcarboxylate)-thiobenzimidazole **2** followed by treatment with ammonia to give the carboxylate thioether **3**. Compound **3** was oxidized to give the carboxylate sulfoxide **4** which is decarboxylated to give omeprazole **5**.

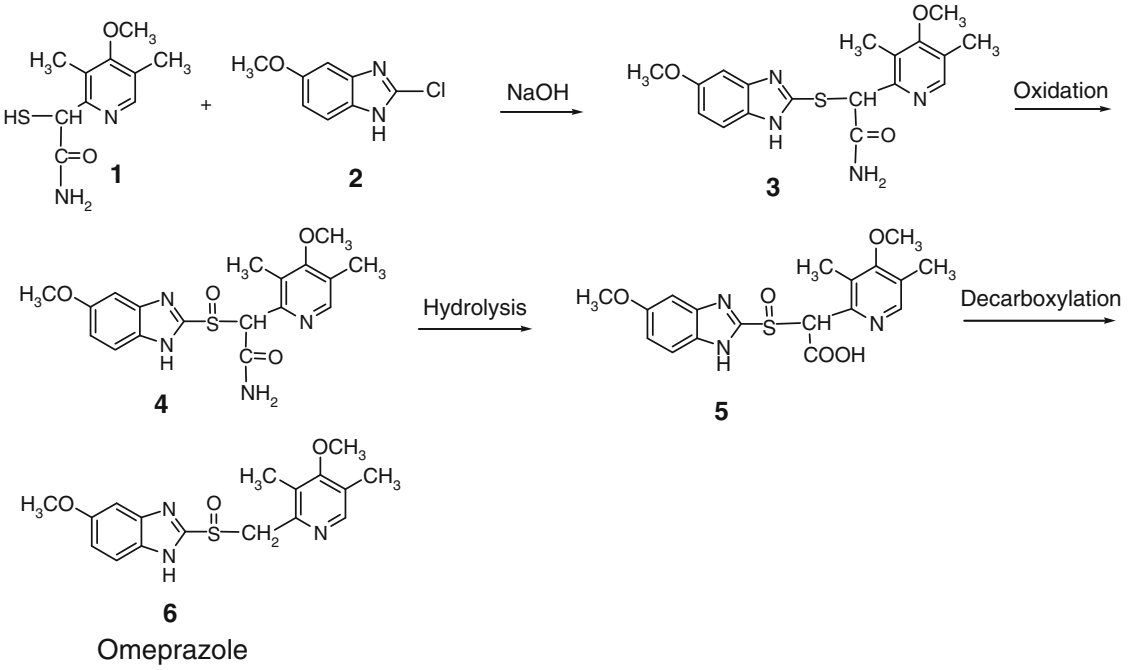


2.2.4. Reaction of 3,5-dimethyl-4-methoxy-2-chloromethylamido pyridine **1** with 5-methoxy-2-mercaptoimidazole **2** in a base to yield the acetamide thioether **3**. Compound **3** was oxidized to the acetamide sulfoxide **4**. Compound **4** was hydrolyzed to yield the carboxylate sulfoxide **5** which was then decarboxylated to give omeprazole **6**.

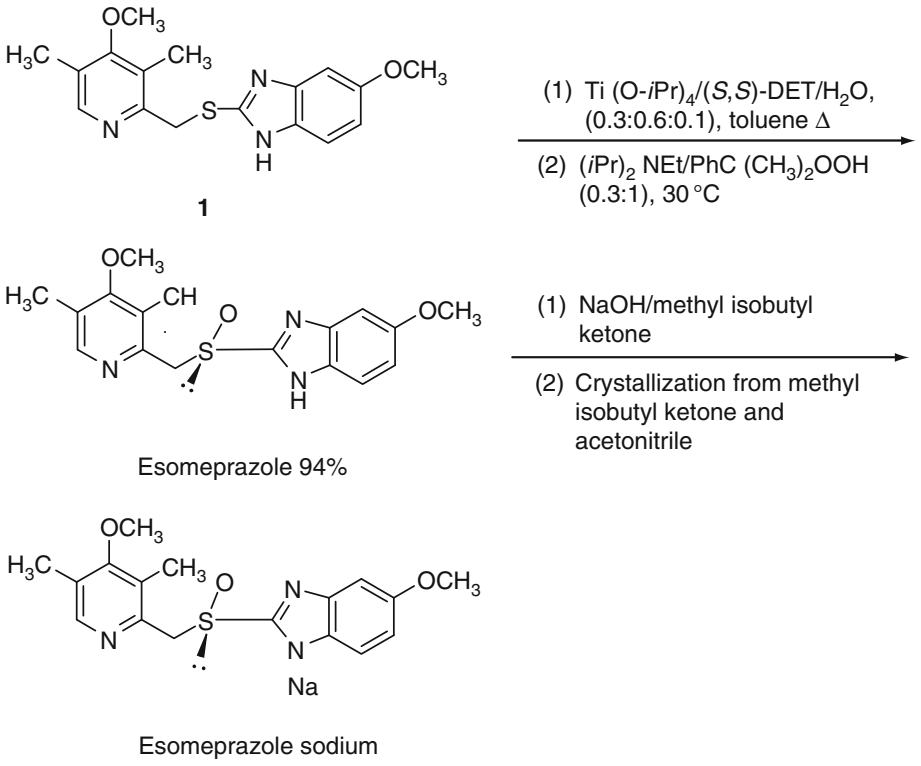


2.2.5. Reaction of 3,5-dimethyl-4-methoxy-2-mercaptomethyl amidopyridine **1** with 5-methoxy-2-chlorobenzimidazole **2** in a base to yield to the acetamide thioether **3**. Compound **3** was oxidized to give the

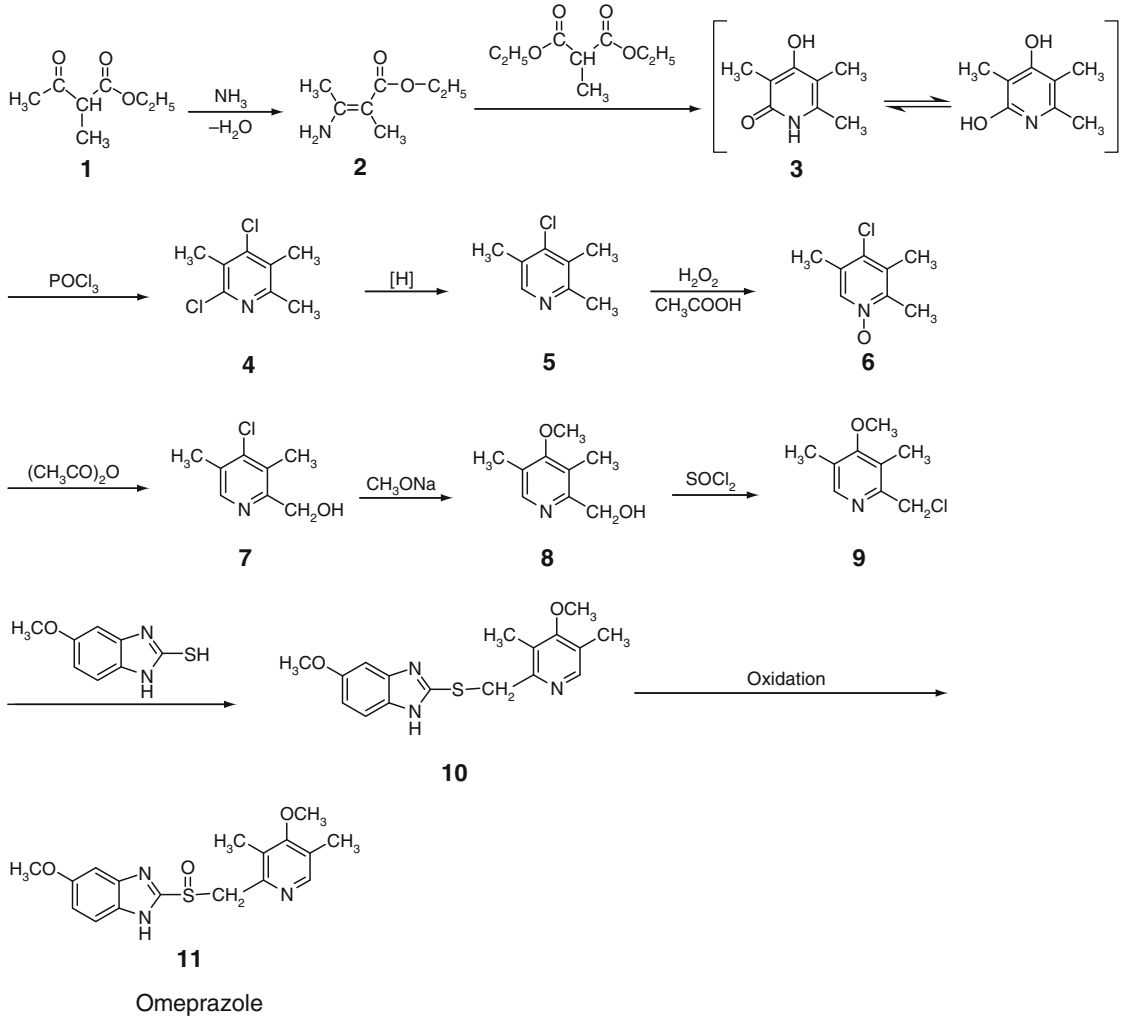
acetamide sulfoxide **4**. Compound **4** was hydrolyzed to yield the carboxylate sulfoxide **5** which was decarboxylated to give omeprazole **6**.



2.3. Cotton *et al.* [14] described an asymmetric synthesis of esomeprazole. Esomeprazole, the (*S*)-enantiomer of omeprazole, was synthesized via asymmetric oxidation of prochiral sulfide 5-methoxy-2-[[(4-methoxy-3,5-dimethyl pyridin-2-yl)methyl]thio]-1*H*-benzimidazole **1**. The asymmetric oxidation was achieved by titanium-mediated oxidation with cumene hydroperoxide in the presence of (*S*,*S*)-diethyl tartarate (DET). The enantioselectivity was provided by preparing the titanium complex in the presence of sulfide **1** at an elevated temperature and/or during a prolonged preparation time and by performing the oxidation of sulfide **1** in the presence of amine. An enantioselectivity of ~94% ee was obtained using this method.



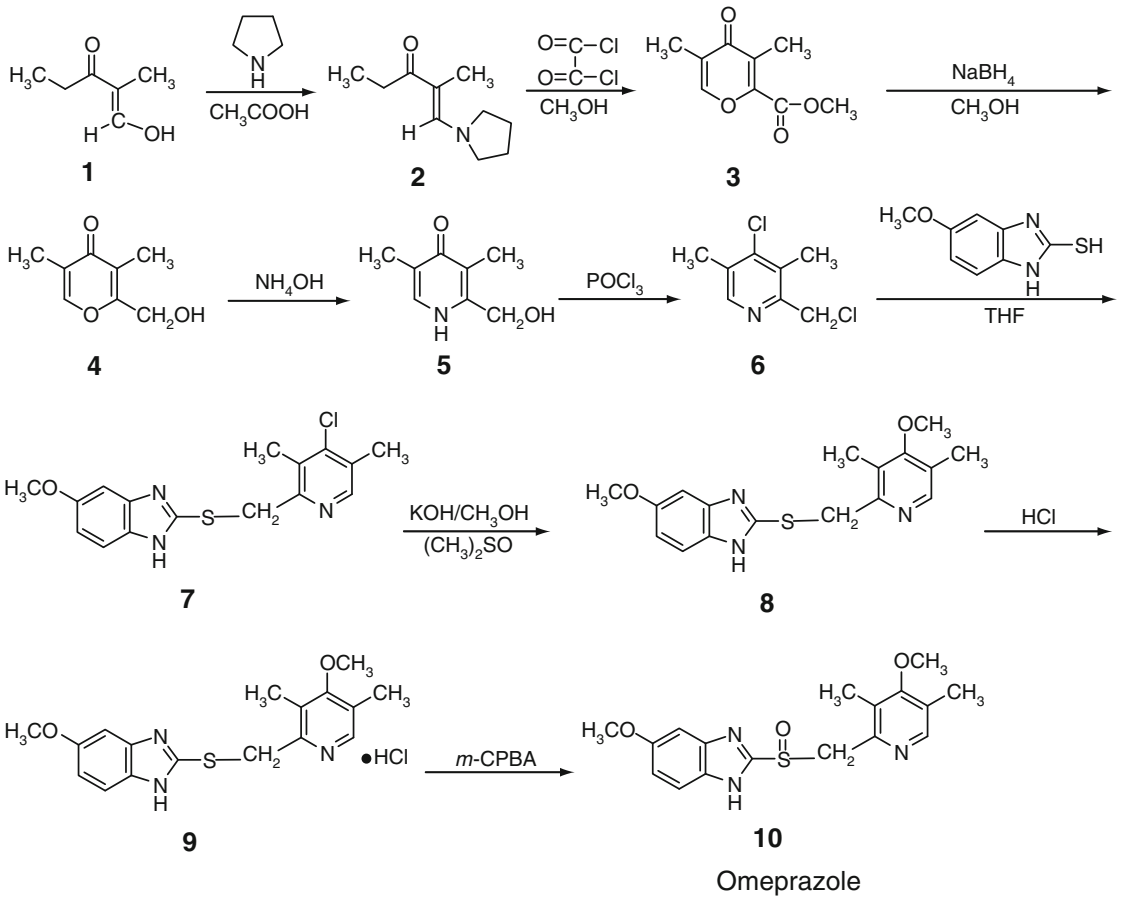
2.4. Omeprazole is obtained [15] by the reaction of acetyl ethyl propionate **1** with ammonia to give ethyl -3-amino-2,3-dimethyl acrylate **2**. Compound **2** was converted to to 2,4-dihydroxy-3,5,6-trimethyl pyridine **3** by treatment with methyl diethylmalonate. Treatment of compound **3** with phosphorous oxychloride produced 2,4-dichloro-3,5,6-trimethyl pyridine **4**. 4-Chloro-3,5,6-trimethyl pyridine **5** was obtained by treatment of compound **4** with hydrogen. On treatment of compound **5** with hydrogen peroxide and acetic acid, 4-chloro-3,5,6-trimethyl-pyridine-*N*-oxide **6** was produced. Treatment of compound **6** with acetic anhydride gave 4-chloro-2-hydroxymethyl-3,5-dimethyl pyridine **7** which was converted to 2-hydroxymethyl-3,5-dimethyl-4-methoxypyridine **8** by treatment with sodium methoxide. Compound **8** was treated with thionyl chloride to produce 2-chloromethyl-3,5-dimethyl-4-methoxypyridine **9**. Compound **9** interacts with 5-methoxy-2-mercaptobenzimidazole to give 5-methoxy 2-[((4-methoxy-3,5-dimethyl-2-pyridinyl)methyl)thio]-1*H*-benzimidazole **10** which is oxidized to omeprazole **11**.



2.5. Baldwin *et al.* [16] used the following scheme for the preparation of omeprazole:

2-Methyl-1-penten-3-one-1-ol **1** and glacial acetic acid in benzene was added to pyrrolidine to give 2-methyl-1-penten-1-[*N*-pyrrolidinyl]-3-one **2**. Compound **2** when treated with oxalyl chloride and methanol was added, 3,5-dimethyl-2-methoxycarbonyl-4-pyrone **3** was produced. Treatment of compound **3** with sodium borohydride in methanol gives 3,5-dimethyl-2-hydroxymethyl-4-pyrone **4**. Compound **4** was converted to 3,5-dimethyl-2-hydroxymethyl-4-pyridone **5** by heating compound **4** with aqueous ammonia in a sealed flask. Compound **5** was converted to 4-chloro-2-chloromethyl-3,5-dimethyl pyridine **6** by treatment with phosphorous oxychloride. Treatment of compound **6** with 5-methoxy-2-mercaptobenzimidazole in tetrahydrofuran gave 2-[2-(4-chloro-3,5-dimethyl pyridinyl)methylthio]-5-methoxy benzimidazole **7**. When compound **7** was treated with potassium hydroxide in dimethyl sulfoxide containing methanol, 2-[2-(3,5-dimethyl-4-methoxypyridinyl)methylthio]-5-methoxy

benzimidazole **8** was obtained. Compound **8** was dissolved in methanol and treated with hydrogen chloride to give the hydrochloride salt **9**. Compound **9** was then converted to omeprazole **10** by treatment with *m*-chloroperbenzoic acid (*m*-CPBA).

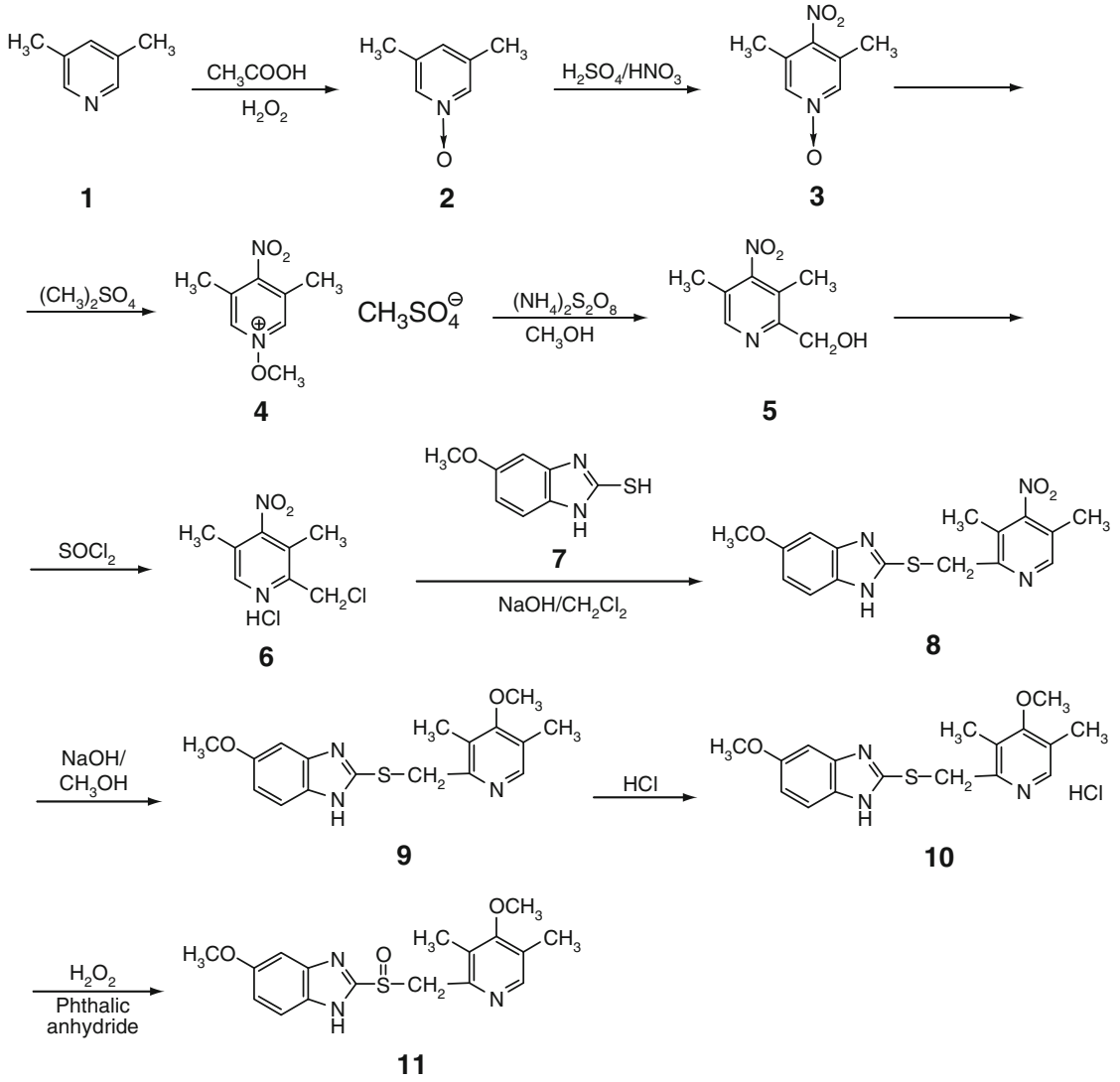


Omeprazole

2.6. Singh *et al.* [17] used the following method for the preparation of omeprazole:

3,5-Dimethyl pyridine **1** was treated with hydrogen peroxide in acetic acid to give 3,5-dimethyl pyridine-*N*-oxide **2**. Compound **2** was converted to 3,5-dimethyl-4-nitropyridine-*N*-oxide **3** by treatment with a mixture of sulfuric and nitric acids. Compound **3** was treated with dimethyl sulfate to give 3,5-dimethyl-4-nitro-1-methoxypyridinium methyl sulfate **4**. Compound **4** was converted to 3,5-dimethyl-4-nitro-2-hydroxy methyl pyridine **5** by treatment with ammonium persulfate and methanol. Compound **5** was treated with thionyl chloride to give 3,5-dimethyl-4-nitro-2-chloromethyl pyridine hydrochloride **6**. When compound **6** was reacted with 5-methoxy-2-mercaptobenzimidazole **7** in sodium hydroxide and dichloromethane, 5-methoxy-2-[(3,5-dimethyl-4-nitro-2-pyridinyl)methylthio]-1*H*-benzimidazole **8** was obtained. Treatment of compound **8** with sodium hydroxide and methanol replaces the 4-nitro group in **8** by a 4-methoxy group to give **9**. Compound **9** was converted to

the hydrochloride salt **10**. Compound **10** was oxidized by hydrogen peroxide in phthalic anhydride to give omeprazole **11.**

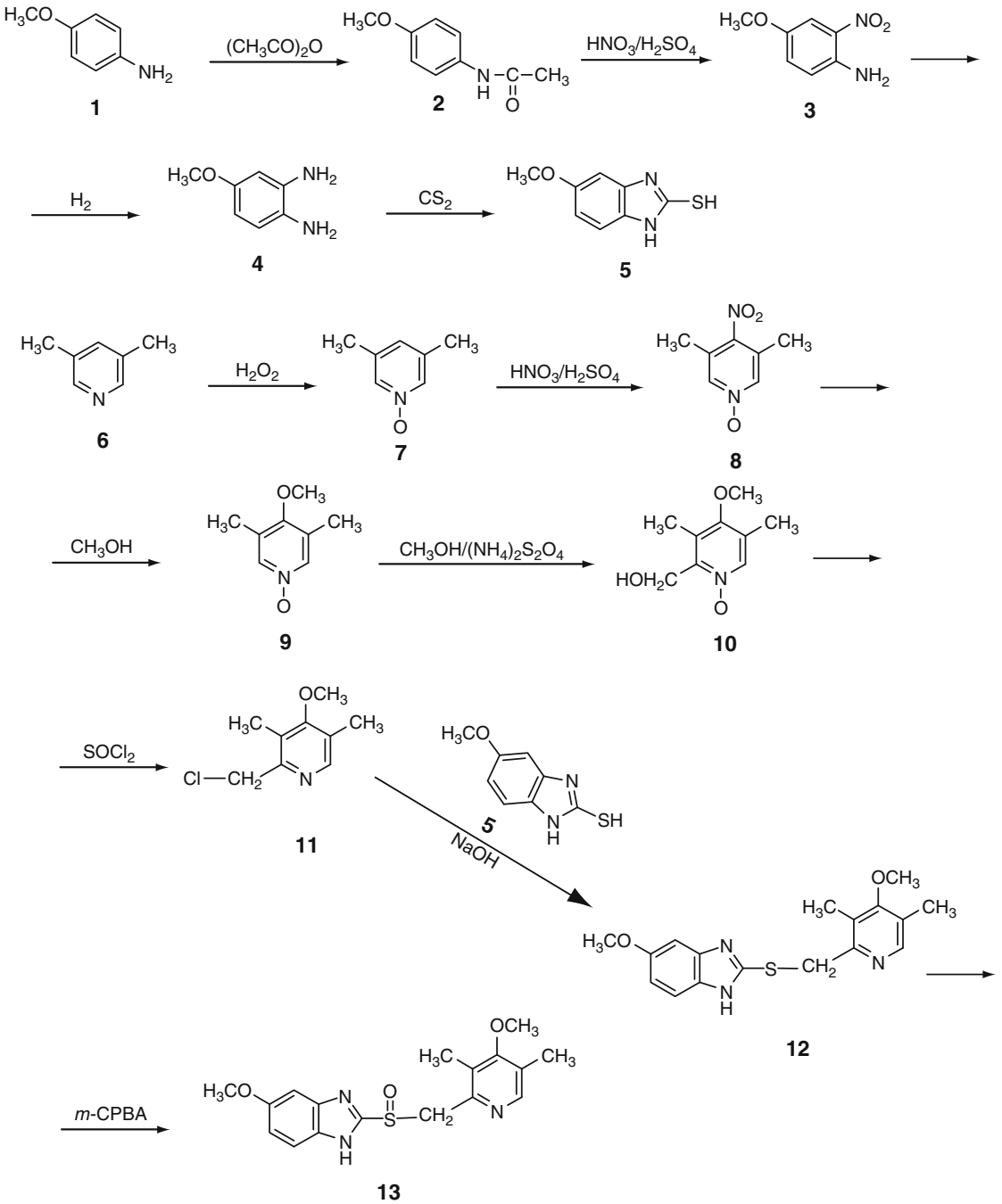


2.7. Liu [18] described the following procedure for the synthesis of omeprazole:

p-Methoxy aniline **1** was converted to 4-methoxyacetanilide **2** by treatment with acetic anhydride. Compound **2** was nitrated with a mixture of nitric and sulfuric acids to produce 4-methoxy-2-nitro-aniline **3**. The nitro group in compound **3** was reduced to the amino group to give 2-amino-4-methoxy aniline **4**. Compound **4** was converted to 5-methoxy-2-mercaptobenzimidazole **5** by treatment with carbon disulfide.

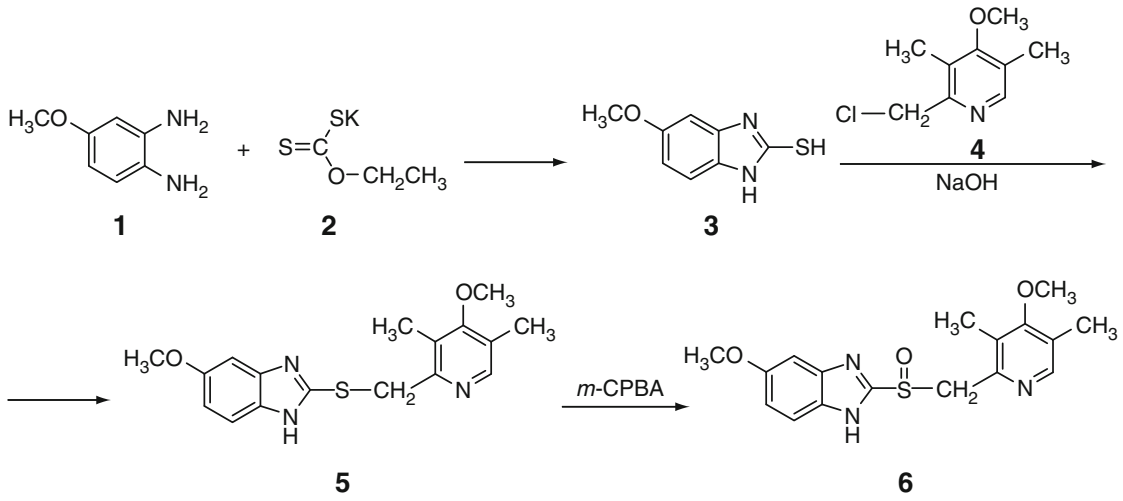
3,5-Dimethyl pyridine **6** was treated with hydrogen peroxide and 3,5-dimethyl pyridine-*N*-oxide **7** was produced. Compound **7** was nitrated with a mixture of nitric and sulfuric acids to give 3,5-dimethyl-4-nitropyridine-*N*-oxide **8**. Compound **8** was converted to 3,5-dimethyl-4-methoxypyridine-*N*-oxide **9** by reaction with methanol. Compound **9** was treated with a mixture of methanol and ammonium dithionite to give 3,5-dimethyl-

4-methoxy-2-hydroxymethyl-pyridine-*N*-oxide **10**. Compound **10** was converted to 3,5-dimethyl-4-methoxy-2-chloromethyl-pyridine **11** by treatment with thionyl chloride. Compound **11** was reacted with compound **5** in sodium hydroxide and 5-methoxy-2-[(3,5-dimethyl-4-methoxy-2-pyridinyl)methylthio]-1*H*-benzimidazole **12** was produced which was oxidized by *m*-CPBA acid to give omeprazole **13**.



2.8. Omeprazole was prepared [19] by reaction of 4-methoxy-*o*-phenylenediamine **1** with potassium ethyl xanthogenate **2** to give 5-methoxy-2-mercapto-1*H*-benzimidazole **3**. Treatment of compound **3** with 3,5-dimethyl-4-methoxy-2-chloromethyl pyridine **4** in sodium hydroxide

gives 5-methoxy-2-[((3,5-dimethyl-4-methoxy-2-pyridinyl)methyl)thio]-1*H*-benzimidazole **5**. Oxidation of compound **5** with 3-chloroperbenzoic acid produced omeprazole **6**.



2.9. Rao *et al.* [20] reviewed the synthetic method used to prepare omeprazole. The advantages and the disadvantages of the various methods are described.

3. PHYSICAL CHARACTERISTICS

3.1. Ionization constant

pK_a = 4.61 and 9.08 [4].

3.2. Solubility characteristics

Omeprazole: Very slightly soluble in water, soluble in alcohol, methanol, and methylene chloride. It dissolves in dilute solution of alkali hydroxides [3].
Omeprazole sodium: Freely soluble in water and alcohol, soluble in propylene glycol, very slightly soluble in methylene chloride [3].

3.3. X-Ray powder diffraction pattern

The X-ray powder diffraction (XPRD) pattern of omeprazole was performed using a Simmon XRD-5000 diffractometer (Fig. 4.1). Table 4.1 shows the values for the scattering angles (° 2θ), the interplanar *d*-spacing (Å), and the relative intensities (%) observed for the major diffraction peaks of a pure sample of omeprazole drug substance.

Omeprazole is known to exist in at least two well-defined polymorphic forms, which have been the subject of patent documentation.

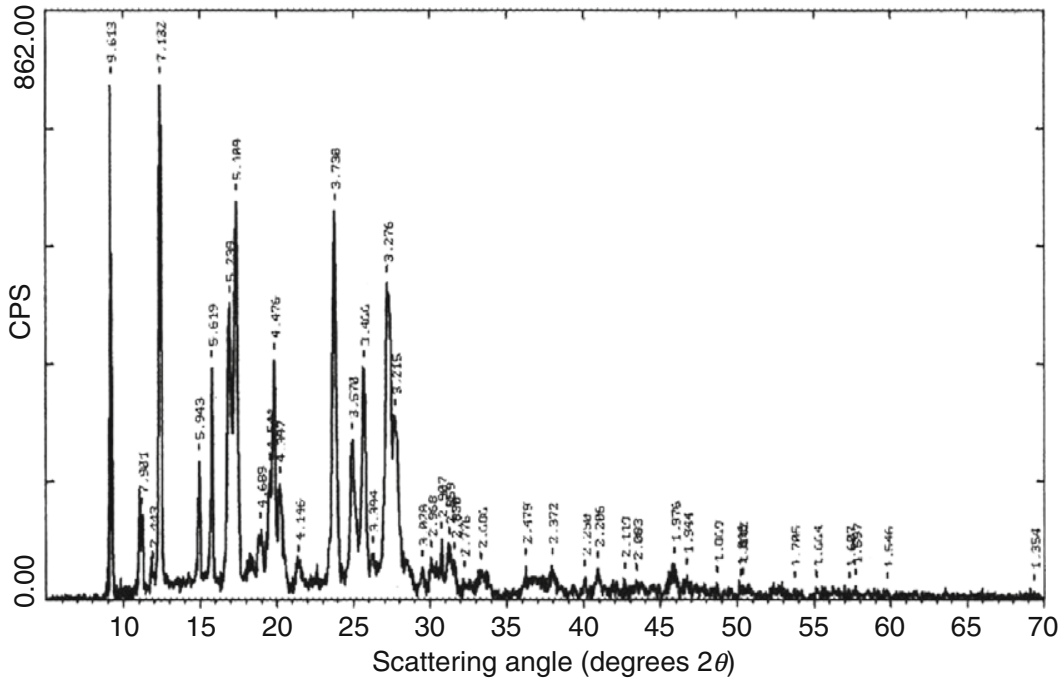


FIGURE 4.1 The X-ray powder diffraction pattern of omeprazole.

TABLE 4.1 The X-ray powder diffraction pattern for omeprazole

Scattering angle (°2 θ)	*d*-Spacing (Å)	Relative intensity (%)	Scattering angle (°2 θ)	*d*-Spacing (Å)	Relative intensity (%)
9.192	9.6133	91.05	31.256	2.8594	9.18
11.190	7.9007	14.44	31.587	2.8301	6.71
11.880	7.4433	5.15	32.214	2.7765	3.54
12.400	7.1321	100.00	33.329	2.6861	4.97
14.895	5.9429	23.63	36.204	2.4791	5.67
15.758	5.6192	39.88	37.907	2.3716	5.76
16.910	5.2388	50.92	40.045	2.2497	3.77
17.344	5.1086	68.42	40.874	2.2060	5.28
18.912	4.6887	12.05	42.640	2.1186	3.34
19.533	4.5410	22.00	43.414	2.0826	2.98
19.820	4.4757	41.18	45.896	1.9756	6.04
20.179	4.3969	19.82	46.691	1.9438	4.05
21.413	4.1462	6.28	48.673	1.8692	2.57
23.781	3.7384	66.89	50.200	1.8158	2.05
24.923	3.5697	27.36	50.323	1.8117	2.41
25.678	3.4665	39.81	53.719	1.7049	1.81
26.238	3.3937	7.86	55.145	1.6641	1.77
27.196	3.2763	54.36	57.289	1.6068	1.74
27.725	3.2149	31.37	57.685	1.5967	2.23
29.471	3.0284	5.42	59.747	1.5465	1.65
30.080	2.9684	6.95	69.354	1.3539	1.30
30.727	2.9073	10.24			

The XRPD patterns and defining peak values for these forms are summarized in Figs. 4.2 and 4.3 and in Tables 4.2 and 4.3 (US Patent 6,150,380).

3.4. Crystal structure

Ohishi *et al.* [21] determined the crystal structure of omeprazole. The crystal structure of omeprazole is triclinic, *P1*, $a = 10.686(5)$ Å, $b = 10.608(7)$ Å, $c = 9.666(6)$ Å, $\alpha = 119.75(5)^\circ$, $\beta = 112.02(5)^\circ$, $\gamma = 68.33(4)^\circ$. $V = 859(1)$ Å^3, $Z = 2$, $D_m = 1.332(2)$, $D_x = 1.335$ g/cm^3, Cu Kα, $\lambda = 1.5418$ Å, $\mu = 18.04$ cm^{-1}, $F(0\ 0\ 0) = 364$, $T = 293$ K, $R = 0.057$ for 1962 observed reflections.

The methylsulfinyl group, which adopts a *trans* conformation, links the pyridine and benzimidazole rings in an almost coplanar orientation. Thus the molecule, as a whole, adopts a nearly extended form. Two centrosymmetrically related molecules form a cyclic dimer by intermolecular N–H···O hydrogen bonding, and the dimers are held together by van der Waals contacts between the neighboring aromatic rings in the crystal structure.

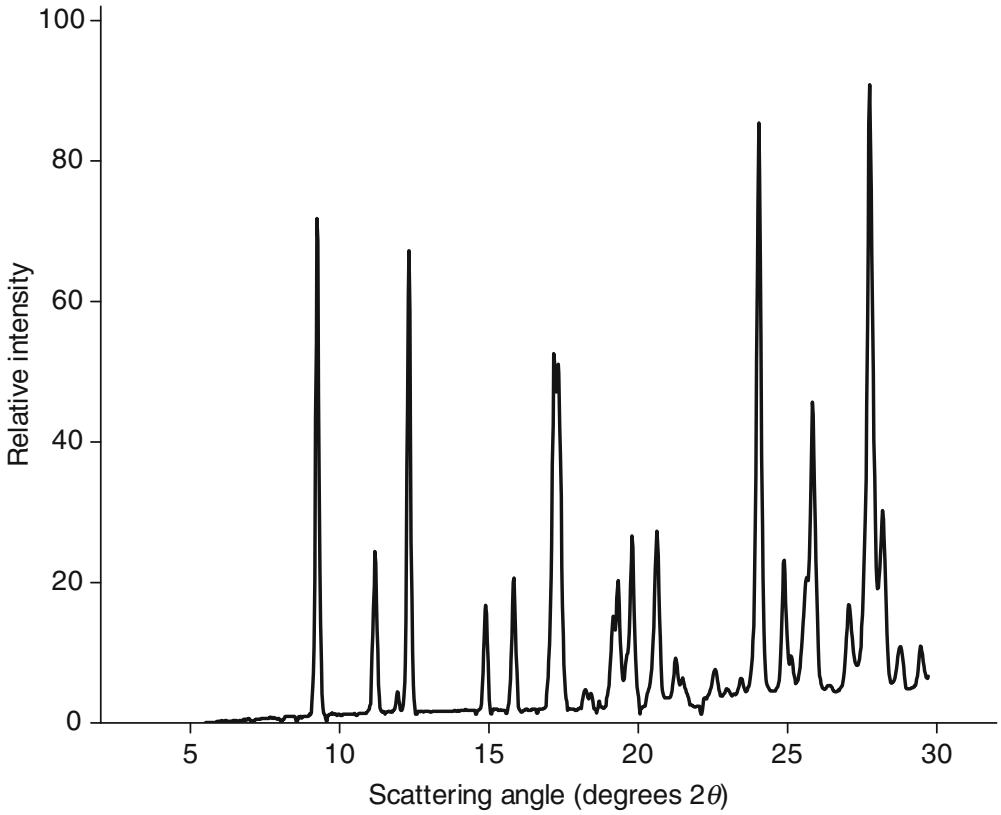


FIGURE 4.2 XRPD pattern of omeprazole, Astra Form-A, scanned and digitized from the pattern disclosed in US patent 6,150,380.

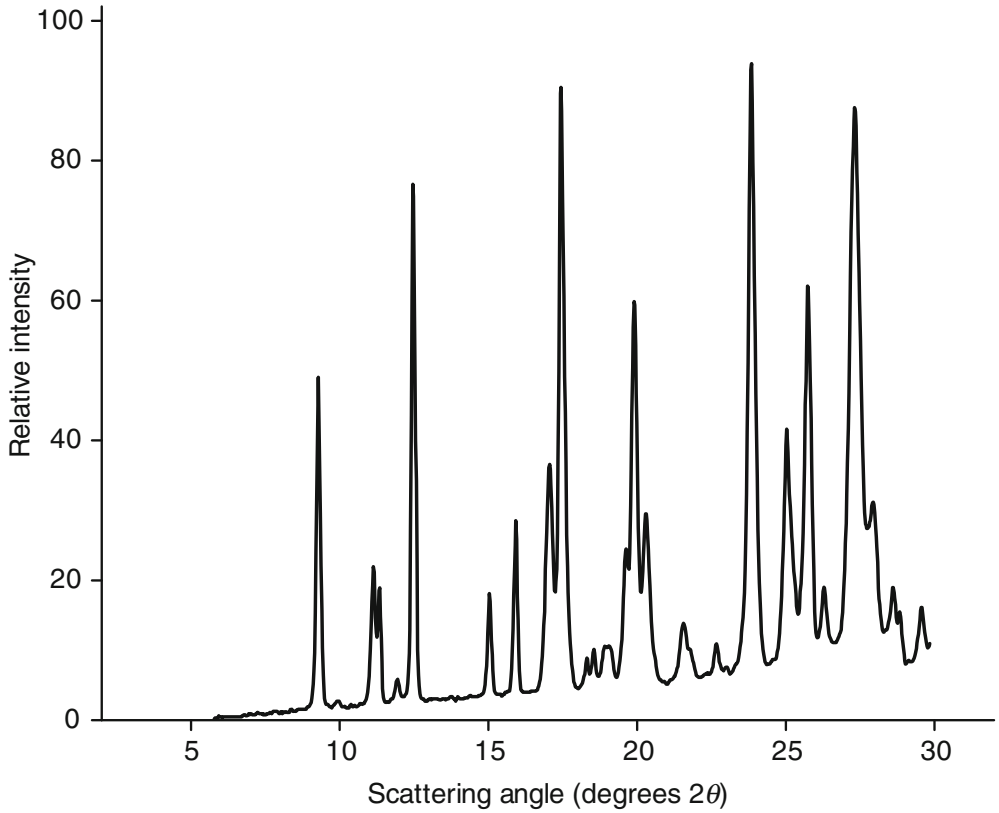
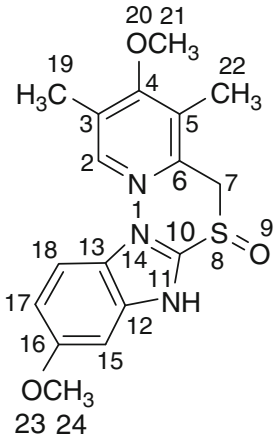


FIGURE 4.3 XRPD pattern of omeprazole, Astra Form-B, scanned and digitized from the pattern disclosed in US patent 6,150,380.

TABLE 4.2 Scattering angles and *d*-spacing of the 10 most intense peaks (US Patents 6,150,380)

Scattering Angle (degrees 2θ)	*d*-Spacing (Å)
9.25	9.557
11.19	7.901
12.32	7.180
14.90	5.941
15.83	5.595
17.18	5.157
20.63	4.302
24.04	3.699
25.84	3.445
27.74	3.214



The molecular structure of omeprazole is presented in Fig. 4.4. Bond distances and angles are presented in Table 4.4, all of which are normal within their e.s.d.'s in comparison with related compounds. The final atomic parameters are listed in Table 4.5.

TABLE 4.3 Scattering angles and *d*-spacing of the 10 most intense peaks (US Patents 6,150,380)

Scattering Angle (degrees 2θ)	*d*-Spacing (Å)
9.28	9.522
12.46	7.099
15.92	5.561
17.05	5.197
17.45	5.079
19.91	4.455
23.84	3.729
25.02	3.555
25.75	3.457
27.32	3.262

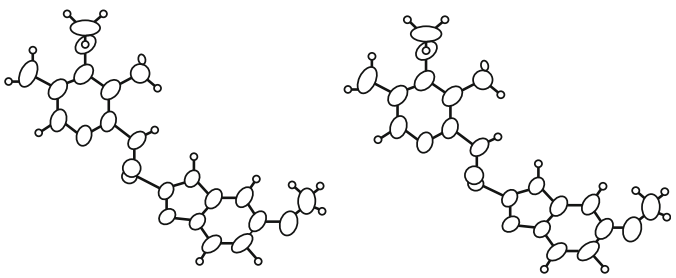


FIGURE 4.4 A stereoscopic view of omeprazole, viewed perpendicular to the pyridine ring [21].

TABLE 4.4 Bond distances (Å) and angles (°) for non-H atoms with e.s.d.'s in parentheses [21]

N(1)–C(2)	1.339(8)	C(10)–N(11)	1.361(7)
N(1)–C(6)	1.319(7)	C(10)–N(14)	1.306(7)
C(2)–C(3)	1.358(9)	N(11)–C(12)	1.396(7)
C(3)–C(4)	1.368(8)	C(12)–C(13)	1.381(7)
C(3)–C(19)	1.53(1)	C(12)–C(15)	1.382(7)
C(4)–C(5)	1.396(7)	C(13)–N(14)	1.376(7)
C(4)–O(20)	1.392(7)	C(13)–C(18)	1.398(8)
C(5)–C(6)	1.387(7)	C(15)–C(16)	1.387(8)
C(5)–C(22)	1.516(8)	C(16)–C(17)	1.377(8)
C(6)–C(7)	1.527(8)	C(16)–O(23)	1.357(7)
C(7)–S(8)	1.815(6)	C(17)–C(18)	1.369(8)
S(8)–O(9)	1.487(4)	O(20)–C(21)	1.421(9)
S(8)–C(10)	1.768(6)	O(23)–C(24)	1.409(8)
C(2)–N(1)–C(6)	117.4(4)	S(8)–C(10)–N(14)	120.7(2)
N(1)–C(2)–C(3)	123.5(4)	N(11)–C(10)–N(14)	115.6(3)
C(2)–C(3)–C(4)	118.0(4)	C(10)–N(11)–C(12)	104.0(3)
C(2)–C(3)–C(19)	121.0(4)	N(11)–C(12)–C(13)	106.1(3)
C(4)–C(3)–C(19)	120.9(4)	N(11)–C(21)–C(15)	130.5(3)
C(3)–C(4)–C(5)	120.9(4)	C(13)–C(12)–C(15)	123.4(3)
C(3)–C(4)–O(20)	121.2(3)	C(12)–C(13)–N(14)	110.8(3)
C(5)–C(4)–O(20)	117.9(3)	C(12)–C(13)–C(18)	120.6(3)
C(4)–C(5)–C(6)	115.6(3)	N(14)–C(13)–C(18)	128.6(3)
C(4)–C(5)–C(22)	120.8(4)	C(10)–N(14)–C(13)	103.6(3)
C(6)–C(5)–C(22)	123.6(4)	C(12)–C(15)–C(16)	114.1(3)
N(1)–C(6)–C(5)	124.5(3)	C(15)–C(16)–C(17)	124.0(4)
N(1)–C(6)–C(7)	115.7(3)	C(15)–C(16)–O(23)	122.4(3)
C(5)–C(6)–C(7)	119.8(3)	C(17)–C(16)–O(23)	113.6(3)
C(6)–C(7)–S(8)	108.7(2)	C(16)–C(17)–C(18)	120.9(4)
C(7)–S(8)–O(9)	105.9(3)	C(13)–C(18)–C(17)	117.0(3)
C(7)–S(8)–C(10)	96.6(3)	C(4)–O(20)–C(21)	114.6(4)
O(9)–S(8)–C(10)	108.0(2)	C(16)–O(23)–C(24)	116.0(4)
S(8)–C(10)–N(11)	123.7(2)		

The molecule takes an extended conformation, in which the pyridine and benzimidazole rings are linked by the methylsulfinyl chain taking a *trans* conformation [C(6)–C(7)–S(8)–C(10) = 179.1(3)°]; the torsion angles N(1)–C(6)–C(7)–S(8), C(5)–C(6)–C(7)–S(8), C(6)–C(7)–S(8)–C(10), and C(7)–S(8)–C(10)–N(14) are −33.6(4)°, 148.3(5)°, 60.9(5)°, and −121.3(5)°, respectively, and the dihedral angle between the aromatic rings is 30.0(2)°. The sulfinyl bond protrudes from the benzimidazole plane.

TABLE 4.5 Fractional atomic coordinates and equivalent isotropic temperature factors (Å^2) for non-H atoms with e.s.d.'s in parentheses [21]

	x	*y*	*z*	B_{eq} [a]
N(1)	0.1549(4)	0.5541(4)	0.9852(5)	6.5(2)
C(2)	0.2194(6)	0.4734(6)	0.0739(7)	7.1(3)
C(3)	0.3401(5)	0.3692(6)	1.0539(7)	6.2(2)
C(4)	0.3972(4)	0.3438(5)	0.9346(6)	6.2(2)
C(5)	0.3343(4)	0.4245(5)	0.8384(6)	5.7(2)
C(6)	0.2131(5)	0.5300(5)	0.8731(6)	5.8(2)
C(7)	0.1411(5)	0.6312(5)	0.7824(7)	6.8(2)
S(8)	−0.0444(1)	0.6751(1)	0.7611(2)	5.94(5)
O(9)	−0.0903(3)	0.5343(3)	0.6413(4)	6.2(1)
C(10)	−0.0916(5)	0.7910(5)	0.6585(6)	5.9(2)
N(11)	−0.0656(4)	0.7435(4)	0.5106(5)	5.8(2)
C(12)	−0.1227(4)	0.8690(5)	0.4758(6)	5.6(2)
C(13)	−0.1783(4)	0.9807(5)	0.6064(6)	5.6(2)
N(14)	−0.1591(4)	0.9295(4)	0.7203(5)	6.2(2)
C(15)	−0.1254(5)	0.8892(5)	0.3440(6)	6.0(2)
C(16)	−0.1882(5)	1.0327(5)	0.3542(6)	6.2(2)
C(17)	−0.2437(5)	1.1469(5)	0.4831(7)	6.8(2)
C(18)	−0.2406(5)	1.1239(5)	0.6119(6)	6.7(2)
C(19)	0.4135(7)	0.2880(9)	1.1666(9)	10.2(4)
O(20)	0.5228(3)	0.2418(4)	0.9104(5)	8.2(2)
C(21)	0.5106(6)	0.0936(6)	0.798(1)	10.3(3)
C(22)	0.3969(6)	0.3954(7)	0.7052(7)	7.9(3)
O(23)	−0.1977(4)	1.0740(4)	0.2377(4)	8.2(2)
C(24)	−0.1520(8)	0.9590(7)	0.0972(7)	8.8(4)

[a] $B_{eq} = \frac{4}{3}\sum_i \sum_j a_i a_j \beta_{ij}$.

The methoxy group attached to the pyridine ring is almost perpendicular to the ring plane [C(3)–C(4)–O(20)–C(21) = 89.5(6)°, C(5)–C(4)–O(20)–C(21) = −93.4(5)°], while that attached to the benzimidazole ring is almost coplanar with the ring [C(15)–C(16)–O(23)–C(24) = 6.0(5)°, C(17)–C(16)–O(23)–C(24) = −175.4(6)°].

The molecules are arrayed along the (2 2 0) plane corresponding to the strongest intensity among the observed reflections. The two molecules which are related to each other by a center of symmetry form a cyclic dimer with an intermolecular N(11)–H···O(9) hydrogen bond [N(x, y, z)···O(−x, 1 − y, 1 − z) = 2.744(6) Å, H···O = 1.78 (7) Å and angle N–H···O = 169(6)° (see Fig. 4.5A)], and the dimer is stabilized by van der Waals contacts between the pyridine and benzimidazole rings;

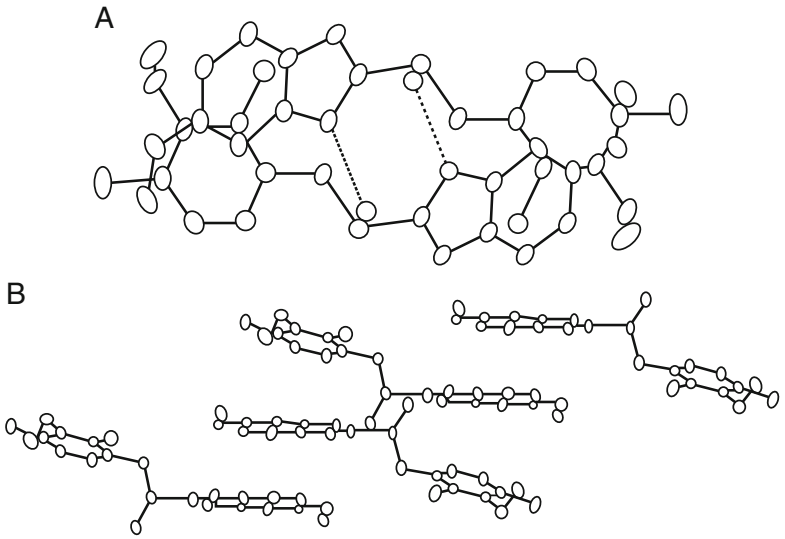


FIGURE 4.5 (A) Cyclic dimer structure formed by N–H···O intermolecular hydrogen bonds represented by dotted lines. (B) Overlapping mode among the neighboring aromatic rings [21].

the average interplanar spacing between the pyridine and benzimidazole rings is 4.13 Å.

On the other hand, the benzimidazole ring in the dimer also forms a stacking interaction with the centrosymmetrically related ring with an average spacing of 3.38 Å (Fig. 4.5B).

3.5. Thermal methods of analysis

3.5.1. Melting range

M.P. 156 °C [3].

3.5.2. Differential scanning calorimetry

The differential scanning calorimetry (DSC) thermogram of omeprazole was obtained using a DuPont 2100 thermal analyzer system. The thermogram shown in Fig. 4.6 was obtained at a heating rate of 10 °C/min, and was run over the range 50–300 °C. Omeprazole was found to melt at 159.65 °C.

3.6. Spectroscopy

3.6.1. Ultraviolet spectroscopy

The ultraviolet (UV) absorption spectrum of omeprazole in methanol (0.0016%, w/v) shown in Fig. 4.7 was recorded using a Shimadzu UV–VIS spectrophotometer 1601 PC. Omeprazole exhibited two maxima at 276 and 302 nm.

Clarke [3] reported the following: Aqueous acid (0.2 M H_2SO_4), 277 and 303 nm; basic, 276 and 305 nm.

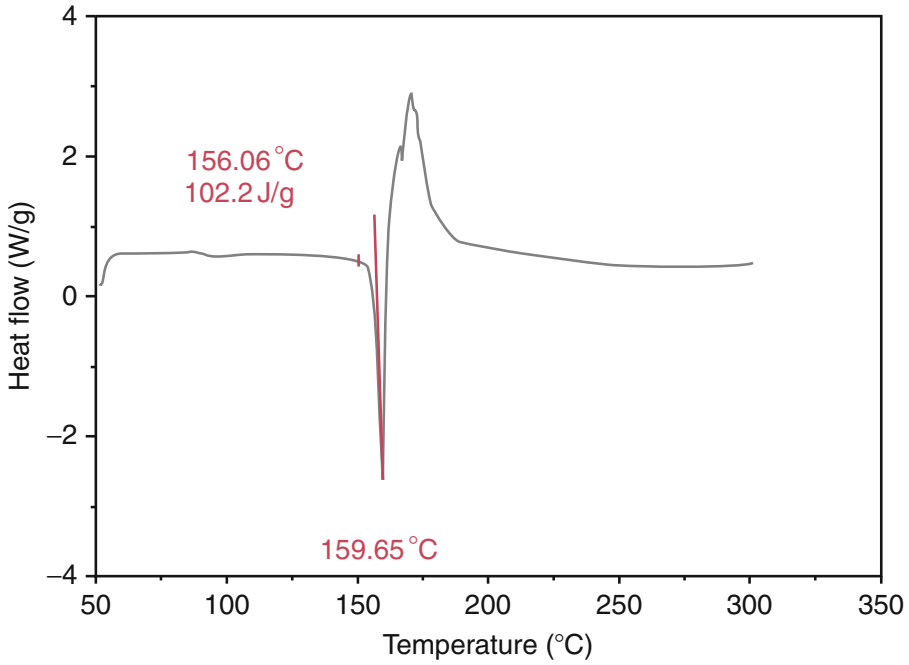


FIGURE 4.6 Differential scanning calorimetry thermogram of omeprazole.

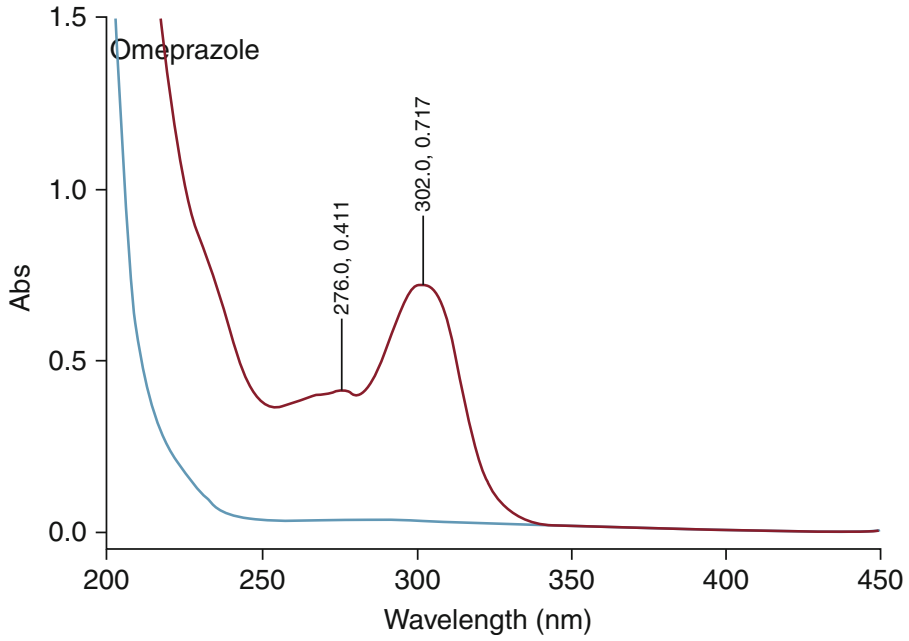


FIGURE 4.7 The UV absorption spectrum of omeprazole.

3.6.2. Vibrational spectroscopy

The infrared (IR) absorption spectrum of omeprazole was obtained in a KBr pellet using a Perkin-Elmer IR spectrophotometer. The IR spectrum is shown in Fig. 4.8, where the principal peaks were observed and the assignments for the major IR absorption bands are listed in Table 4.6.

Clarke [3] reported that principal peaks are at wavenumbers 1625, 1205, 1015 cm^{-1} (KBr disc).

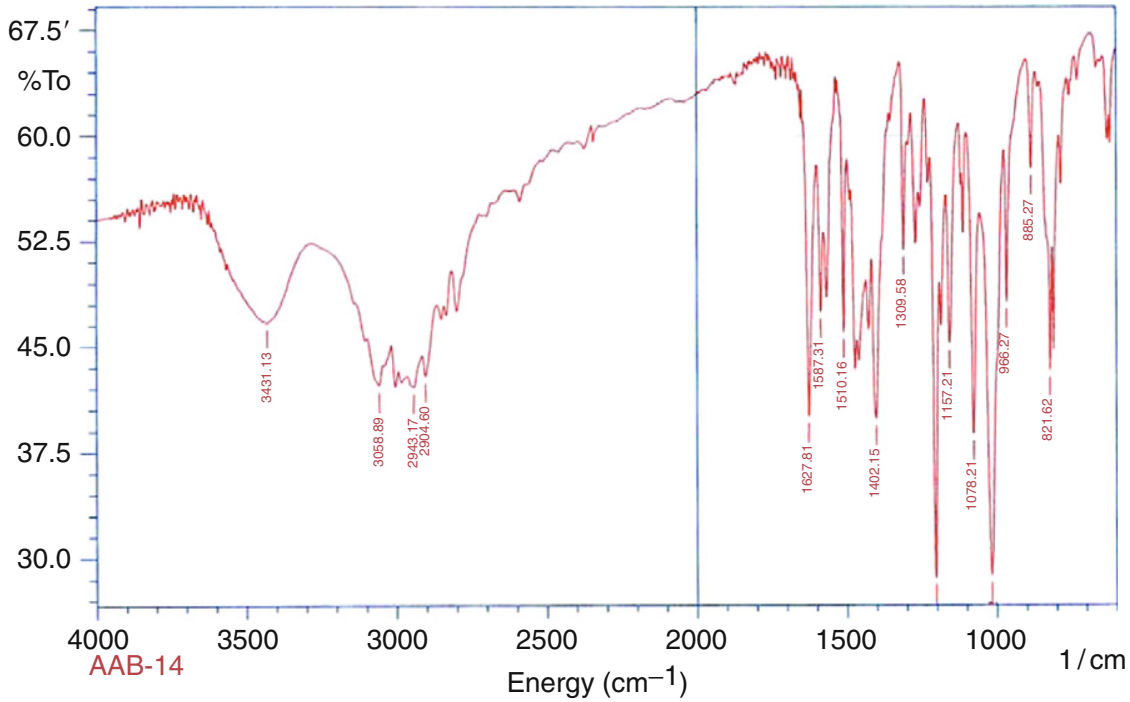


FIGURE 4.8 The infrared absorption spectrum of omeprazole.

TABLE 4.6 Vibrational assignments for omeprazole infrared absorption bands

Frequency (cm^{-1})	Assignment
3431	N–H stretch
3058	Aromatic C–H stretch
2943 and 2904	C–H stretch
1627	C=C stretch
1587	C=N stretch
1510	CH_2 bending
1402 and 1309	CH bending
1157	C=O stretch
1070	C=S stretch
966, 885, and 821	C–H bending

3.6.3. Nuclear magnetic resonance spectrometry

3.6.3.1. ^1H NMR spectrum The proton nuclear magnetic resonance (^1H NMR) spectrum of omeprazole were obtained using a Bruker Instrument operating at 300, 400, or 500 MHz. Standard Bruker Software was used to execute the recording of DEPT, COSY, and HETCOR spectra. The sample was dissolved DMSO-d_6 and all resonance bands were referenced to tetramethylsilane (TMS) as internal standard. The ^1H NMR spectra of omeprazole are shown in Figs. 4.9–4.12 and the COSY ^1H NMR is

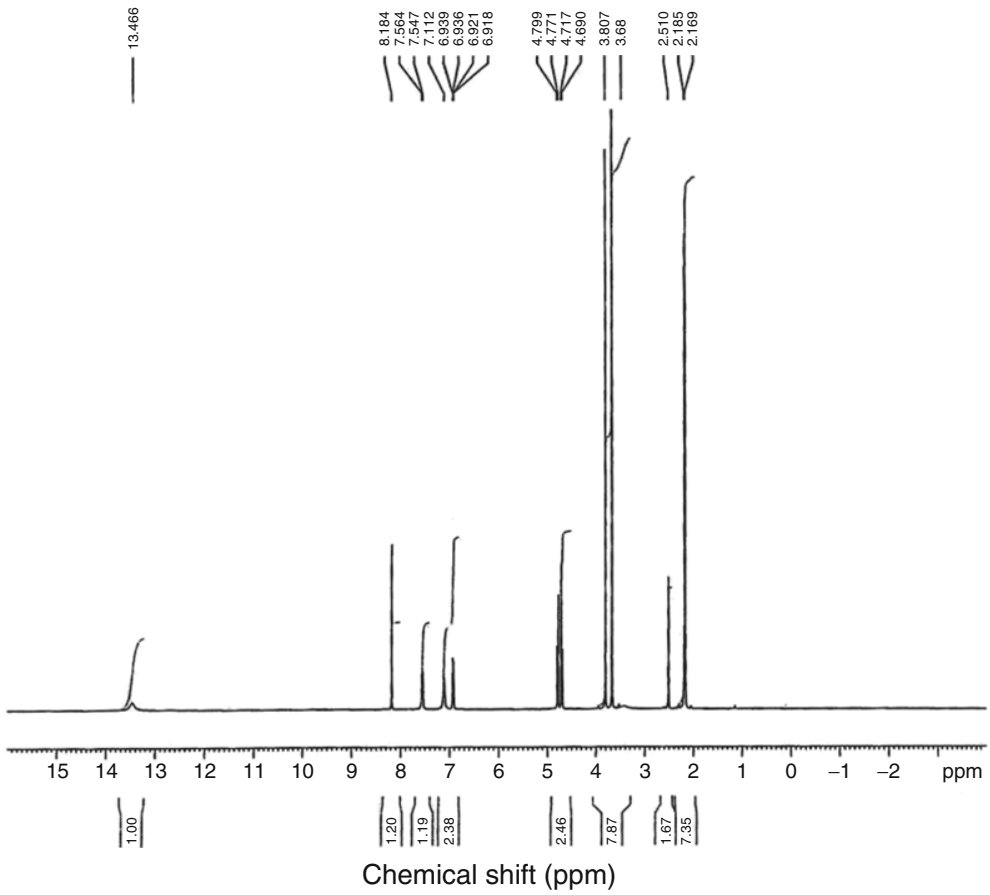


FIGURE 4.9 The ^{1}H NMR spectrum of omeprazole in DMSO-d_6.

shown in Fig. 4.13. The ^{1}H NMR assignments for omeprazole are listed in Table 4.7.

3.6.3.2. ^{13}C NMR spectrum The ^{13}C NMR spectra of omeprazole were obtained using a Bruker Instrument operating at 75, 100, or 125 MHz. The sample was dissolved in DMSO-d_6 and TMS was added to function as the internal standard. The ^{13}C NMR spectra are shown in Figs. 4.14 and 4.15 and the HSQC and the HMBC NMR are shown in Figs. 4.16 and 4.17, respectively. The DEPT 135 are shown in Figs. 4.18 and 4.19, respectively. The assignments for the observed resonance bands associated with the various carbons are listed in Table 4.8.

Claramunt *et al.* [22] used a ^{1}H and ^{13}C NMR to study the tautomerisim of omeprazole in solution. The tautomeric equilibrium constant, $K_T = 0.59$ in tetrahydrofuran at 195 K, is in favor of the 6-methoxy tautomer. The assignment of the signals was made by comparison with its two *N*-methyl derivatives in acetone-d_6 and through theoretical calculations of the absolute shieldings (GIAO/DFT/6-3111++G**).

Claramunt *et al.* [23] recorded the ^{13}C and ^{15}N CPMAS spectra of solid sample of omeprazole and all signals assigned. The sample consists uniquely of the 6-methoxy tautomer.

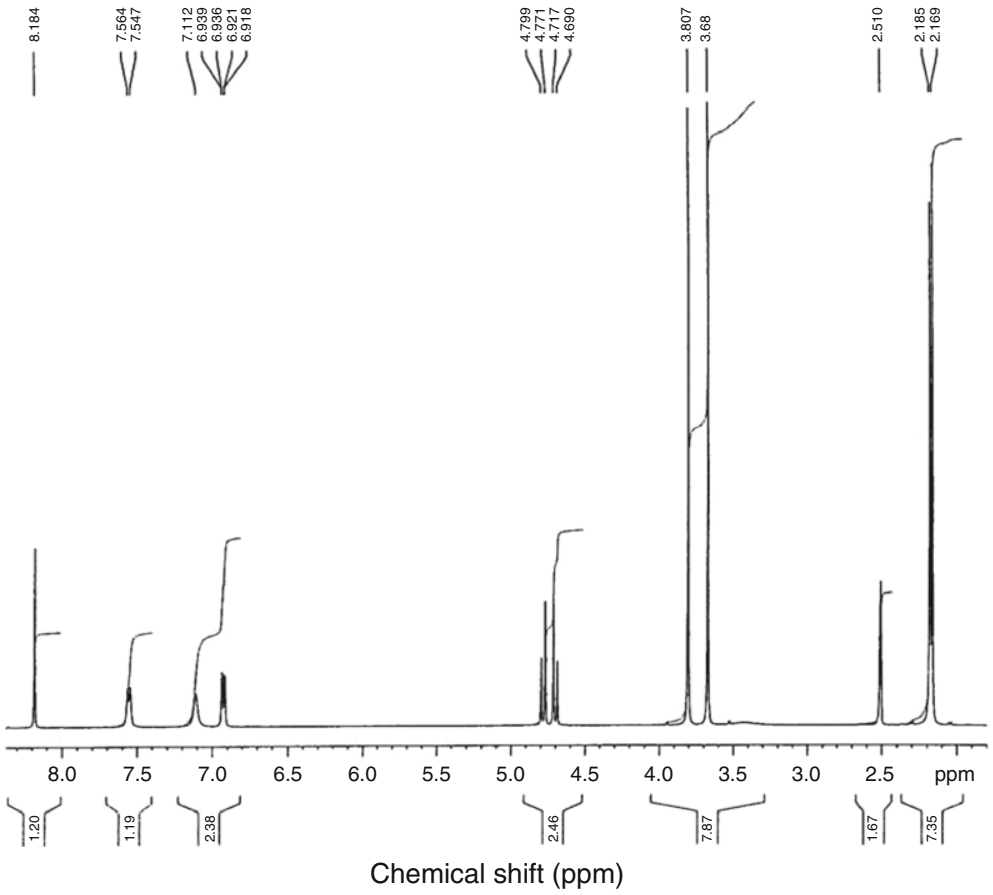


FIGURE 4.10 Expanded ^{1}H NMR spectrum of omeprazole in DMSO-d_6.

3.7. Mass spectrometry

The mass spectrum of omeprazole was obtained using a Shimadzu PQ-5000 mass spectrometer. The parent ion was collided with helium as the carrier gas. Figure 4.20 shows the mass fragmentation pattern of the drug substance (Table 4.9).

Clarke [3] reported that principal ions are at m/z 151, 136, 121, 120, 180, 297, 77, and 93.

4. METHODS OF ANALYSIS

4.1. Compendial methods

4.1.1. European Pharmacopoeia methods [24]

4.1.1.1. Omeprazole Omeprazole contains less than 99% and not more than the equivalent of 101% of 5-methoxy-2-[[(*RS*)-(4-methoxy-3,5-dimethyl-pyridine-2-yl)methyl]sulfinyl]-1*H*-benzimidazole, calculated with reference to the dried substance.

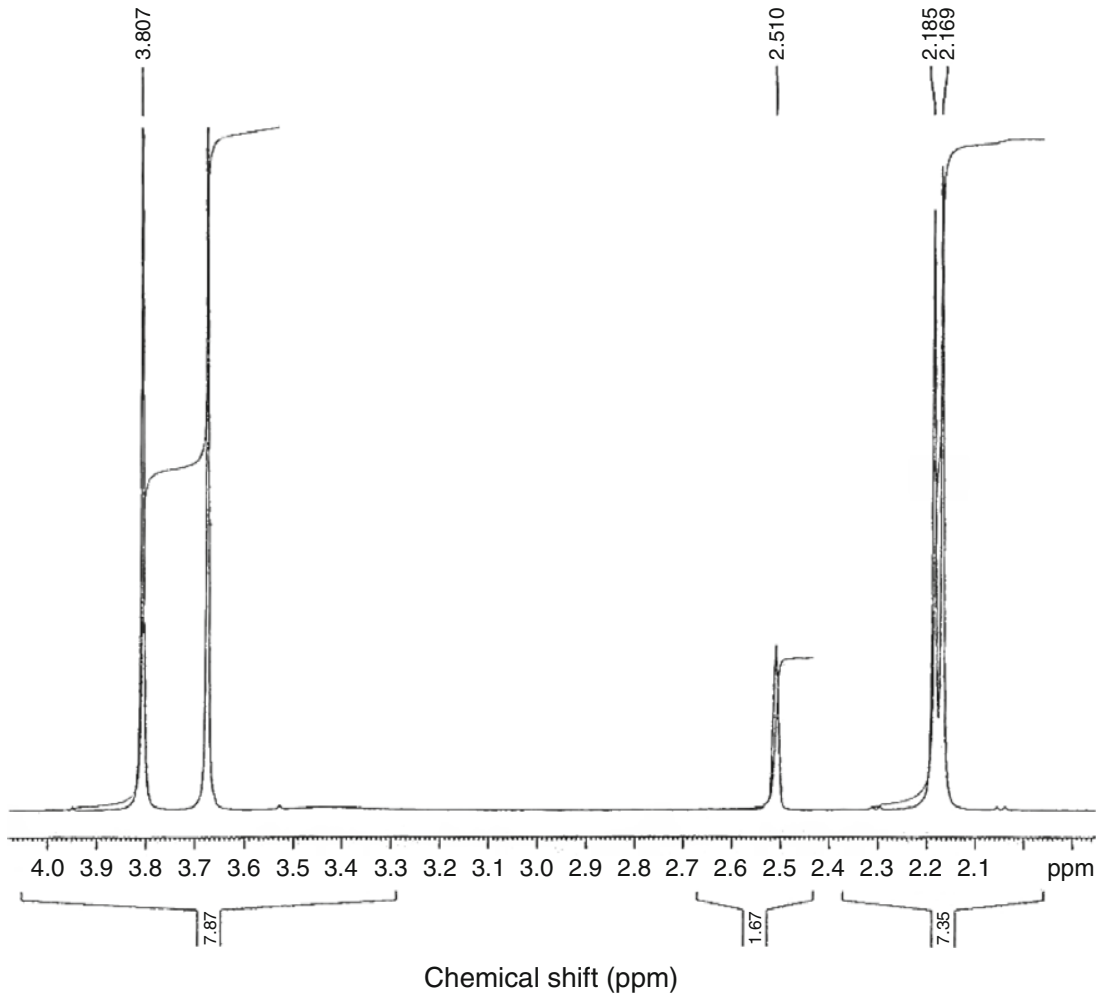


FIGURE 4.11 Expanded ^{1}H NMR spectrum of omeprazole in DMSO-d_6.

- *Identification*

  Test A: Dissolve 2 mg in 0.1 M *sodium hydroxide* and dilute to 100 ml with the same solution. Examined between 230 and 350 nm, according to the general method (2.2.25), the solution shows two absorption maxima, at 276 and 305 nm. The ratio of the absorbance measured at the maximum at 305 nm to that measured at the maximum at 276 nm is 1.6–1.8.

  Test B: Examine by IR absorption spectrophotometry, according to the general method (2.2.24), comparing with the spectrum obtained with *omeprazole CRS*. If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

  Test C: Examine the chromatograms obtained in the test for omeprazole impurity C. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). Place the plate in a tank saturated with vapor of *acetic acid R*. The spots rapidly turn brown.

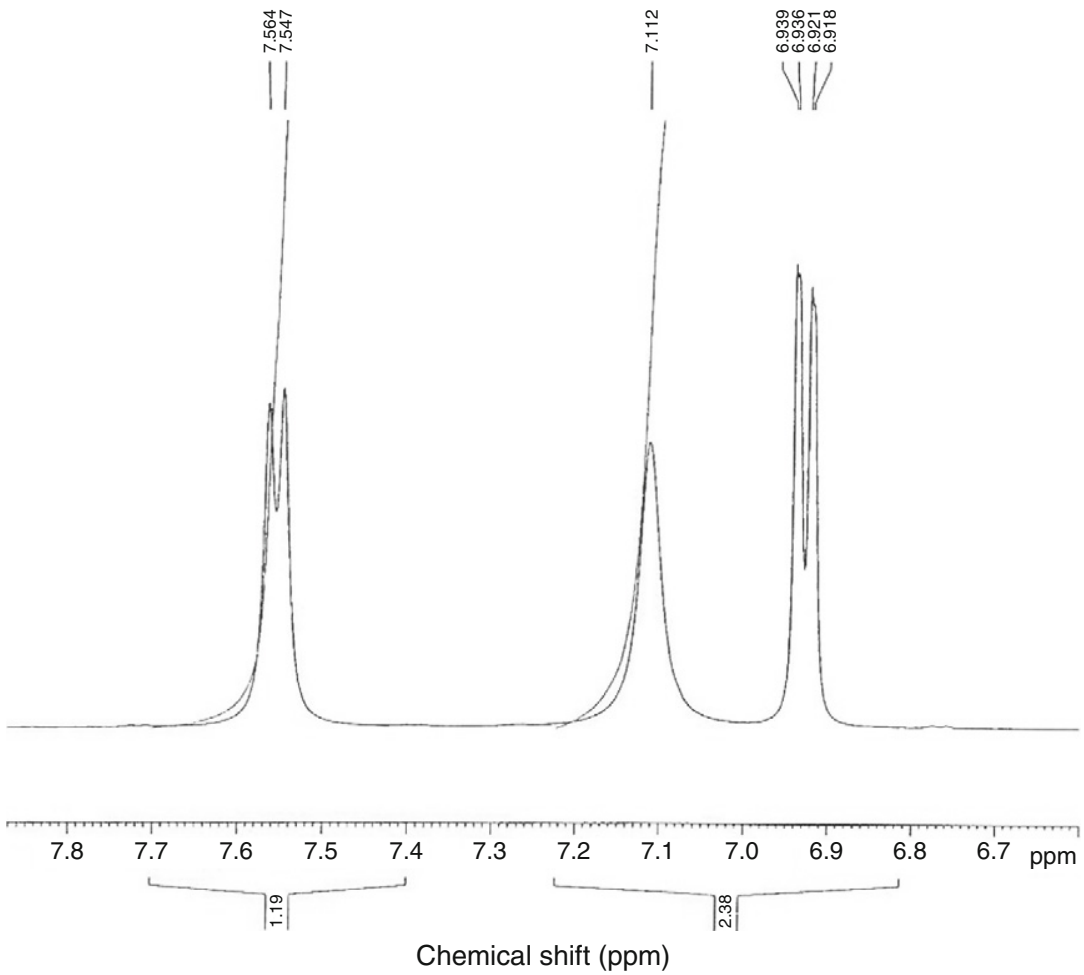


FIGURE 4.12 Expanded ^{1}H NMR spectrum of omeprazole in DMSO-d_6.

- *TESTS*

  Solution S: Dissolve 0.5 g of omeprazole in *methylene chloride R* and dilute to 25 ml with the same solvent.

  Appearance of solution: When this test is carried out according to the general method (2.2.1), solution S is clear.

  Absorbance: When this test is carried out according to the general procedure (2.2.25), the absorbance of solution S measured at 440 nm is not more than 0.1 (this limit corresponds to 0.035% of omeprazole impurity F or omeprazole impurity G).

  Omeprazole impurity C: Examine by thin-layer chromatography (TLC), according to the general procedure (2.2.27), using a TLC *silica gel* F_{254} *R*.
- *Test solution (a)*. Dissolve 0.1 g of omeprazole in 2 ml of a mixture of equal volumes of *methanol R* and *methylene chloride R*.
- *Test solution (b)*. Dilute 1 ml of test solution (a) to 10 ml with *methanol R*.
- *Reference solution (a)*. Dissolve 10 mg of *omeprazole CRS* in 2 ml of *methanol R*.
- *Reference solution (b)*. Dilute 1 ml of test solution (a) to 10 ml with a mixture of equal volumes of *methanol R* and *methylene chloride R*. Dilute 1 ml of this solution to 100 ml with a mixture of equal volumes of *methanol R* and *methylene chloride R*.

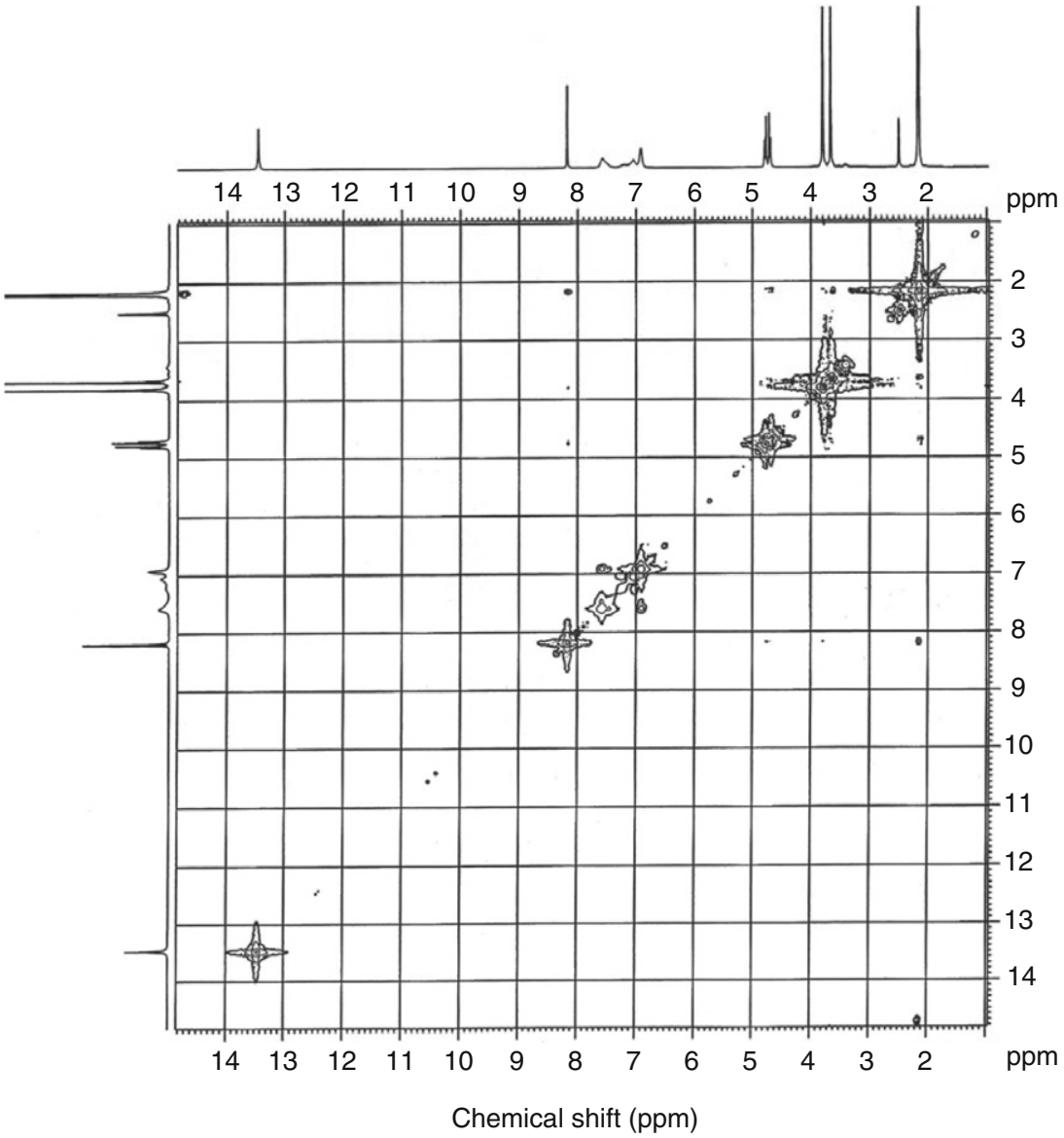
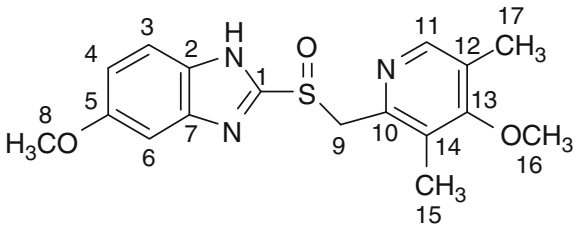


FIGURE 4.13 COSY ^{1}H NMR spectrum of omeprazole in DMSO-d_6.

Apply to the plate 10 μl of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *2-propanol R*, 40 volumes of *methylene chloride R* previously shaken with *concentrated ammonia R* (shake 100 ml of *methylene chloride R* with 30 ml of *concentrated ammonia R* in a separating funnel; allow the layers to separate and use the lower layer) and 40 volumes of *methylene chloride R*. Allow the plates to dry in air. Examine in UV light at 254 nm. Any spot in the chromatogram obtained with test solution (a) with a higher R_f value than that of the spot due to omeprazole is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.1%).

Related substances. Examine by liquid chromatography, according to the general procedure (2.2.29).

TABLE 4.7 Assignments of the resonance bands in the ^{1}H NMR spectrum of omeprazole



Chemical shift (ppm, relative to TMS)	Number of protons	Multiplicity[a]	Assignment (proton at carbon number)
2.17	3	s	15 or 17
2.19	3	s	15 or 17
2.51	DMSO	s	
3.68	3	s	8 or 16
3.81	3	s	8 or 16
4.68, 4.76	2	2d, $J = 13.5$ Hz	9
6.92–6.94	1	d	4
7.11	1	s	6
7.55–7.56	1	d	3
8.18	1	s	11
13.47	1	s	NH

[a] s, singlet; m, multiplet; d, doublet.

- *Test solution*. Dissolve 3 mg of omeprazole in the mobile phase and dilute to 25 ml with the mobile phase.
- *Reference solution (a)*. Dissolve 1 mg of *omeprazole CRS* and 1 mg of *omeprazole impurity D CRS* in the mobile phase and dilute to 10 ml with the mobile phase.
- *Reference solution (b)*. Dilute 1 ml of the test solution to 100 ml with the mobile phase. Dilute 1 ml of this solution to 10 ml with the mobile phase.

The chromatographic procedure may be carried out using:

– a stainless-steel column 0.15 m long and 4 mm in internal diameter packed with *octylsilyl silica gel for chromatography R* (5 μm).
– as mobile phase, at a flow-rate of 1 ml/min, a mixture of 27 volumes of *acetonitrile R* and 73 volumes of a 1.4-g/l solution of *disodium hydrogen phosphate R* previously adjusted to pH 7.6 with *phosphoric acid R*.
– as detector a spectrophotometer set at 280 nm.

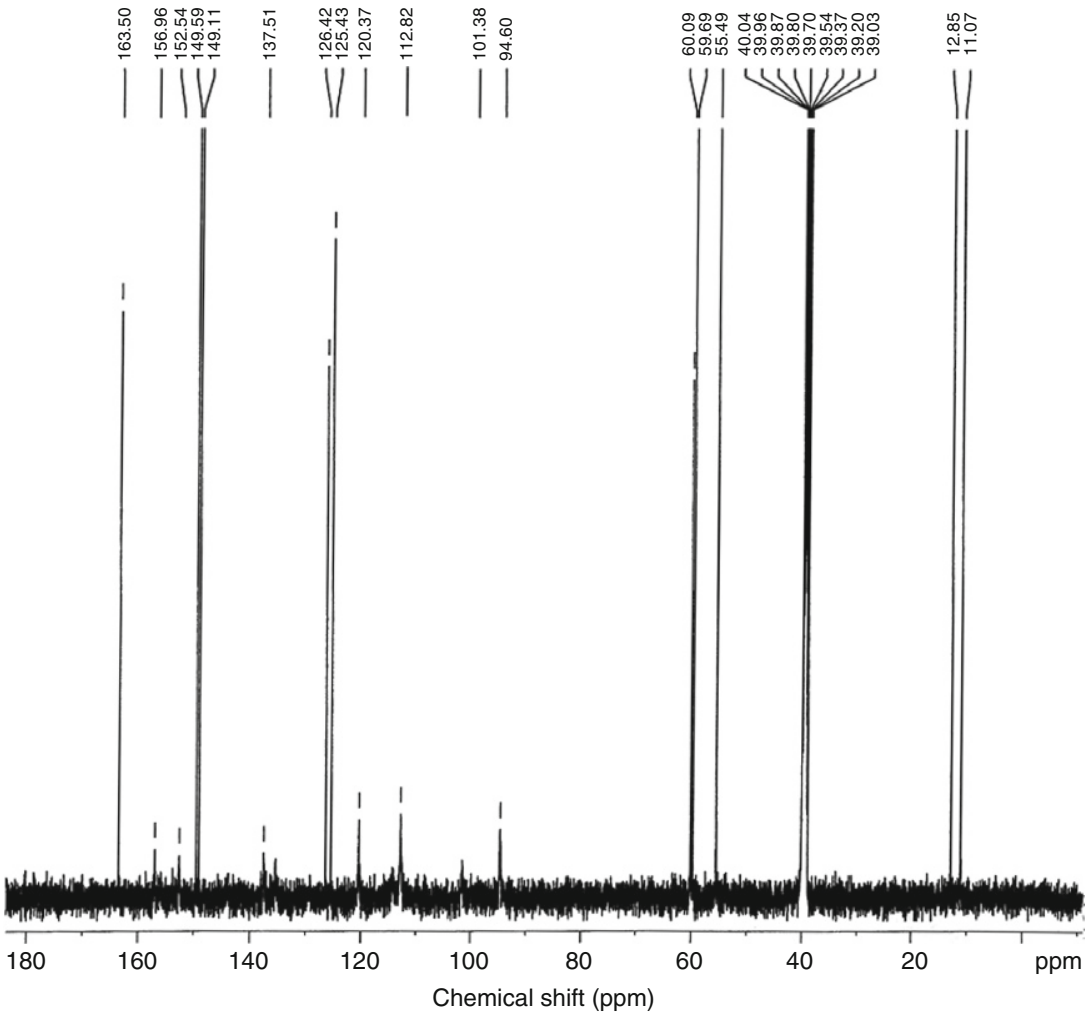


FIGURE 4.14 ^{13}C NMR spectrum of omeprazole in DMSO-d_6.

When the chromatograms are recorded under the prescribed conditions, the retention time of omeprazole is about 9 min and the relative retentions of impurities A, E, D, and B are about 0.4, 0.6, 0.8, and 0.9, respectively. Inject separately 40 μl of each solution and continue the chromatography for three times the retention time of omeprazole. Where applicable, adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 15% of the full scale of the recorder. The test is not valid unless the chromatogram obtained with reference solution (a), the resolution between the peaks corresponding to omeprazole impurity D and omeprazole is greater than 3. If necessary, adjust the pH of the mobile phase or the concentration of *acetonitrile R*; an increase in the pH will improve the resolution. The area of any peak due to impurities A, B, D, and E or any other peak, apart from the principal peak, in the chromatogram obtained with the test solution is not greater than the area of the peak in the chromatogram obtained with reference solution (b) (0.1%).

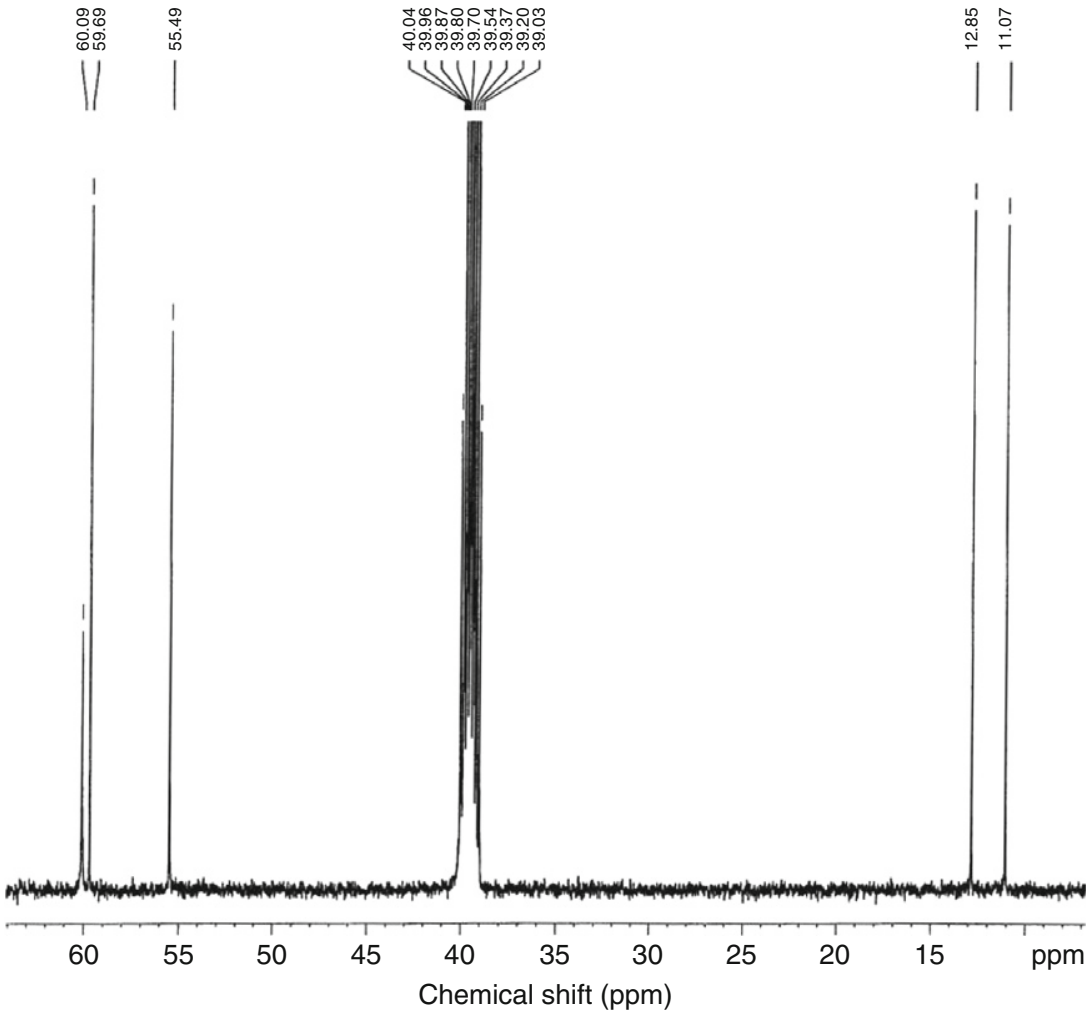


FIGURE 4.15 Expanded ^{13}C NMR spectrum of omeprazole in DMSO-d_6.

Residual solvents. Examine by head-space gas chromatography, according to the general procedure (2.2.28), using the standard addition method. The content of chloroform is not more than 50 ppm and the content of methylene chloride is not more than 100 ppm.

The chromatographic procedure may be carried out using:

- a fused-silica column 30 m long and 0.32 mm in internal diameter coated with a 1.8-μm film of cross-linked *poly-[(cyanopropyl)(phenyl)] [dimethyl] siloxane R*.
- *nitrogen for chromatography R* as the carrier gas.
- a flame-ionization detector.
- a suitable head-space sampler.

Place 0.5 g of omeprazole in a 10-ml vial. Add 4 ml of *dimethylacetamide R* and stopper the vial. Equilibrate the vial at 80 °C for 1 h.

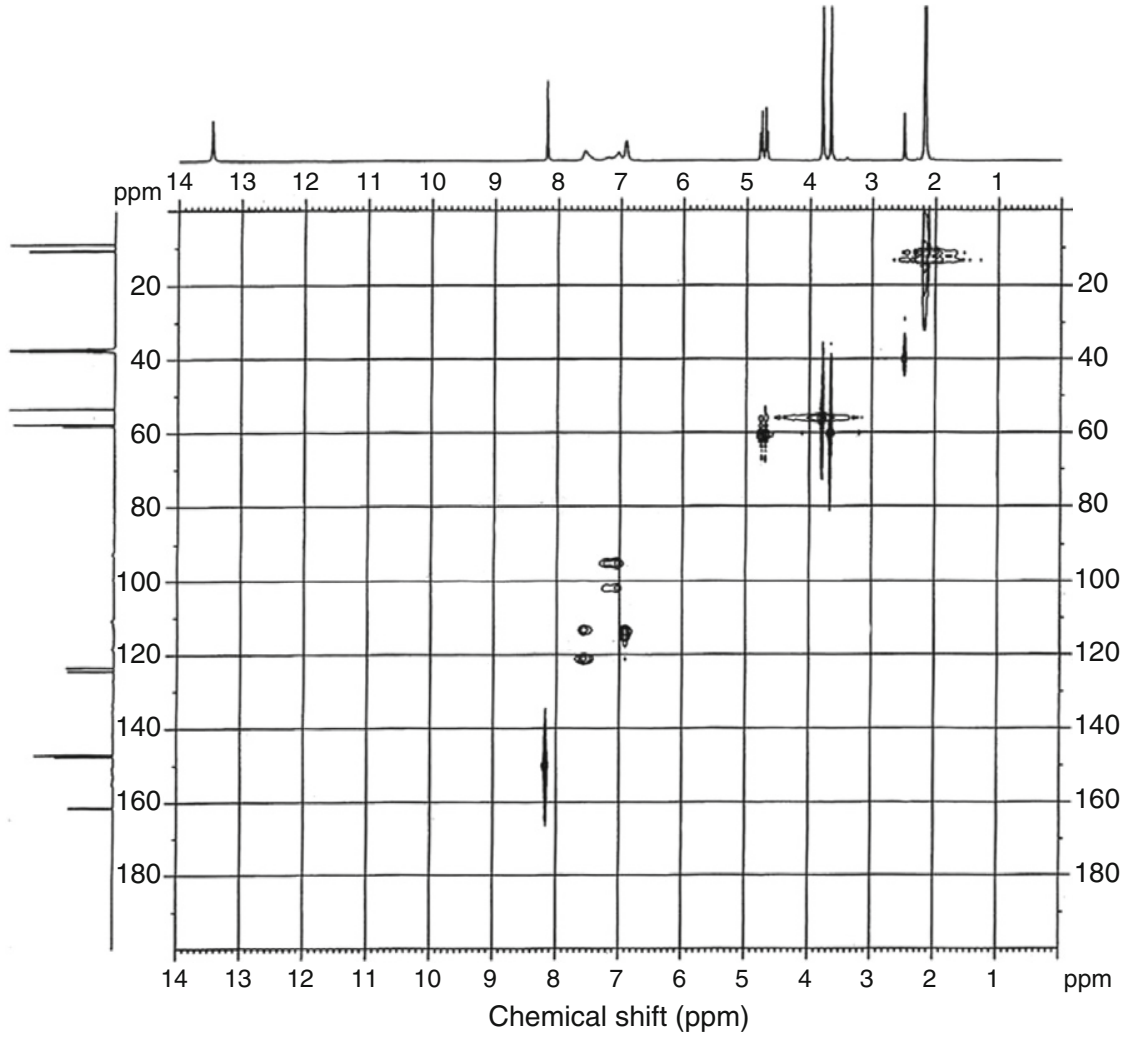


FIGURE 4.16 The HSQC NMR spectrum of omeprazole in DMSO-d_6.

Loss on drying. This test should be carried out according to the general procedure (2.2.32). Maximum 0.2%, determined on 1 g by drying under high vacuum at 60 °C for 4 h.

Sulfated ash. This test should be carried out according to the general procedure (2.4.14). Maximum 0.1%, determined on 1 g.

- *Assay*

Dissolve 1.1 g of omeprazole in a mixture of 10 ml of *water R* and 40 ml of *ethanol R* 96%. Titrate with 0.5 M *sodium hydroxide*, determining the end point potentiometrically as described in the general procedure (2.2.20).

1 ml of 0.5 M *sodium hydroxide* is equivalent to 0.1727 g of $C_{17}H_{19}N_3O_3S$.

- *Storage*

Store in an airtight container, protected from light, at a temperature between 2 and 8 °C.

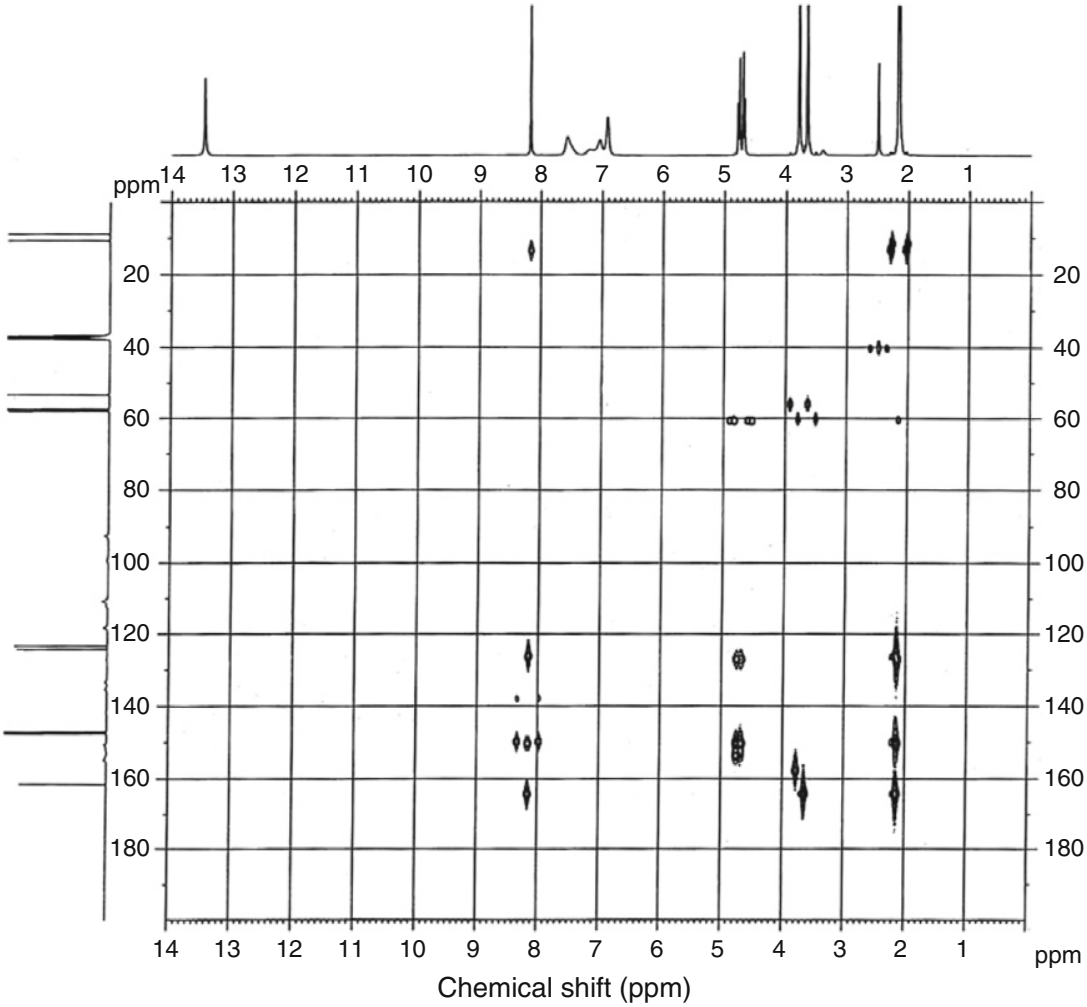
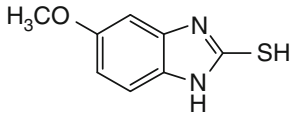


FIGURE 4.17 The HMBC NMR spectrum of omeprazole in DMSO-d_6.

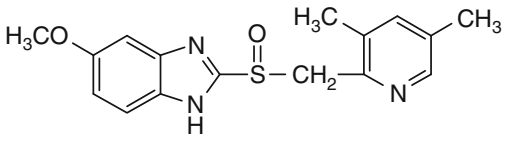
- *Impurities*

  Specified impurities A, B, C, D, E, F, G. Other detectable impurities H, I.

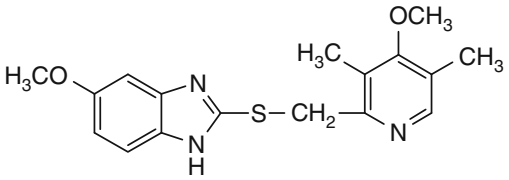
A. 5-Methoxy-1*H*-benzimidazole-2-thiol



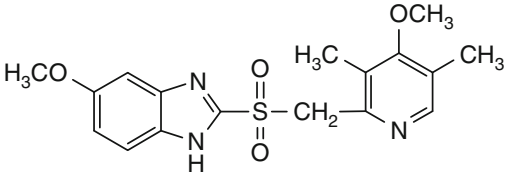
B. 2-(*RS*)-[[(3,5-dimethylpyridin-2-yl)methyl]sulphinyl]-5-methoxy-1*H*-benzimidazole



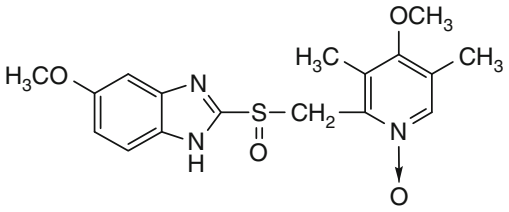
C. 5-Methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl] sulfanyl]-1*H*-benzimidazole (*ufiprazole*)



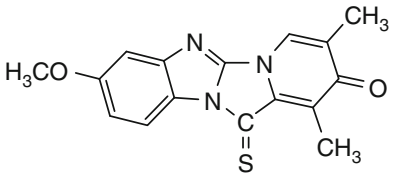
D. 5-Methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl] sulfonyl]-1*H*-benzimidazole (*omeprazole sulfone*)



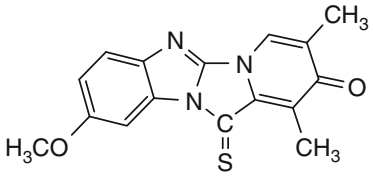
E. 4-Methoxy-2-[[(*RS*)-(5-methoxy-1*H*-benzimidazole-2-yl)sulfinyl] methyl]-3,5-dimethyl-pyridine-1-oxide



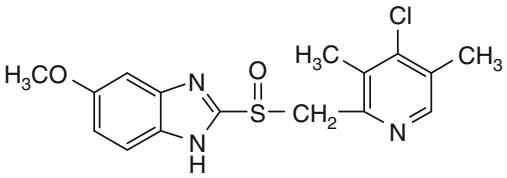
F. 8-Methoxy-1,3-dimethyl-12-thioxopyrido[1′,2′:3,4]imidazo[1,2-*a*] benzimidazol-2(12*H*)-one



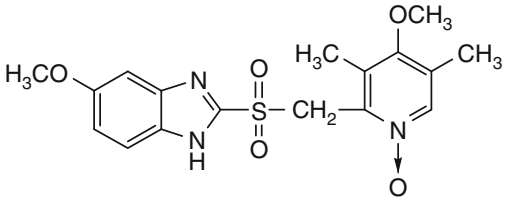
G. 9-Methoxy-1,3-dimethyl-12-thioxopyrido[1′,2′:3,4]imidazo[1,2-*a*] benzimidazol-2(12*H*)-one



H. 2-[(*RS*)-[(4-chloro-3,5-dimethylpyridin-2-yl)methyl]sulfinyl]-5-methoxy-1*H*-benzimidazole



I. 4-Methoxy-2-[[(5-methoxy-1*H*-benzimidazol-2-yl)sulfonyl] methyl]-3,5-dimethyl-pyridine-1-oxide



4.1.1.2. Omeprazole sodium

- *Definition*

Omeprazole sodium contains not less than 98% and not more than the equivalent of 101% of sodium 5-methoxy-2[(*RS*)-[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfinyl]-1*H*-benzimidazole, calculated with reference to the anhydrous substance.

- *Characters*

A white or almost white powder, hygroscopic, freely soluble in water and in alcohol, soluble in propylene glycol, very slightly soluble in methylene chloride.

- *Identification*

Test A: Dissolve 2 mg of omeprazole sodium in 0.1 M *sodium hydroxide* and dilute to 100 ml with the same solvent. Examined between 230 and 350 nm, as directed in the general procedure (2.2.25), the solution shows two absorption maxima, at 276 and 305 nm. The ratio of the absorbance measured at the maximum at 305 nm to that measured at the maximum at 276 nm is 1.6–1.8.

Test B: Examine the chromatograms obtained in the test for omeprazole impurity C. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). Place the plate in a tank saturated with vapor of *acetic acid R*. The spots rapidly turn brown.

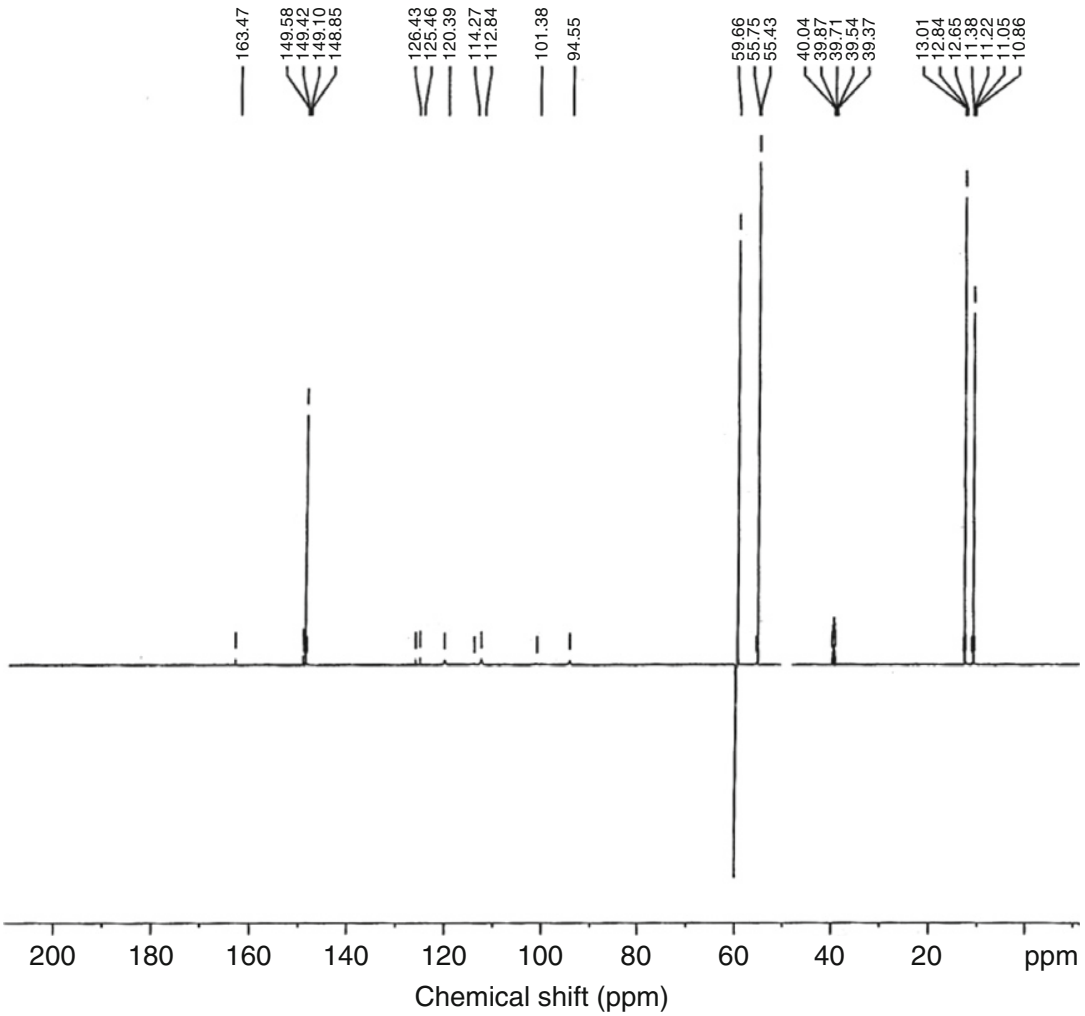


FIGURE 4.18 The DEPT 135 ^{13}C NMR spectrum of omeprazole in DMSO-d_6.

Test C: Ignite 1 g of omeprazole and cool. Add 1 ml of *water R* to the residue and neutralize with *hydrochloric acid R*. Filter and dilute the filtrate to 4 ml with *water R*. 0.1 ml of the solution gives reaction (b) of sodium, this test should be carried according to the general procedure (2.3.1).

- *TESTS*

Solution S: Dissolve 0.5 g of omeprazole sodium in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution: Carry out this test as directed in the general procedure (2.2.1), solution S is clear and not more intensely colored than reference solution B_6 (*Method II* in the general procedure (2.2.2)).

pH: This test should be carried out according to the general procedure (2.2.3). The pH of solution S is 10.3–11.3

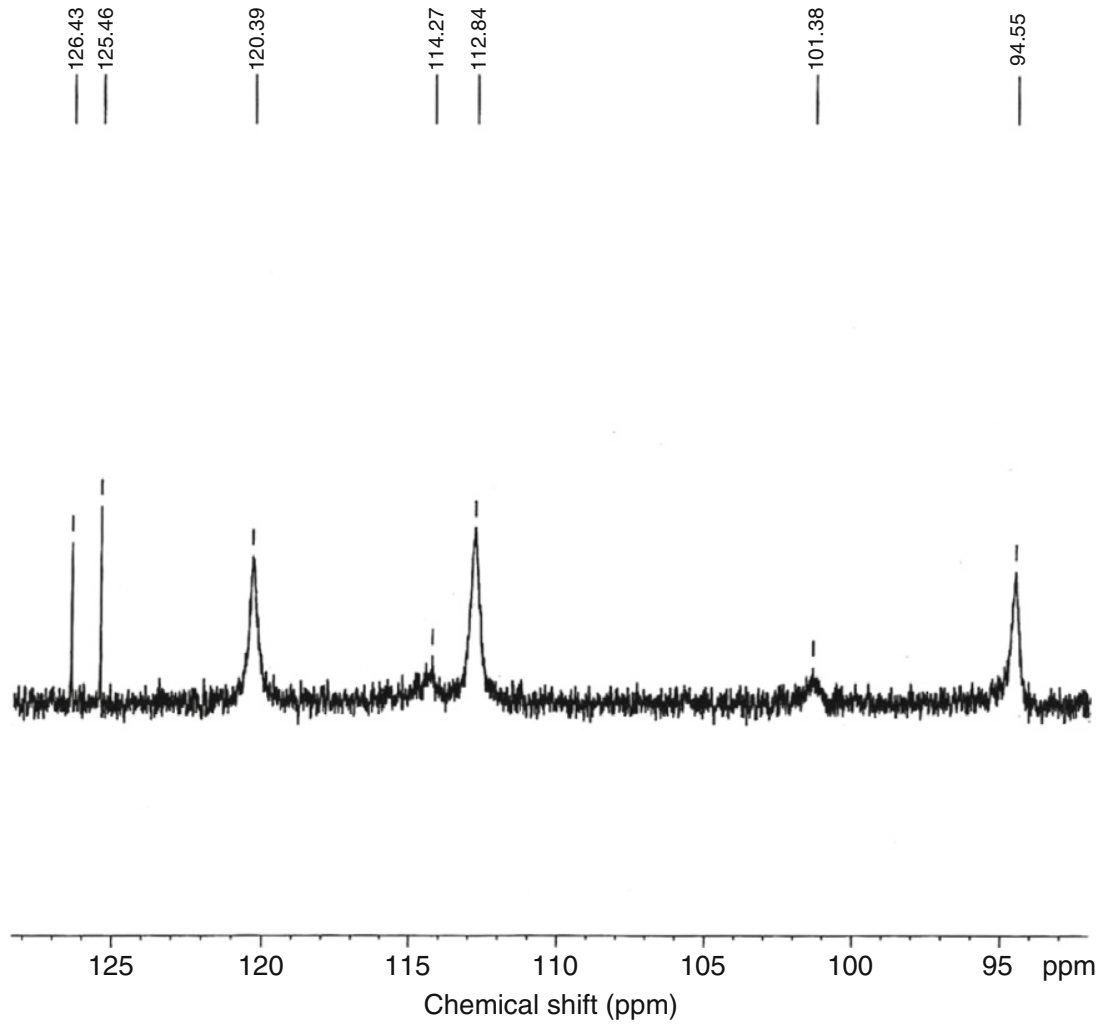


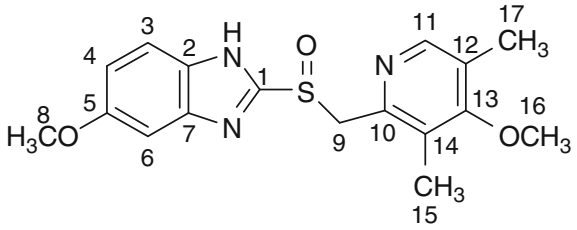
FIGURE 4.19 Expanded DEPT 135 ^{13}C NMR spectrum of omeprazole in DMSO-d_6.

Omeprazole impurity C: Examine by TLC, as directed in the general procedure (2.2.27), using *silica gel* HF_{254} *R* as the coating substance.

- *Test solution (a)*. Dissolve 0.1 g of omeprazole sodium in 2 ml of *methanol R*.
- *Test solution (b)*. Dilute 1 ml of test solution (a) to 10 ml with *methanol R*.
- *Reference solution (a)*. Dissolve 9 mg of *omeprazole CRS* in 2 ml of *methanol R*.
- *Reference solution (b)*. Dilute 1 ml of test solution (b) to 100 ml *with methanol R*.

Apply separately to the plate 10 μl of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *2-propanol R*, 40 volumes of *methylene chloride R* previously shaken with *concentrated ammonia R* (shake 100 ml of *methylene chloride R* with 30 ml of *concentrated ammonia R* in a separating funnel, allow the layers to separate and use the lower layer) and 40 volumes of *methylene chloride R*. Allow the plate to dry in air.

TABLE 4.8 Assignments of the resonance bands in the ^{13}C NMR spectra of omeprazole



Chemical shift (ppm relative to TMS)	Assignment at carbon number
11.07	15 or 17
12.85	15 or 17
55.49	8 or 16
59.69	8 or 16
60.09	9
94.55, 101.38, 112.84, 114.27, 120.39, 125.46, 126.43, 148.85, 149.10, 149.42, 149.58, 163.47	8 quaternary and 4 protonated carbons

Examine in the UV light at 254 nm. Any spot in the chromatogram obtained with test solution (a) with a higher R_f value than that of the spot corresponding to omeprazole is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.1%).

Related substances. Examine by liquid chromatography, as directed in the general procedure (2.2.29).

- *Test solution*. Dissolve 3 mg of omeprazole sodium in the mobile phase and dilute to 25 ml with the mobile phase.
- *Reference solution (a)*. Dissolve 1 mg of *omeprazole CRS* and 1 mg of *omeprazole impurity D CRS* in the mobile phase and dilute to 10 ml with the mobile phase.
- *Reference solution (b)*. Dilute 1 ml of the test solution to 100 ml with the mobile phase. Dilute 1 ml of this solution to 10 ml with the mobile phase.

The chromatography may be carried out using:

– a stainless-steel column 0.15 m long and 4 mm in internal diameter packed with *octylsilyl silica gel for chromatography R* (5 μm).

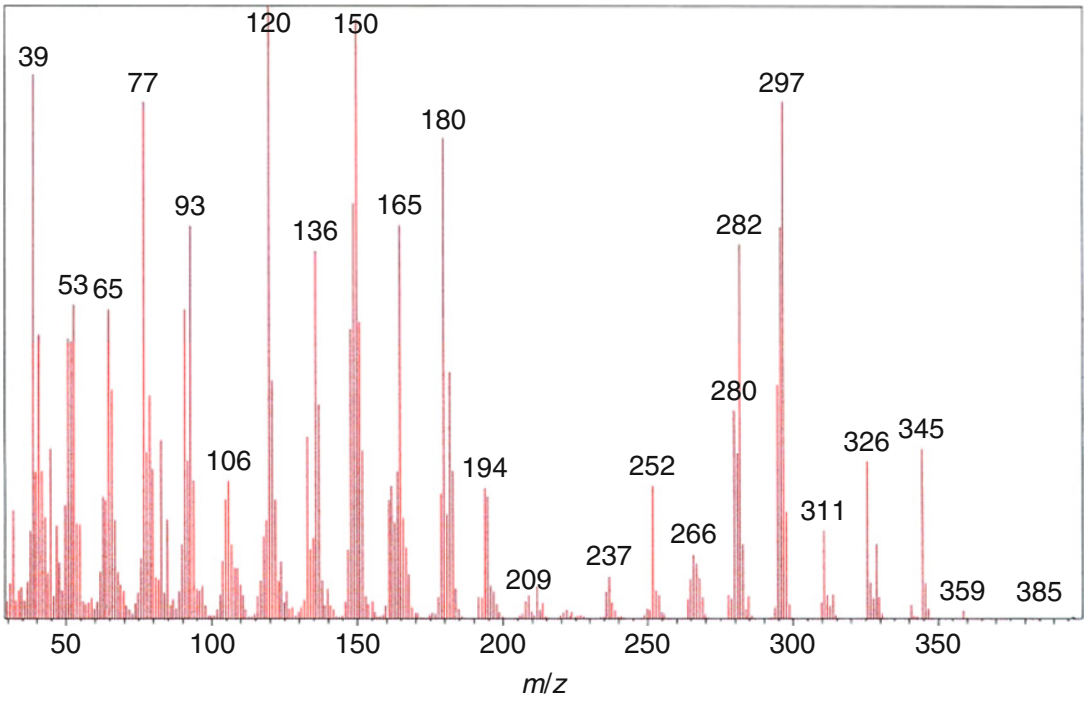


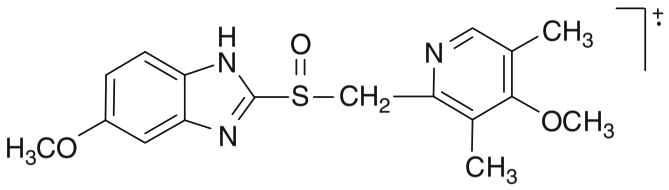
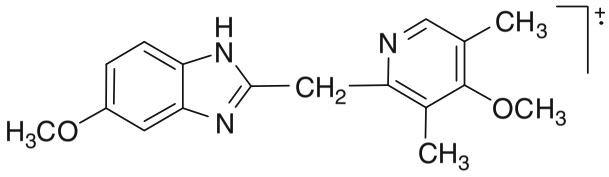
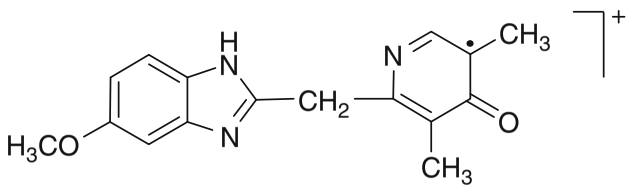
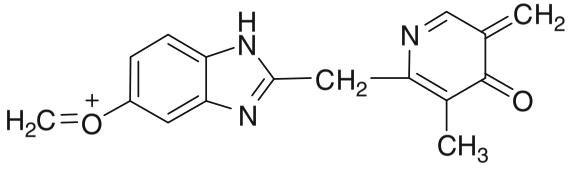
FIGURE 4.20 Mass spectrum of omeprazole in DMSO-d_6.

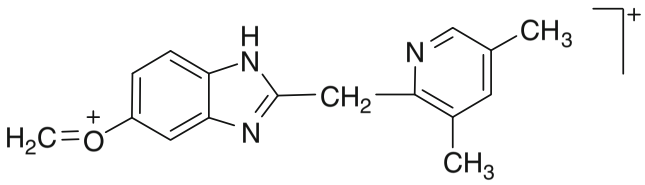
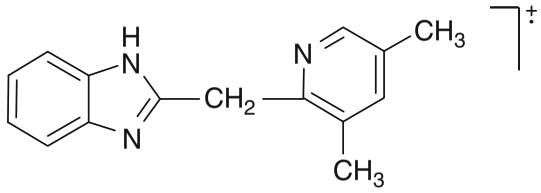
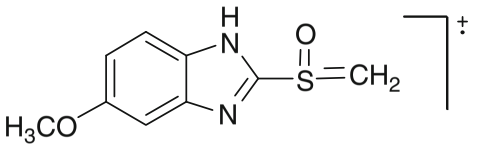
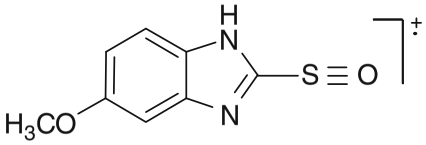
– as mobile phase at a flow-rate 1 ml/min a mixture of 27 volumes of *acetonitrile R* and 73 volumes of a 1.4-g/l solution of *disodium hydrogen phosphate R*, previously adjusted to pH 7.6 with *phosphoric acid R*.
– as detector a spectrophotometer set at 280 nm.

When the chromatograms are recorded in the prescribed conditions, the retention time of omeprazole is about 9 min and the relative retention time of omeprazole impurity *D* is about 0.8. Inject separately 40 μl of each solution and continue the chromatography for three times the retention time of omeprazole. Adjust the sensitivity of the detector so that the height of the principal peak in the chromatogram obtained with reference solution (b) is not less than 15% of the full scale of the recorder. The test is not valid unless the chromatogram obtained with reference solution (a), the resolution between the peaks corresponding to omeprazole impurity *D* and omeprazole is greater than 3. If necessary adjust the pH of the mobile phase or the concentration of *acetonitrile R*, an increase in the pH will improve the resolution. The area of any peak apart from the principal peak in the chromatogram obtained with the test solution is not greater than the area of the peak in the chromatogram obtained with reference solution (b) (0.1%).

Heavy metals: This test should be carried out as directed in the general procedure (2.4.8). One gram of omeprazole sodium complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

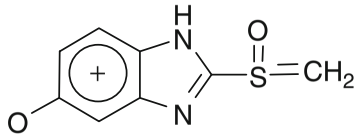
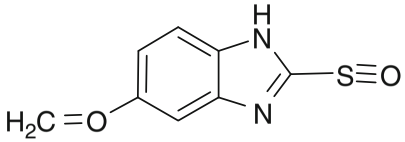
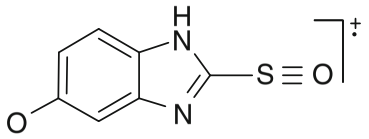
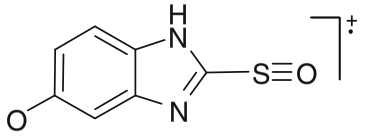
TABLE 4.9 Mass spectral fragmentation pattern of omeprazole

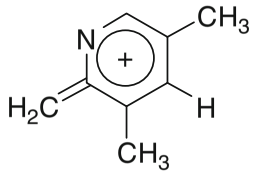
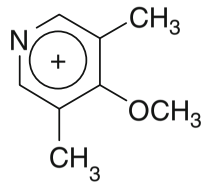
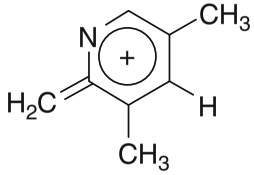
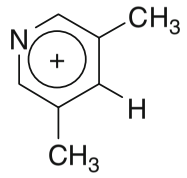
		Fragment	
m/z	Relative intensity (%)	Formula	Structure
345	6	$C_{17}H_{19}N_3O_3S$	
297	6	$C_{17}H_{19}N_3O_2$	
282	61	$C_{16}H_{16}N_3O_2$	
280	34	$C_{16}H_{14}N_3O_2$	

266	10	$C_{16}H_{16}N_3O$	
237	7	$C_{15}H_{15}N_3$	
209	4	$C_9H_9N_2O_2S$	
195	18	$C_8H_7N_2O_2S$	

(*continued*)

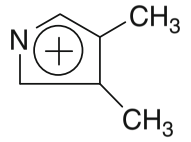
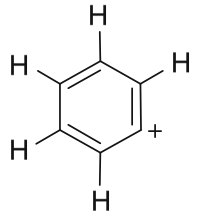
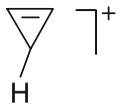
TABLE 4.9 (*continued*)

		Fragment	
m/z	Relative intensity (%)	Formula	Structure
194	20	$C_8H_6N_2O_2S$	 or 
180	79	$C_7H_4N_2O_2S$	
165	64	$C_7H_5N_2OS$	

150	98	$C_9H_{12}NO$	
136	60	$C_8H_{10}NO$	
120	100	$C_8H_{10}N$	
106	22	C_7H_8N	

(*continued*)

TABLE 4.9 (*continued*)

m/z	Relative intensity (%)	Fragment	
		Formula	Structure
93	64	C_6H_7N	
77	85	C_6H_5	
65	50	C_5H_5	
39	89	C_3H_3	

Water: This test should be carried out as directed in the general procedure (2.5.12). 4.5–10%, determined on 0.3 g by the semimicro determination of water.

• *Assay*

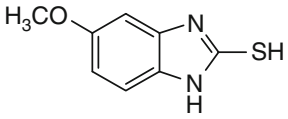
Dissolve 0.3 g of omeprazole sodium in 50 ml of *water R*. Titrate with 0.1 M *hydrochloric acid*, determining the end point potentiometrically as directed in the general procedure (2.2.20). One milliliter of 0.1 M *hydrochloric acid* corresponds to 36.74 of $C_{17}H_{18}N_3NaO_3S$.

• *Storage*

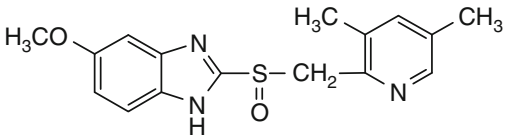
Store omeprazole sodium in an airtight container, protected from light.

• *Impurities*

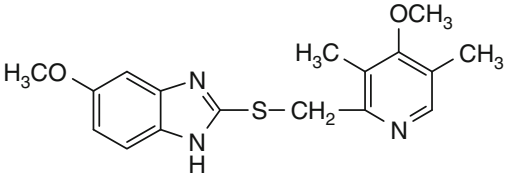
A. 5-Methoxy-1*H*-benzimidazole-2-thiol



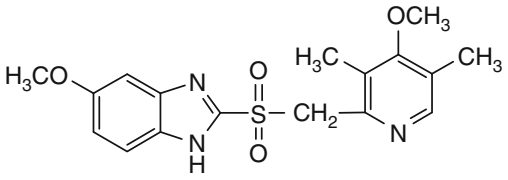
B. 2-[(*RS*)-[(3,5-dimethylpyridin-2-yl)methyl]sulfinyl]-5-methoxy-1*H*-benzimidazole



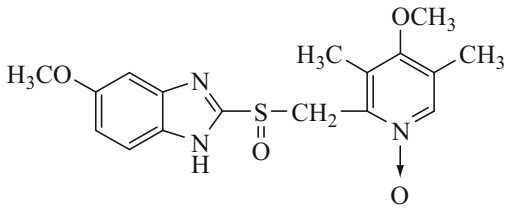
C. 5-Methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]thio]-1*H*-benzimidazole (*ufiprazole*)



D. 5-Methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl] sulfonyl]-1*H*-benzimidazole (*omeprazole sulfone*)



E. 4-Methoxy-2-[[(*RS*)-(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl] methyl]-3,5-dimethyl-pyridine-1-oxide



4.1.2. United States Pharmacopeia (USP) methods [25]

4.1.2.1. Omeprazole Omeprazole contains not less than 98% and not more than 102% of $C_{17}H_{19}N_3O_3S$, calculated on the dried basis.

Packaging and storage: Preserve in tight containers and store in a cold place, protected from moisture.

USP Reference Standards, general procedure ⟨11⟩: *USP Omeprazole RS*.

- *Identification*

  Test A: The R_f value of the principal spot observed in the chromatogram of the *Identification solution* corresponds to that of the principal spot observed in the chromatogram of the *Standard solution* containing 0.15 mg of *USP Omeprazole RS* per ml, obtained as directed in the test for *Chromatographic purity*, *Method 1*.

  Test B: *Infrared absorption*—Carry out this test as directed in the general procedure ⟨197 K⟩. The IR absorption spectrum of a potassium bromide dispersion of omeprazole previously dried, exhibits maxima only at the same wavelength as that of similar preparation of *USP Omeprazole RS*.

  Completeness of solution—This test should be carried out as directed in the general procedure ⟨641⟩. Meets the requirements, a solution in methylene chloride containing 20 mg/ml being used.

  Color of solution—Determine the absorbance of the solution prepared for the *Completeness of solution* test at 440 nm, in 1-cm cells, using methylene chloride as the blank: the absorbance is not greater than 0.1.

  Loss on drying—Carry out this test as directed in the general procedure ⟨731⟩. Dry omeprazole in vacuum at 60 °C for 4 h: it loses not more than 0.5% of its weight.

  Residue on ignition—Carry out this test as directed in the general procedure ⟨281⟩: not more than 0.1%.

  Heavy metals—Carry out this test as directed in the general procedure ⟨231⟩, *Method II*, 0.002%.

Organic volatile impurities—Carry out this test as directed in the general procedure <467>, *Method IV*: meet the requirements.
Solvents—Use dimethylacetamide.

- *Chromatographic purity*

METHOD 1

Solvents: Prepare a mixture of dichloromethane and methanol (1:1).
Standard solutions: Dissolve an accurately weighed quantity of *USP Omeprazole RS* in *Solvent*, and mix to obtain *Standard solution A* having a known concentration of about 0.5 mg/ml. Dilute this solution quantitatively with *Solvent* to obtain *Standard solution B* and *Standard solution C* having known concentrations of about 0.15 and 0.05 mg/ml, respectively.
Test solution: Prepare a solution of Omeprazole in *Solvent* containing 50 mg/ml.
Identification solution: Dilute a volume of the *Test solution* quantitatively with *Solvent* to obtain a solution containing 0.25 mg/ml.
Procedure: Separately apply 10 µl of the *Test solution*, the *Identification solution*, and each of the *Standard solutions* to a TLC plate (see *Chromatography*, in the general procedure ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spot to dry, and develop the chromatogram in a solvent system consisting of a mixture of ammonia-saturated dichloromethane, dichloromethane, and isopropanol (2:2:1) until the solvent front has moved about three-fourths of the length of the plate.

[**Note:** Prepare ammonia-saturated dichloromethane as follows. Shake 100 ml of dichloromethane with 30 ml of ammonium hydroxide in a separatory funnel, allow the layers to separate, and use the lower layer]. Remove the plate from the developing chamber, mark the solvent front, allow the solvent to evaporate, and examine the plate under short-wavelength UV light: the chromatograms show principal spots at about the same R_f value. Estimate the intensities of any secondary spots observed in the chromatogram of the *Test solution* by comparison with the spots in the chromatograms of the *Standard solutions*: no secondary spot from the chromatogram of the *Test solution* is larger or more intense than the principal spot obtained from *Standard solution B* (0.3%), and the sum of the intensities of all secondary spots obtained from the *Test solution* is not more intense than the principal spot obtained from *Standard solution A* (1%).

METHOD 2

Diluent: Use *Mobile phase*.
Phosphate buffer, *Mobile phase*, *System suitability solution*, and *Chromatographic system*: Proceed as directed in the *Assay*.

Test solution: Dissolve an accurately weighed quantity of omeprazole in *Diluent* to obtain a solution containing about 0.16 mg/ml [**Note:** Prepare this solution fresh].

Procedure: Inject equal volumes (about 40 μl) of the *Test solution* and *Diluent* into the chromatograph, and allow the *Test solution* to elute for not less than two times the retention time of omeprazole. Record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of omeprazole taken by the formula:

$$100 \times (r_i/r_s)$$

in which r_i is the peak response for each impurity and r_s is the sum of the responses of all the peaks: not more than 0.3% of any individual impurity is found, and the sum of all impurities is not more than 1%.

- *Assay*

  Phosphate buffer: Dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 300 ml of water, dilute with water to 1000 ml, and mix. Dilute 250 ml of this solution with water to 1000 ml. If necessary, adjust the pH with phosphoric acid to 7.6.

  Mobile phase: Prepare a filtered and degassed mixture of *Phosphate buffer* and acetonitrile (3:1). Make adjustment if necessary (see *system suitability* under *Chromatography*, in the general procedure ⟨621⟩).

  Diluent: Prepare a mixture of 0.01 M sodium borate and acetonitrile (3:1).

  Standard preparation: Dissolve an accurately weighed quantity of *USP Omeprazole RS* in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.2 mg/ml.

  Assay preparation: Transfer about 100 mg of Omeprazole, accurately weighed, to a 50-ml volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix. Transfer 5 ml of this solution to a 50-ml volumetric flask, dilute with *Diluent* to volume, and mix.

  System suitability solution: Dilute a volume of *Standard preparation* with *Diluent* to obtain a solution containing about 0.1 mg of *USP Omeprazole RS* per ml.

  Chromatographic system (see *Chromatography*, in the general procedure ⟨621⟩): The liquid chromatography is equipped with a 280-nm detector and a 4.6 mm × 15-cm column that contains 5-μm packing L7. The flow-rate is about 0.8 ml/min. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the capacity factor, k', is not less than 6; the column efficiency is not less than 3000 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation (RSD) for replicate injections is not more than 1%.

Procedure: Separately inject equal volumes (about 20 μl) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{17}H_{19}N_3O_3S$ in the portion of omeprazole taken by the formula:

$$500 \times C(r_U/r_s)$$

in which C is the concentration (in mg/ml) of *USP Omeprazole RS* in the *Standard preparation*, and r_U and r_s are the peak responses obtained from the *Assay preparation* and *Standard preparation*, respectively.

4.1.2.2. Omeprazole delayed-release capsules Omeprazole Delayed-Release Capsules contain not less than 90% and not more than 110% of the labeled amount of omeprazole ($C_{17}H_{19}N_3O_3S$).

Packaging and storage: Preserve in tight, light-resistant container. Store between 15 and 30 °C.

Labeling: When more than one *Dissolution test* is given, the labeling states the *dissolution test* used only if *Test 1* is not used.

USP Reference Standards; general procedure ⟨11⟩: *USP Omeprazole RS*.

Identification: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution. Carry out this test as directed in the general procedure ⟨711⟩.

TEST 1
– ACID RESISTANCE STAGE

Medium: 0.1 N hydrochloric acid; 500 ml.

Apparatus 2: 100 rpm.

Time: 2 h.

pH 7.6 Phosphate buffer, *Mobile phase*, and *Chromatographic system*: Proceed as directed for *Buffer stage*.

Standard solution: Transfer about 50 mg of *USP Omeprazole RS*, accurately weighed, to a 250-ml volumetric flask, dissolve in 50 ml of alcohol, dilute with 0.01 M sodium borate solution to volume, and mix. Transfer 10 ml of this solution into a 100-ml volumetric flask, add 20 ml of alcohol, dilute with 0.01 M sodium borate solution to volume, and mix.

Test solution: After 2 h, filter the *Medium* containing the pellets through a sieve with an aperture of not more than 0.2 mm. Collect the pellets on the sieve, and rinse them with water. Using approximately 60 ml of 0.01 M sodium borate solution, carefully transfer the pellets quantitatively to a 100-ml volumetric flask. Sonicate for about 20 min until the pellets are broken up. Add 20 ml of alcohol to the flask, dilute with 0.01 M sodium

borate solution to volume, and mix. Dilute an appropriate amount of this solution with 0.01 M sodium borate solution to obtain a solution having a concentration of about 0.02 mg/ml. At level L_1, test 6 units. Test 6 additional units at level L_2, and at level L_3, an additional 12 units are tested. Continue testing through the three levels unless the results conform at either L_1 or L_2.

Procedure: Separately inject equal volumes (about 20 μl) of the *Standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of omeprazole ($C_{17}H_{19}N_3O_3S$) dissolved in the *medium* by the formula:

$$T - CD\,(r_U/r_s)$$

in which T is the labeled quantity (in mg) of omeprazole in the capsule, C is the concentration (in mg/ml) of *USP Omeprazole RS* in the *standard solution*, D is the dilution factor used in preparing the *test solution*, and r_U and r_s are the omeprazole peak responses obtained from *Test solution* and the *Standard solution*, respectively.

Tolerances: Level L_1: no individual value exceeds 15% of omeprazole dissolved. Level L_2: the average of 12 units is not more than 20% of omeprazole dissolved, and no individual unit is greater than 35% of omeprazole dissolved. Level L_3: the average of 24 units is not more than 20% of omeprazole dissolved, not more than 2 units are greater than 35% of omeprazole dissolved, and no individual unit is greater than 45% of omeprazole dissolved.

– BUFFER STAGE

Medium: pH 6.8 phosphate buffer, 900 ml.

Proceed as directed for *Acid resistance stage* with a new set of capsules from the same batch. After 2 h, add 400 ml of 0.235 M dibasic sodium phosphate to the 500 ml of 0.1 N hydrochloric acid medium in the vessel. Adjust, if necessary, with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of 6.8 $\pm$ 0.05.

Apparatus 2: 100 rpm.

At the end of 30 min, determine the amount of $C_{17}H_{19}N_3O_3S$ dissolved in pH 6.8 phosphate buffer by employing the following method:

pH 10.4, 0.235 M Dibasic sodium phosphate: Dissolve 33.36 g of anhydrous dibasic sodium phosphate in 1000 ml of water, and adjust with 2 N sodium hydroxide to a pH of 10.4 $\pm$ 0.1.

pH 6.8 Phosphate buffer: Add 400 ml of 0 N hydrochloric acid to 320 ml of pH 10.4, 0.235 M *Dibasic sodium phosphate*, and adjust with 2 N hydrochloric acid or 2 N sodium hydroxide, if necessary, to a pH of 6.8 $\pm$ 0.05.

pH 7.6 Phosphate buffer: Dissolve 0.718 g of monobasic sodium phosphate and 4.49 g of dibasic sodium phosphate in 1000 ml of water. Adjust with 2 N hydrochloric acid or 2 N sodium hydroxide, if necessary, to a pH of 7.6 ± 0.1. Dilute 250 ml of this solution with water to 1000 ml.

Mobile phase: Transfer 340 ml of acetonitrile to a 1000-ml volumetric flask, dilute with *pH 7.6 Phosphate buffer* to volume, and pass through a membrane filter having a 0.5-μm or finer porosity. Make adjustments, if necessary (see *System Suitability* under *Chromatography*, in the general procedure ⟨621⟩).

Standard solution 1 (for Capsules labeled 10 mg): Dissolve an accurately weighed quantity of *USP Omeprazole RS* in alcohol to obtain a solution having a known concentration of about 2 mg/ml. Dilute with *pH 6.8 Phosphate buffer* quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.01 mg/ml. Immediately add 2 ml of 0.25 M sodium hydroxide to 10 ml of this solution, and mix. [**Note:** Do not allow the solution to stand before adding the sodium hydroxide solution.]

Standard solution 2 (for Capsules label 20 mg and 40 mg): Proceed as directed for *Standard solution 1*, except to obtain a solution having a known concentration of about 0.02 mg/ml before mixing with 2 ml of 0.25 M sodium hydroxide.

Test solution 1 (for Capsules containing 10 and 20 mg): Immediately transfer 5 ml of the solution under test to a test tube containing 1 ml of 0.25 M sodium hydroxide. Mix well, and pass through a membrane filter having a 1.2-μm or finer porosity. Protect from light.

Test solution 2 (for Capsules labeled 40 mg): Immediately transfer 5 ml of the solution under test to a test tube containing 2 ml of 0.25 M sodium hydroxide and 5 ml of *pH 6.8 Phosphate buffer*. Mix well, and pass through a membrane filter having a 1.2-μm or finer porosity. Protect from light.

Chromatographic system (see *Chromatography*, in the general procedure ⟨621⟩): The liquid chromatograph is equipped with a 280-nm detector and a 4 mm × 12.5-cm analytical column that contains 5-μm packing L7. The flow-rate is about 1 ml/min. Chromatograph the appropriate *Standard solution* and record the peak responses as directed for *Procedure*: the column efficiency is not less than 2000 theoretical plates, and the RSD for replicate injections is not more than 2%.

Procedure: Separately inject equal volumes (about 20 μl) of the appropriate *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of omeprazole ($C_{17}H_{19}N_3O_3S$) dissolved by the formula:

$$VCD\,(r_U/r_s)$$

in which V is the volume of *Medium* in each vessel, C is the concentration (in mg/ml) of *USP Omeprazole RS* in the appropriate *standard solution*, D is the dilution factor used in preparing the appropriate *test solution*, and r_U and r_s are the omeprazole peak responses obtained from the appropriate *Test solution* and the *Standard solution*, respectively.

Tolerances: For Capsules labeled 10 and 20 mg, not less than 75% (Q) of the labeled amount of $C_{17}H_{19}N_3O_3S$ is dissolved in 30 min. For capsules labeled 40 mg, not less than 70% (Q) of the labeled amount of $C_{17}H_{19}N_3O_3S$ is dissolved in 30 min. The requirements are met if the quantities dissolved from the product conform to *Acceptance Table*.

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

– ACID RESISTANCE STAGE

Medium: 0.1 N hydrochloric acid; 900 ml.
Apparatus 1: 100 rpm.
Time: 2 h.
Procedure: After 2 h, remove each sample from the basket, and quantitatively transfer into separate volumetric flasks to obtain a solution having a final concentration of about 0.2 mg/ml. Proceed as directed for the *Assay preparation* in the *Assay*, starting with ''Add about 50 ml of *Diluent*.'' Calculate the quantity, in mg, of omeprazole ($C_{17}H_{19}N_3O_3S$) dissolved in the *Medium* by the formula:

$$T - CD\,(r_U/r_s)$$

in which T is the assayed quantity (in mg) of omeprazole in the capsule, C is the concentration (in mg/ml) of *USP Omeprazole RS* in the *Standard solution*, D is the dilution factor used in preparing the *Test solution*, and r_U and r_s are the omeprazole peak responses obtained from *Test solution* and *Standard solution*, respectively.

Tolerances: It complies with *Acceptance Table* 4.10.

TABLE 4.10 Acceptance Table

Level	Criterion
L_1	The average of the 6 units is not more than 10% of omeprazole dissolved
L_2	The average of the 12 units is not more than 10% of omeprazole dissolved
L_3	The average of the 24 units is not more than 10% of omeprazole dissolved

– BUFFER STAGE

Medium: pH 6.8 0.05 M phosphate buffer; 900 ml (see *Reagents*, *Indicators*, and *Solutions*).
Apparatus 1: 100 rpm.
Time: 45 min.
Procedure: Proceed as directed for *Acid resistance stage* with a new set of capsules from the same batch. After 2 h, replace the acid *Medium* with the buffer *Medium* and continue the test for 45 more minutes. Determine the amount of $C_{17}H_{19}N_3O_3S$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 305 nm on portions of the solutions under test passed through 0.2-μm nylon filter, in comparison with a Standard solution having a known concentration of *USP Omeprazole RS* and the same *Medium*.
Tolerances: It complies with *Acceptance* Table 4.10 under *Dissolution*, in the general procedure ⟨711⟩. Not less than 75% (Q) of the labeled amount $C_{17}H_{19}N_3O_3S$ is dissolved in 45 min.

Uniformity of dosage units, general procedure ⟨905⟩: meet the requirements.

- *Chromatographic purity*

  Diluent, *Solution A*, *Solution B*, *Mobile phase*, and *Chromatographic system*: Proceed as directed in the *Assay*.
  Standard solution: Prepare as directed for the *Standard preparation* in the *Assay*.
  Test solution: Use the *Assay preparation*.
  Procedure: Separately inject equal volumes (about 10 μl) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure all the peak responses. Calculate the percentage of each impurity in the portion of capsules taken by the formula:

$$10 \times (C/A)(1/F)(r_i/r_s)$$

  in which C is the concentration (in μg/ml) of *USP Omeprazole RS* in the *Standard solution*, A is the quantity (in mg) of omeprazole in the portion of capsules taken, as determined in the *Assay*, F is the relative response factor (see Table 4.11 for values), r_i is peak response for each impurity obtained from the *Test solution*, and r_s is the peak response for omeprazole obtained from *Standard solution*. In addition to not exceeding the limits for each impurity in Table 4.11, not more than 2% of total impurities is found.

TABLE 4.11 Limits for impurity

Name	Relative retention time	Relative response factor, *F*	Limit (%)
Thioxopyrido conversion product[a]	0.33	1.6	0.5
5-Methoxy-1*H*-benzimidazole-2-thiol	0.64	3.1	0.5
Any other individual impurity	–	1.0	0.5

[a] Formed in the solution from two isomers: 1,3-dimethyl-8-methoxy-12-thioxopyrido[1′,2′:3,4]imidazo-[1,2-*a*]benzimidazol-2-(12*H*)-one and 1,3-dimethyl-9-methoxy-12-thioxopyrido[1′,2′:3,4]imidazo[1,2-*a*]benzimidazole-2(12*H*)-one.

- *Assay*

  Diluent: Dissolve 7.6 g of sodium borate decahydrate in about 800 ml of water. Add 1 g of edetate disodium, and adjust with 50% sodium hydroxide solution to a pH of 11 ± 0.1. Transfer the solution to a 2000-ml volumetric flask, add 400 ml of dehydrated alcohol, and dilute with water to volume.

  Solution A: Prepare a filtered and degassed solution of 6 g of glycine in 1500 ml of water. Adjust with 50% sodium hydroxide solution to a pH of 9, and dilute with water to 2000 ml.

  Solution B: Use a filtered and degassed mixture of acetonitrile and methanol (85:15).

  Mobile phase: Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatograpic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography*, in the general procedure ⟨621⟩).

  Standard preparation: Dissolve, by sonicating, an accurately weighed quantity of *USP Omeprazole RS* in *Diluent*, and dilute quantitatively, and stepwise, if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.2 mg/ml.

  Assay preparation: Weigh and mix the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the mixture, equivalent to about 20 mg of omeprazole, to a 100-ml volumetric flask, add about 50 ml of *Diluent*, and sonicate for 15 min. Cool, dilute with *Diluent* to volume, mix, and pass through a membrane filter having 0.45 μm or finer porosity. [**Note:** Bubbles may form just before bringing the solution to volume. Add a few drops of dehydrated alcohol to dissipate the bubbles if they persist for more than a few minutes].

Chromatographic system (see *Chromatography*, in the general procedure ⟨621⟩: The liquid chromatography is equipped with a 305-nm detector and a 4.6 mm × 15-cm column that contains 5-μm base-deactivated packing L7. The flow-rate is about 1.2 ml/min. The chromatograph is programmed as follows:

Time (min)	Solution A (%)	Solution B (%)	Elution
0–20	88 → 40	12 → 60	Linear gradient
20–21	40 → 88	60 → 12	Linear gradient
21–25	88	12	Isocratic

Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 20,000 theoretical plates; the tailing factor is not less than 0.8 and not more than 2; and the RSD for replicate injections is not more than 2%.

Procedure: Separately inject equal volumes (about 10 μl) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of omeprazole ($C_{17}H_{19}N_3O_3S$) in the portion of Capsules taken by the formula:

$$DC(r_U/r_s)$$

in which D is the dilution factor of the *Assay preparation*, C is the concentration (in mg/ml) of *USP Omeprazole RS* in the *Standard preparation*, and r_U and r_s are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

4.2. Reported methods of analysis

4.2.1. Spectrophotometry

Dhumal *et al.* [26] described an individual UV spectrophotometric assay method for the analysis of omeprazole from separate pharmaceutical dosage forms. Powdered tablets, equivalent to 50 mg of the drug, were sonicated with 35 ml of 0.1 M sodium hydroxide for 5 min and diluted to 50 ml with 0.1 M sodium hydroxide. The solution was filtered and a 2-ml portion of the filtrate was diluted to 200 ml with 0.1 M sodium hydroxide before the absorbance of the solution was measured at 305 nm versus 0.1 M sodium hydroxide. Beer's law was obeyed for 6–25 μg/ml of omeprazole. Coefficient of variation was 3.1%. Recovery was quantitative.

Sastry *et al.* [27] described four simple and sensitive spectrophotometric methods for the assay of omeprazole in pure and in dosage forms based on the formation of chloroform soluble ion-associated under specified experimental conditions. Four acidic dyes: Suprachen Violet 3B (SV 3B, method A), Tropaeolin 000 (TP 000, method B), Boromocresol Green (BCG, method C), and Azocarmine G (AG, method D) are utilized.

The extracts of the ion-associates exhibit absorption maxima at 590, 420, 500, and 540 nm for methods A, B, C, and D, respectively. Beer's law and the precision and accuracy of the methods are checked by UN reference method.

Ozaltin and Kocer [28] used a derivative spectroscopic method for the determination of omeprazole in pharmaceuticals. Capsule contents were powdered and a sample equivalent to one capsule content was sonicated with 10 ml ethanol, diluted to 100 ml with 0.1 M borate buffer of pH 10 and further diluted as necessary. Spectra were recorded at 50 nm/min with a 3-nm slit width second-order derivative curves were obtained for 200–400 nm using $\Delta\lambda = 31.5$ and $N = 9$. The calibration graph for peak-to-peak measurements between 303 and 310 nm were linear for 0.2–40 μg/ml omeprazole and the RSDs were 1.09–4.55%. Mean recovery was 100.7%. Result agreed with those obtained by polarography.

Tuncel and Dogrukol-Ak [29] developed a flow-through spectrophotometric method for the determination of omeprazole in pharmaceutical preparations containing enteric-coated pellets. Sample was dissolved in 100 ml 0.1 M sodium hydroxide and filtered. Portions were analyzed by flow-through spectrophotometry using a Spectrophoresis 100 system with 75 μm fused-silica capillaries with detection at 305 nm. Samples were pumped through the system for 2 min. Results were compared with those obtained by standard spectrophotometry at 305 nm. Detection limits was 8 μM omeprazole and the calibration graph was linear. The pellet matrix did not interfere. RSD was 1.9% ($n = 6$).

Karlsson and Hermansson [30] used chemometrics for optimization of chiral separation of omeprazole and one of its metabolites on immobilized α1-acid glycoprotein. Plasma was centrifuged at 2500 rpm and a portion (20–50 μl) was injected into a 5-μm Chiral-AGP column (10 cm $\times$ 4 mm) with α1-acid glycoprotein immobilized to silica as a chiral stationary phase and acetonitrile–phosphate buffer of pH 5.7–7.2 as mobile phase (1 ml/min). Detection of omeprazole and its main metabolite, hydroxylated omeprazole, was performed at 302 nm. A statistical model was developed for the optimization of the operational parameters. The experimental data were evaluated with multivariate analyses; column temperature and acetonitrile concentration were the most important variables for the enantioseparations. Complete enantiomeric separation for omeprazole and hydoxylated omeprazole was obtained within 15 min.

El-Kousy and Bebawy [31] described two stability-indicating spectrophotometric methods for the determination of omeprazole in the presence of its photodegradation products. In the first method, omeprazole from capsules or vials were dissolved in acetonitrile/water (1:1) and UV–VIS spectrophotometry used to determine the first-, second-, and third-derivative absorption curves between 200 and 400 nm. The level of omeprazole was assayed from the values of ordinates of the three curves at 290.4,

320.6, and 311.6 nm using calibration curves from standard solutions with concentrations of 3–25 μg/ml. In the second method, omeprazole in tablets was dissolved in chloroform and reacted with 0.2% chloranil solution in chloroform by heating at 70 °C for 20 min. The absorption at 377 nm was measured against a reagent blank. The level of omeprazole was determined against a calibration curve obtained with standard solutions of 8–55 μg/ml.

Wahbi *et al.* [32] used a spectrophotometric method for the determination of omeprazole in pharmaceutical formulations. The compensation method and other chemometric methods (derivative, orthogonal function, and difference spectrophotometry) have been applied to the direct determination of omeprazole in its pharmaceutical preparations. The method has been validated; the limits of detection was 3.3×10^{-2} μg/ml. The repeatability of the method was found to be 0.3–0.5%. The linearity range is 0.5–3.5 μg/ml. The method has been applied to the determination of omeprazole in its gastro-resistant formulation. The difference spectrophotometric (ΔA) method is unaffected by the presence of acid induced degradation products, and can be used as a stability-indicating assay method.

Karljikovic-Rajic *et al.* [33] developed a first-derivative UV spectrophotometry, applying the zero-crossing method, for the determination of omeprazole and omeprazole sulfone in methanol/4% ammonia, where sufficient spectral resolution of the drug and corresponding impurity were obtained, using the amplitudes $^1D_{304}$ and $^1D_{307}$, respectively. The method showed good linearity in the ranges (μg/ml) 1.61–17.2 for omeprazole and 2.15–21.50 for omeprazole sulfone and also good accuracy and precision. The experimentally determined values of the limit of detection (μg/ml) were 1.126 and 0.76 for omeprazole and omeprazole sulfone, respectively.

Salama *et al.* [34] developed and validated a spectrophotometric method for the determination of omeprazole and pantoprazole sodium via their metal chelates. The procedures were based on the formation of 2:1 chelates of both drugs with different metal ions. The colored chelates of omeprazole in ethanol were determined spectrophotometrically at 411, 339, and 523 nm using iron(III), chromium(III), and cobalt(II), respectively. Regression analysis of Beer's plots showed good correlation in the concentration ranges 15–95, 10–60, and 15–150 μg/ml of pure omeprazole using iron(III), chromium(III), and cobalt(II), respectively.

Riedel and Leopold [35] investigated the degradation of omeprazole in organic polymer solutions and aqueous dispersions of enteric-coating polymers by UV spectroscopy. Data were compared with those obtained in a previous high-performance liquid chromatographic (HPLC) study. For comparative purposes the cationic Eudragit RS 100 and the monomeric acid acetic acid were included in this study. The discolorations of

the degraded omeprazole solutions were analyzed by visible spectroscopy. UV–VIS spectra were recorded after preparation of the solutions and after 180 min of storage. The change of absorption was calculated as the difference of the absorption values at 305 nm. Degradation of omeprazole depends on the amount of acidic groups in the polymer structure.

Yang *et al.* [36] studied omeprazole samples from different sources and in different forms spectrophotometrically to obtain pK_a values. In the neutral to alkaline pH region, two consistent pK_a values of 7.1 and 14.7 were obtained from various samples. The assignment of these pK_a values were realized by comparison with the prototropic properties of *N*(1)-methylated omeprazole substituted on the nitrogen at the 1-position of the benzimidazole ring, which was found to have a pK_a value of 7.5. The omeprazole pK_a of 14.7 is assigned to the dissociation of the hydrogen from the 1-position of the benzimidazole ring and the pK_a of of 7.1 is assigned to the dissociation from the protonated pyridine nitrogen of omeprazole. The results presented are at variance with those of earlier work.

4.2.2. Colorimetry

Sastry *et al.* [37] used a spectrophotometric method for the determination of omeprazole in pharmaceutical formulation. The content of omeprazole capsules were powdered and dissolved in methanol or aqueous sodium hydroxide. Omeprazole was determined by the following:

1. Mixing aqueous sample with 2 ml 10 mM 1,10-phenanthroline solution and 1.5 ml 3 mM ferric chloride, heating on a boiling water bath for 30 min, cooling to room temperature, mixing with 2 ml 20 mM phosphoric acid, dilution to 10 ml with water and absorbance measurement at 515 nm.
2. Mixing aqueous samples with 1 ml 0.088% *N*-bromosuccinimide (NBS) and 1 ml 5% acetic acid, dilution to 10 ml with water, equilibration for 20 min, addition of 1 ml 0.3% 4-(methylamino)-phenol sulfate, equilibration for 2 min, addition of 2 ml 0.2% sulfanilamide, dilution to 25 ml with water, and absorbance measurement at 520 nm within 10–30 min.
3. Mixing methanolic samples with 2 ml methanolic 0.5% *p*-dimetyylamino benzaldehyde and 2 ml sulfuric acid with cooling and agitation, dilution to 10 ml with methanol and absorbance measurement at 420 nm within 20 min. Beer's law was obeyed from 0.5–5, 4–20, and 12.5–125 μg/ml of omeprazole, for the three methods, respectively, and the corresponding molar absorptivities (ε) were 42,800, 6400, and 2380. The relative standard derivations ($n = 6$) were 0.29–0.41% and recoveries were 98.17–100.1%.

Sastry *et al.* [38] described four spectrophotometric methods for the determination of omeprazole in bulk form and in pharmaceutical formulations.

Method A: Sample was oxidized with ferric chloride and reacted with 3-methyl-2-benzothiazolinone hydrochloride and the absorbance was measured at 660 nm.
Method B: Sample was oxidized with chloramine T, reacted with *m*-aminophenol and the absorbance was measured at 420 nm.
Method C: Sample was oxidized with excess NBS and the consumed NBS was determined by observing the decrease in color intensity using Celestine blue with measurement of absorbance at 540 nm.
Method D: Sample was reacted with Folin-Ciocalteu reagent and the absorbance was measured at 770 nm.

Beer's law was obeyed for 1–10, 2–32, 0.4–2.4, and 0.8–10 μg/ml for methods A, B, C, and D, respectively (ε = 21,000, 11,900, 75,800, and 28,500, respectively). The detection limits were 0.074, 0.104, 0.023, and 0.039 μg/ml, respectively. The corresponding RSDs were 0.69%, 0.53%, 0.73%, and 0.48%. The method was applied to pharmaceuticals and recoveries were 98.7–100.1%.

4.2.3. Argentometry

Zhang *et al.* [39] carried out studies on the determination of omeprazle by argentometry. Sample (0.3 gm) was dissolved in 20 ml ethanol and 6 ml ammonia reagent (mainly ammonium hydroxide) and 50 ml 0.05 M silver nitrate was added. The mixture was heated at $\leq$50 °C for 15 min, cooled and the solution was diluted to 100 ml with water and filtered. Portions (50 ml) of the filtrate were mixed with 3 ml nitric acid and the mixture was heated until fuming ceased. On cooling, 25 ml water and 2 ml ammonium iron(III) sulfate indicator were added and the excess silver nitrate was titrated against with 50 mM ammonium thiocyanate as titrant until a reddish brown end point was observed, providing an indirect method for determining omeprazole. The average recovery of omeprazole was 99.9% with an RSD was 0.21%. Results were compared with those obtained by HPLC.

4.2.4. Electrochemical analysis

4.2.4.1. Voltammetry Pinzauti *et al.* [40] designed an adsorptive stripping voltammetric method for the determination of omeprazole. The method was optimized using a multivariate procedure and was used to analyze dosage capsules. A 100 μl of sample solution containing 1.4 μg/ml omeprazole was added to 10 ml 0.01 M potassium chloride of supporting electrolyte in a voltammetric cell and the accumulation was carried out at 0 V onto a hanging mercury drop electrode (Ag/AgCl reference electrode, Pt wire auxillary electrode) for 68 s at a stirring speed of 400 rev/min. The stirrer was then stopped and the voltammogram was recorded after 10 s by applying a differential-pulse potential scan from $-$0.7 to $-$1.5 V at

40 mV/s and 70 mV pulse amplitude. The calibration graph was linear for 2.88–48.9 μg/ml of omeprazole and the detection limit was 2.25 μg/ml. The mean recovery of omeprazole in capsules was 101.9% with an RSD ($n = 5$) of 2%.

Radi [41] used an anodic voltammetric assay method for the analysis of omeprazole and lansoprazole on a carbon paste electrode. The electrochemical oxidations of the drugs have been studied at a carbon paste electrode by cyclic and differential-pulse voltammetry in Britton–Robinson buffer solutions (0.04 M, pH 6–10). The drug produced a single oxidation step. By differential-pulse voltammetry, a linear response was obtained in Britton–Robinson buffer pH 6 in a concentration range from 2×10^{-7} to 5×10^{-5} M for lansoprazole or omeprazole. The detection limits were 1×10^{-8} and 2.5×10^{-8} M for lansoprazole and omeprazole, respectively. The method was applied for the analysis of omeprazole in capsules. The results were comparable to those obtained by spectrophotometry.

Qaisi *et al.* [42] studied the acid decomposition of omeprazole in the absence of thiol using a differential-pulse polarography at the static mercury drop electrode. Reactions were monitored, using differential-pulse polarography at the static mercury drop electrode, in solutions buffered to pH values ranging from 2 to 8. The fast, sensitive, and selective electrochemical technique facilitated to repeat recordings of successive voltammograms [peak current (nA) versus peak potential (volts versus Ag/AgCl saturated with 3 M KCl)]. The differential-pulse polarographic signals of omeprazole and its degradation products, believed to be due sulfur functional group (the principal site for electrode reaction), gave advantages over the previously employed UV detection technique. The latter primarily relied on pyridine and benzimidazole analytical signals, which are common reaction products of proton pump inhibitors in aqueous acidic solutions. After peak identification, the resulting current (nA)–time (s) profiles, demonstrated that omeprazole undergoes degradation to form two main stable compounds, the first is the cyclic sulfenamide (D^+), previously believed to be the active inhibitor of the H^+/K^+-ATPase, the second is omeprazole dimer. This degradation is highly dependent on pH. Unlike previous studies which reported that the lifetime of D^+ is few seconds, the cyclic sulfenamide (D^+) was found to be stable for up to 5–20 min. The results further indicated that omeprazole converts into the cyclic sulfenamide in an irreversible reaction, consequently, D^+ and sulfenic acid (an intermediate which rapidly converts into D^+) were not interconvertable. It is suggested that the sulfenic acid is the active inhibitor *in vivo*. The omeprazole reactions, in the absence of thiol, were not as complicated as were previously reported.

Yan [43] investigated the electrochemical behavior of omeprazole on a glassy carbon electrode by cyclic voltammetry and differential-pulse

voltammetry. Omeprazole was found to give a sensitive oxidation peak at +0.74 V in the acetic acid/sodium acetate buffer solution (pH 5.10) under the differential-pulse voltammetric mode. The peak current was linear with the concentration of omeprazole in the range 1–20 mg/l. Based on which, a differential-pulse voltammetric method for the determination of omeprazole with the detection limit of 0.19 mg/l has been developed. The method has been used for determination of omeprazole concentration in omeprazole enteric-coated tablets, the recovery was found to be in the range of 99.3–102%. The mechanism for this electrochemical reaction at the glassy carbon electrode was also discussed in this study.

4.2.4.2. Polarography McClean *et al.* [44] used fluorimetry, UV spectrophotometry, liquid chromatography, and differential-pulse polarography, to study the degradation of omeprazole in 10 mM hydrochloric acid, and the subsequent reactions of the respective degradation products with 2-mercaptoethanol. Omeprazole and its degradation products could also be determined in pharmaceutical formulations or biological fluids, by differential-pulse polarography in Britton–Robinson buffer solution of pH 9. Calibration graphs were linear up to 100 μM and detection limits were 0.07 μM omeprazole and 0.08 μM SK&F 95601. For 0.4 and 100 μM drug the respective RSD ($n = 6$) were 4.28% and 0.55% for omeprazole and 6.11% and 1.15% for SK&F 95601.

Ames and Kovacic [45] studied the electrochemistry of omeprazole, active metabolites and a bound enzyme model, with possible involvement of electron transfer in the antiulcer action of the drug. The active metabolites cyclic sulfenamide and sulfur radical entities, exhibited reduction potentials of −0.3 and −0.2 V, respectively. The value for the bound enzyme model was −0.7 V and that for omeprazole was >−1.4 V. The results lend credence to the hypothesis that electron transfer comprises part of the mode of action in addition to H^+/K^+-ATPase inhibition.

Ozaltin and Temizer [46] used a differential-pulse polarographic method for the determination of omeprazole in pharmaceutical preparations. Various polarographic techniques were investigated and the best results were obtained using differential-pulse polarography in a borate buffer of pH 9. A sensitive well-defined peak was observed at −1.28 V versus Ag/AgCl; no other peaks were observed in the range −0.2 to −2.0 V. The detection limit was 0.1 μM omeprazole and the calibration graph was linear from 0.2 to 20 μM. The RSD of the calibration plot was 3.92%. The method was applied to two different commercial preparations and was accurate, sensitive, cheap, and easy to apply.

Dogrukol-Ak and Tuncel [47] determined omeprazole in capsules by polarographic techniques. An enteric-coated pellet was mixed with one drop of 1 M sodium hydroxide, made up to 100 ml with deoxygenated water then vigorously shaken. Analysis was carried out with a

polarographic system comprising a Polaropulse PRG-5 instrument; dual function EGMA cell, and HG, Pt-wire and Ag/AgCl as working, auxiliary and reference electrodes, respectively. The supporting electrolyte was 0.2 M potassium chloride–ethanol (9:1) in a buffer solution of 0.2 M sodium acetate or 0.2 M sodium dihydrogen phosphate adjusted with 2 M hydrochloric acid or 2 M sodium hydroxide; 10 ml was placed in to the polarographic cell with the sample then purified. Nitrogen was passed through the solution for 10 min. Polarography was carried out by scanning cathodically from 0 to −2000 mV. Results were comparable to with data obtained from a standard spectrophotometric technique. This method was accurate, practical, rapid, and free from interference; and could be applied to routine analysis.

Knoth *et al.* [48] studied the electrochemical behavior of omeprazole with the aid of the direct-current and differential-pulse polarography. Omeprazole was determined in Britton–Robinson buffers pH 7–9 up to a concentration of 10^{-5} M. The mechanism of the reduction process on the dropping mercury electrode is elucidated. With the consumption of two electrons and two protons, omeprazole will be reduced to 5-methoxy-2-[(3,5-dimethyl-4-methoxypyridin-2-yl)methylthio]-1*H*-benzimidazole which will be cleaved with the uptake of two further electrons and two protons into 4-methoxy-2,3,5-trimethyl pyridine and 2-mercapto-5-methoxybenzimidazole.

Oelschlaeger and Knoth [49] described a differential-pulse polarographic procedure for the determination of omeprazole in individual enteric-coated capsules and dry ampoules. The pellet from a hand-opened capsule was disintegrated by ultrasonication for 5 min in 25 ml methanol. For 20–40 mg capsules, 2 and 1 ml of the suspension, respectively, was withdrawn and diluted with methanol to 50 ml. After the addition of 9 ml of Britton–Robinson buffer of pH 7, 1 ml of the solution was subjected to differential-pulse polarography from −800 to −1200 mV at 16.67 mV/s. The method can also be applied to methanolic solutions of the contents of single omeprazole ampoules. The method was validated by HPLC and used to check the uniformity of Antra 20 and 40 and Gastroloc capsules and Antra pro infusion ampoules according to the German Pharmacopeia (DAB 1996 V.5.2.).

Belal *et al.* [50] used an anodic polarographic method for the determination of omeprazole and lansoprazole in pure form and in pharmaceutical dosage forms. The study was carried out in Britton–Robinson buffer over the pH range 4.1−11.5. In Britton–Robinson buffer of pH 7, well-defined anodic waves were produced with diffusion–current constant (I_d) of 1.7 ± 0.01 ($n = 6$) and 1.66 ± 0.01 ($n = 8$) for lansoprazole and omeprazole, respectively. The current–concentration plots were rectilinear over the ranges of 4−24, 2−16 μg/ml using direct current (DC_t) mode for lansoprazole and omeprazole, respectively, and over the range 2−18,

0.4−12 μg/ml using the differential-pulse polarographic mode for lansoprazole and omeprazole, respectively. The detection limits ($S/N = 2$) using differential-pulse polarographic modes were 0.2 μg/ml (5.41×10^{-7} M) and 0.05 μg/ml (1.45×10^{-7} M) for lansprazole and omeprazole, respectively. The method was applied to the analysis of the two drugs in their commercial capsules. The average percent recoveries were compared with those obtained by reference methods, with satisfactory standard deviations. The method is simple, accurate, and stability-indicating.

El-Enany *et al.* [51] studied the alternating current (AC_t) polarographic behavior of omeprazole in Britton–Robinson buffers over the pH range 4.1–11.5. In Britton–Robinson buffer of pH 9.6 and 10.5, well-defined AC_t peaks were obtained for omeprazole. The current–concentration plot was rectilinear over the range 0.2–10 μg/ml. The minimum detection limit ($S/N = 2$) was 0.01 μg/ml (2.9×10^{-5} M). The method was applied to the analysis of the drug in its commercial capsules. The average percent recovery was favorably compared to those obtained by reference methods. The pathway for the electrode reaction for the drug involved reduction of the sulfonyl group into the corresponding thiol group at the dropping mercury electrode. The advantages of the method were time saving and more sensitive than other voltammetric method. The method was applied to analysis of lansoprazole.

Cao and Zeng [52] used of an oscillopolarographic method for the determination and the electrochemical behavior of omeprazole. Portions of standard omeprazole solution were treated with 1 ml 1 M ammonia/ammonium chloride at pH 8.9 and the solution was diluted with water to 10 ml. The diluted solution was subjected to single sweep oscillopolarography with measurement of the derivative reduction peak at −1.105 V versus saturated calomel electrode. The calibration graph was linear from 0.5 to 10 μM omeprazole with a detection limit of 0.2 μM. The method was applied to the analysis of omeprazole in capsules with recoveries of 100–118.6% and RSD of 6.78%. The electrochemical behavior of omeprazole at the mercury electrode was also investigated.

4.2.5. Chromatography

4.2.5.1. Thin-layer chromatography Mangalan *et al.* [53] used of an HPTLC method for the detection and determination of omeprazole in plasma levels. Plasma was extracted three times with dichloromethane at pH 6.5–7 and the combined extracts were evaporated to dryness at 60 °C. The residue was dissolved in dichloromethane and the solution was analyzed by TLC on aluminium-packed plates precoated with Silica gel 60 F_{254} with the upper organic layer of butanol–ammonium hydroxide–water (14:1:15) as mobile phase. The spots were observed by fluorescence quenching under UV light illumination at 280 nm; the total area of each

spot was determined with use a dual wavelength scanner. The calibration graph was rectilinear for 0.1–1 mg of omeprazole. The recovery was 87% and coefficients of variation were 4.1–8.2%.

Ray and Kumar-De [54] described an HPTLC method and a TLC method for the rapid quantification and identification of omeprazole. Ground powder (omeprazole powders, capsules, or tablets) equivalent of 25 mg omeprazole was warmed for 10 min with shaking with 25 ml methanol. After cooling the solution was made up to 50 ml with methanol, mixed and filtered. The filtrate was spotted (2 μl) on to HPTLC plates (20 $\times$ 20 cm) coated with Kieselgel 60 GF_{254} activated at 110 °C for 30 min. The plates were developed to 16 cm with methanol–water (2:1) as mobile phase. After development, the plates were dried in warm air and the spots were visualized at 302 nm. The calibration graph was linear from 2.5 to 10 μg omeprazole and the RSD was $\leq$0.60%. The recovery was 99.42%. Results compared well with those obtained by elution of the spots with methanol following TLC and spectrophotometric detection at 302 nm.

Dogrukol-Ak *et al.* [55] determined omeprazole in pharmaceutical preparations by a TLC densitometric method. Pellets from eneric coated capsules were finely powdered and dissolved in ethanolic 0.05 M potassium hydroxide with sonication. Four microliters of the solution was subjected to TLC on a silica gel FG_{254} plates with chloroform–methanol–25% ammonia (97.5:2.5:1) as mobile phase and densitometric detection of omeprazole ($R_f = 0.46$) at 302 nm. Calibration graphs were linear for 0.42−1.68 μg omeprazole; the detection limit was 25 ng. In the determination of omeprazole in 20 mg Omeprazit, Omeprol, and Losec capsules, the found amounts were 20.2, 20.3, and 19.8 mg omeprazole, respectively, with corresponding RSD 1.9, 1.8, and 1.6% ($n = 8$). The results agree with those of UV spectrophotometry.

Agbaba *et al.* [56] developed an HPTLC method for the determination of omeprazole, pantoprazole, and their impurities omeprazole sulfone and *N*-methylpantoprazole in pharmaceutical. The mobile phase chloroform–2-propanol:25% ammonia–acetonitrile (10.8:1.2:0.3:4), enables good resolution of large excesses of the drugs from the possible impurities. Regression coefficients ($r > 0.998$), recovery (90.7–120.0%), and detection limit (0.025–0.05%) were validated and found to be satisfactory. The method is convenient for quantitative analysis and purity control of the compounds.

4.2.5.2. High-performance liquid chromatography Persson *et al.* [57] determined omeprazole and three of its metabolites, the sulfone, the sulfide, and the hydroxy metabolite, in plasma and urine by liquid chromatographic methods. The compounds are extracted from the biological sample and the extract is subjected to liquid chromatographic separation, either directly or after evaporation of the organic solvent and dissolution

in a polar phase. The effluent from the column is UV-monitored at 302 nm and the quantitative evaluation performed by electronic integrator.

Amantea and Narang [58] used a reversed-phase HPLC method for the quantitation of omeprazole and its metabolites. Plasma was mixed with the internal standard (the 5-methyl analog of omeprazole), dichloromethane, hexane, and 0.1 M carbonate buffer (pH 9.8). After centrifugation, the organic phase was evaporated to dryness and the residue was dissolved in the mobile phase [methanol–acetonitrile–0.025 M phosphate buffer of pH 7.4 (10:2:13)] and subjected to HPLC at 25 °C on a column (15 cm × 4.6 mm) of Beckman Ultrasphere C8 (5 μm) with a guard column (7 cm × 2.2 mm) of Pell C8 (30–40 μm). The mobile phase flow-rate was 1.1 ml/min with detection at 302 nm. The calibration graphs are linear for $\leq$200 ng/ml, and the limits of detection were 5, 10, and 7.5 ng/ml for omeprazole, its sulfone, and its sulfide, respectively. The corresponding recoveries were 96.42% and 96% and the coefficients of variation ($n = 5$ or 6) were 3.0–13.9%.

Shim *et al.* [59] developed an HPLC method, with column switching, for the determination of omeprazole in plasma. The plasma samples were injected onto a Bondapak Phenyl/Corasil (37–50 μm) precolumn and polar plasma components were washed with 0.06 M borate buffer. After valve switching, the concentrated drug were eluted in the back-flush mode and separated on a μ-Bondapack C_{18} column with acetonitrile–phosphate buffer as the mobile phase. The method showed excellent precision, accuracy, and speed with detection limit of 0.01 μg/ml. Total analysis time per sample was less than 20 min and the coefficients of variation for intra- and interassay were less than 5.63%. The method has been applied to plasma samples from rats after oral administration of omeprazole.

Balmer *et al.* [60] separated the two enantiomers of omeprazole on three different stationary phases with immobilized protein, viz, Chiral-AGP with α-1 acid glycoprotein, Ultron ES-OVM with ovomucoid, and BSA–DSC with BSA cross-linked into 3-aminopropyl silica using *N*-succinimidyl carbonate. The mobile phase (1 ml/min) was phosphate buffer solution with 3−10% 2-propanol as the organic modifier. The enantiomers of omeprazole were separated on Chiralpak AD, an amylose-based chiral stationary phase, with ethanol–hexane (1:4) as mobile phase (1 ml/min).

Kang *et al.* [61] developed an advanced and sensitive HPLC method for the determination of omeprazole in human plasma. After omeprazole was extracted from plasma with diethylether, the organic phase was transferred to another tube and trapped back with 0.1N sodium hydroxide solution. The alkaline aqueous layer was injected into a reversed-phase C_8 column. Lansoprazole was used as the internal standard. The mobile phase consisted of 30% of acetonitrile and 70% of 0.2 M potassium dihydrogen phosphate, pH 7. Recoveries of the analytes and internal

standard were 75.48%. The coefficients of variation of intra- and interday assay were <5.78 and 4.59 for plasma samples. The detection limit in plasma was 2 ng/ml. The method is suitable for the study of the kinetic disposition of omeprazole in the body.

Motevalian *et al.* [62] developed a rapid, simple, and sensitive HPLC assay method for the simultaneous determination of omeprazole and its major metabolites in human plasma using a solid-phase extraction procedure. Eluent (50 μl) was injected on a μBondapak C_{18} reversed-phase column (4.6 mm $\times$ 250 mm, 10 μm). The mobile phase consisted of 0.05 M phosphate buffer (pH 7.5) and acetonitrile (75:25) at a flow-rate of 0.8 ml/min. UV detection was at 302 nm. Mean recovery was greater than 96% and the analytical responses were linear over the omeprazole concentration range of 50–2000 ng/ml. The minimum detection limits were 10, 10, and 15 ng/ml for omeprazole, omeprazole sulfone, and hydroxyomeprazole, respectively. The method was used to determine the plasma concentration of the respective analytes in four healthy volunteers after an oral dose of 40 mg of omeprazole.

Garcia-Encina *et al.* [63] validated of an automated system using online solid-phase extraction and HPLC with UV detection, to determine omeprazole in human plasma. The extraction was carried out using C_{18} cartridges. After washing, omeprazole was eluted from the cartridge with mobile phase onto an Inertsil ODS-2 column. The developed method was selective and linear for drug concentrations ranging between 5 and 500 ng/ml. The recovery of omeprazole ranged from 88.1% to 101.5% and the limit of quantitation was 5 ng/ml. This method was applied to determine omeprazole in human plasma samples from bioequivalence studies.

Castro *et al.* [64] reported a comparison between derivative spectrophotometric and liquid chromatographic methods for the determination of omeprazole in aqueous solutions during stability studies. The first derivative procedure was based on the linear relationship between the omeprazole concentration and the first derivative amplitude at 313 nm. The first derivative spectra were developed between 200 and 400 nm ($\Delta\lambda = 8$). This method was validated and compared with the official HPLC method of the USP. It showed good linearity in the range of concentration studied (10$-$30 μg/ml), precision (repeatability and interday reproducibility), recovery, and specificity in stability studies. It also seemed to be 2.59 times more sensitive than the HPLC method. These results allowed to consider this procedure as useful for rapid analysis of omeprazole in stability studies since there was no interference with its decomposition products.

Persson and Andersson [65] reviewed the unusual effects in liquid chromatographic separations of enantiomers on chiral stationary phases with emphasis on polysaccharide phases. On protein phases and Pirkle phases, reversal of the elution order between enantiomers due to

variation and temperature and mobile phase composition has been reported. Most of the nonanticipated observations have dealt with the widely used polysaccharide phases. Reversed retention order and other stereoselective effects have been observed from variation of temperature, organic modifier, and water content in nonpolar organic mobile phases.

Cass *et al.* [66] used a polysaccharide-based column on multimodal elution for the separation of the enantiomers of omeprazole in human plasma. Amylose tris (3,5-dimethylphenylcarbamate) coated onto APS-Hypersil (5 μm particle size and 120 Å pore size) was used under normal, reversed-phase, and polar-organic conditions for the enantioseparation of six racemates of different classes. The chiral stationary phase was not altered when going from one mobile phase to another. All compounds were enantioresolved within the elution modes with excellent selectivity factor. The separation of the enantiomers of omeprazole in human plasma in the polar-organic mode of elution is described.

Sluggett *et al.* [67] used an HPLC method with coulometric detection for the determination of omeprazole. A sensitive HPLC method for the analysis of omeprazole and three related benzimidazole with coulometric detection was carried out at $+0.8$ V using a porous C electrode. The linear range is $0.01-10$ μg/ml. The method has a high degree of precision; the RSD of omeprazole at a concentration of 1.06 μg/ml was 0.7% ($n = 4$). The cyclic voltammogram of omeprazole is consistent with the hydrodynamic voltammogram exhibiting a single major irreversible oxidative wave with a peak potential at $+1.105$ V. The response factors for the four compounds are similar indicating that the oxidative process does not involve the S moiety exclusively. The data are most consistent with oxidation primarily of the benimidazole groups. The method was applied to the determination of omeprazole in a paste formulation.

Dubuc *et al.* [68] described a rapid HPLC method for the separation and determination of omeprazole extracted from human plasma. Omeprazole and the internal standard (H 168/24) were extracted from plasma samples by solid-phase extraction using a polymeric sorbent-based cartridge. The separation was accomplished under reversed phase conditions using an Eclipse XDB-C8 Rapid Resolution (4.6 $\times$ 50 mm) column. The mobile phase consisted of 23% acetonitrile and 77% of 30.4 mM disodium hydrogen phosphate and 1.76 mM potassium dihydrogen phosphate solution, pH 8.4, in which a gradient elution was used to linearly change solvent composition to 33% acetonitrile and 67% phosphate buffer during the first minute. Absorbance was monitored at 302 nm for omeprazole and at 294 nm for the internal standard and the total analysis time was 4 min.

Gonzalez *et al.* [69] presented a new simple and reliable HPLC method for measuring omeprazole and its two main metabolites in plasma. Omeprazole, hydroxyomeprazole, and omeprazole sulfone were extracted from

plasma samples with phosphate buffer and dichloromethane–ether (95:5). HPLC separation was achieved using an Ultrasphere ODS C_{18} column. The mobile phase was acetonitrile–phosphate buffer (24:76, pH 8) containing nonylamine at 0.015%. Retention times were 9.5 min for omeprazole, 3.25 min for hydroxyomeprazole, 7.4 min for omeprazole sulfone, and 6.27 min for internal standard (phenacetin). Detection (UV at 302 nm) of analytes was linear in the range from 96 to 864 ng/ml.

Cheng *et al.* [70] used a microdialysis technique coupled to a validated microbore HPLC system to monitor the levels of protein-unbound omeprazole in rat blood, brain, and bile, constructing the relationship of the time course of the presence of omeprazole. Microdialysis probes were simultaneously inserted into the jugular vein toward right atrium, the brain striatum, and the bile duct of the male Sprague–Dawley rats for biological fluid sampling after the administration of omeprazole (10 mg/kg) through the femoral vein. The concentration–response relationship from the present method indicated linearity ($r^2 > 0.995$) over a concentration range of 0.01–50 μg/ml for omeprazole. Intra- and interassay precision and accuracy of omeprazole fell well within the predefined limit of acceptability. Following omeprazole administration, the blood-to-brain coefficient of distribution was 0.15, which was calculated as the area under the concentration versus time curve in the brain divided by the area under the curve in blood. The blood-to-bile coefficient of distribution was 0.58.

Cass *et al.* [71] described a direct injection HPLC method, with column-switching, for the determination of omeprazole enantiomers in human plasma. A restricted access media of bovine serum albumin octyl column has been used in the first dimension for separation of the analyte from the biological matrix. The omeprazole enantiomers were eluted from the restricted access media column onto an amylose tris (3,5-dimethylphenylcarbamate) chiral column by the use of a column-switching valve and the enantioseparation was performed using acetonitrile–water (60:40) as eluent. The analytes were detected by their UV absorbance at 302 nm. The validated method was applied to the analysis of the plasma samples obtained from 10 Brazilian volunteers who received a 40-mg oral dose of racemic omeprazole and was able to quantify the enantiomers of omeprazole in the clinical samples analyzed.

Schubert *et al.* [72] developed and validated a liquid chromatographic method for the determination of omeprazole in powder for injection and in pellets. The analyses were performed at room temperature on a reversed-phase C_{18} column of 250 mm $\times$ 4.6 mm (5 μm). The mobile phase, composed of methanol–water (90:10) was pumped at a constant flow-rate of 1.5 ml/min. Detection was performed on a UV detector at 301 nm. The method was validated in terms of linearity, precision, accuracy, and ruggedness.

Orlando and Bonato [73] presented a practical and selective HPLC method for the separation and quantification of omeprazole enantiomers in human plasma. C_{18} solid-phase extraction cartridges were used to extract the enantiomers from plasma samples and the chiral separation was carried out on a Chiralpak AD column protected with a CN guard column, using ethanol–hexane (70:30) as the mobile phase, at a flow-rate of 0.5 ml/min. The detection was carried out at 302 nm. The method is linear in the range of 10–1000 ng/ml for each enantiomer, with a quantification limit of 5 ng/ml. Precision and accuracy, demonstrated by within-day and between-day assays, were lower than 10%.

Rezk *et al.* [74] developed and validated a reversed-phase HPLC assay method for the simultaneous quantitative determination of omeprazole and its three metabolites in human plasma. The method provides excellent chromatographic resolution and peak shape for the four components and the internal standard within a 17-min run time. The simple extraction method results in a clean baseline and relatively high extraction efficiency. The method was validated over the range of 2–2000 ng/ml. The resolution and analysis for the four analytes; omeprazole, hydroxyomeprazole, omeprazole sulfone, and omeprazole sulfide and the internal standard utilized a Zorbax C18 (15 cm $\times$ 3 mm, 5 μm) with a Zorbax C18 (12.5 cm $\times$ 4.6 mm) guard column. The mobile phase consisted of two components. Mobile phase A was 22 mM phosphate monobasic, adjusted to a pH of 6 with diluted sodium hydroxide. This solution was filtered through a 0.45-μm membrane filter, then mixed as 900 ml buffer to 100 ml methanol. Mobile phase B was composed of 100 ml of the phosphate buffer as mobile phase A, mixed with 800 ml of acetonitrile, 100 ml of methanol, and 100 μl of trifluoroacetic acid with an initial flow-rate of 0.55 ml/min and detection at 302 nm.

Shimizu *et al.* [75] described a column-switching HPLC method for the simultaneous determination of omeprazole and its two main metabolites, 5-hydroxyomeprazole and omeprazole sulfone, in human plasma. Omeprazole and its two metabolites and lansoprazole as an internal standard were extracted from 1 ml of alkalinized plasma sample using diethyl ether–dichloromethane (45:55). The extract was injected into a column I (TSK-PW precolumn, 10 μm, 35 mm $\times$ 4.6 mm) for cleanup and column II (Inertsil ODS-80A column, 5 μm, 150 mm $\times$ 4.6 mm) for separation. The mobile phase consisted of phosphate buffer–acetonitrile (92:8, pH 7) for cleanup and phosphate buffer–acetonitrile–methanol (65:30:5, pH 6.5) for separation, respectively. The peak was detected with a UV detector set at a wavelength of 302 nm, and total time for chromatographic separation was approximately 25 min. The validated concentration ranges of this method were 3–2000 ng/ml for omeprazole, 3–50 ng/ml for 5-hydroxyomeprazole, and 3–1000 ng/ml for omeprazole sulfone. Mean recoveries were 84.3% for omeprazole, 64.3% for 5-hydroxyomeprazole, and 86.1%

for omeprazole sulfone. Intra- and interday coefficient variations were less than 5.1 and 6.6 for omeprazole, 4.6% and 5.0% for hydroxyomeprazole and 4.6% and 4.9% for omeprazole sulfone at the different concentrations. The limits of quantification were 3 ng/ml for omeprazole and its metabolites. This method was suitable for use in pharmacokinetic studies in human volunteers.

Zarghi *et al.* [76] developed an HPLC method, using a monolithic column, for quantification of omeprazole in plasma. The method is specific and sensitive with a quantification limit of 10 ng/ml. Sample preparation involves simple, one-step extraction procedure, and analytical recovery was complete. The separation was carried out in reversed-phase conditions using a Chromolith Performance (RP-18e, 100 × 4.6 mm) column with an isocratic mobile phase consisting of 0.01 mol/l disodium hydrogen phosphate buffer–acetonitrile (73:27) adjusted to pH 7.1. The wavelength was set at 302 nm. The calibration curve was linear over the concentration range 20–1500 ng/ml. The coefficients of variation for intra- and interday assay were found to be less than 7%.

Jia *et al.* [77] validated an HPLC method without solvent extraction and using UV detection at 302 nm for the determination of omeprazole in rat plasma. Plasma sample after pretreatment with acetonitrile to effect deproteinization were dried under nitrogen at 40 °C and reconstituted with mobile phase. The apparatus used was an Agilent 1100 quaternary pump, with a variable wavelength detector, thermostated autosampler, and column thermostat. A Hypersil ODS_2 C_{18} column (250 mm × 4.6 mm, 5 μm) was fitted with a Phenomenex guard column packed with octadecyl C_{18}. The mobile phase comprised 50 mM potassium dihydrogen phosphate buffer (pH 7.1, contained 0.7% triethylamine) and acetonitrile (75:25), the detection wavelength was 302 nm. Analyses were run at a flow-rate of 1 ml/min at 25 °C and the samples were quantified using peak areas. The standard calibration curve for omeprazole was linear ($r^2 = 0.999$) over the concentration range of 0.02–3 μg/ml. The intra- and interday assay variability range was 4.8–9.2% and 5.2–10.3% individually. This method has been applied to a pharmacokinetic study of omeprazole in rats.

Pearce and Lushnikova [78] used semipreparative HPLC method for the isolation of the three omeprazole metabolites produced by the fungi. Incubation of *Cunninghamella elegans* ATCC 9245 and omeprazole allowed putative fungal metabolite to be isolated in sufficient quantities for structural elucidation. The metabolites structures were identified by a combination of LC/MS and NMR spectrometric experiments. These isolates are used as reference standards in the confirmatory analysis of mammalian metabolites of omeprazole. In the LC/MS and LC/MS/MS analysis, components were separated by reversed-phase HPLC on a Hypersyl HyPurity (15 cm × 4.6 mm, 5 μm) column. The mobile phase consisted

of 10 mM ammonium hydroxide in water (solvent A) and 1 mM ammonium hydroxide in 90% methanol (solvent B). A linear, one-step solvent gradient was applied, changing an initial composition of 85% solvent A to a final composition of 10% solvent A in 14 min. The injection volume was 10 μl, the solvent flow-rate was 0.25 ml/min and the total run time for each sample was 15 min. Metabolite isolation was carried out by reversed-phase HPLC on a semipreparative scale. A Hypersil HyPurity Elite C_{18} column (15 cm $\times$ 10 mm, 5 μm) column was used. The mobile phase consisted of 10 mM ammonium hydroxide in water (solvent A) and 1 mM ammonium hydroxide in 90% methanol (solvent B). A linear, three-step solvent gradient was applied, maintaining an initial composition of 75% solvent A for 1 min, changing to 40% solvent A over the next 15 min, then to 30% solvent A in the next 2 min and finally to 10% solvent A in the next 2 min. Injection volume was 2 ml, the solvent flow-rate was 1 ml/min., the UV detection wavelength was at 302 nm and the total run time for each sample loading was 21 min.

El-Sherif *et al.* [79] developed and validated a reversed-phase HPLC method for the quantitative determination of omeprazole and two other proton pump inhibitors in the presence of their acid-induced degradation products. The drugs were monitored at 280 nm using Nova-Pak C_{18} column and mobile phase consisting of 0.05 M potassium dihydrogen phosphate–methanol–acetonitrile (5:3:2). Linearity range for omeprazole was 2–36 μg/ml. The recovery of omeprazole was 100.50 $\pm$ 0.8%, and the minimum detection was 0.54 μg/ml. The method was applied to the determination of pure, laboratory prepared mixtures, and pharmaceutical dosage forms. The results were compared with the official USP method for omeprazole.

Linden *et al.* [80] developed a simple HPLC-diode array detector method using a reverse phase column and isocratic elution for the simultaneous determination of omeprazole, 5-hydroxyomeprazole, and omeprazole sulfone. The method was used to study CYP_2C_{19} and CYP_3A_4 genetic polymorphisms using omeprazole as the probe drug in a group of Brazilian volunteers. Omeprazole, 5-hydroxyomeprazole, and omeprazole sulfone were extracted from plasma samples with Tris buffer pH 9.5 (0.2 mol/l) and ethyl acetate. HPLC separation was achieved using a Shim-Pack RP-18e (15 cm $\times$ 4.6 mm, 5 μm) column with acetonitrile–phosphate buffer, pH 7.6 (24:76) as mobile phase and total run time for 15 min. Retention times were 2.7 min for internal standard, sulpiride, 4.1 min for 5-hydroxyomeprazole, 11.6 min for omeprazole, and 12.6 min for omeprazole sulfone. Detection (UV at 302 nm) of analytes was linear in the range from 25 to 1000 ng/ml. Extraction recoveries were in the range of 64.3–73.2% for all analytes. A group of 38 Brazilian healthy volunteers was phenotyped with this method, after a single oral dose of 20 mg of omeprazole. The method presented adequate accuracy and precision,

with a limit of quantification of 25 ng/ml for omeprazole and metabolites, which allowed the identification of ultra-rapid metabolizers for both CYP_2C_{19} and CYP_3A_4 and took advantage of the selective identification offered by diode-array detectors.

Sivasubramanian and Anilkumar [81] described a simple reversed-phase HPLC method for the determination of omeprazole and domperidone from tablet formulations. The analysis was carried out on a Hypersil ODS C_{18} (15 cm $\times$ 4.6 mm, 5 μm) column using a mobile phase of methanol– 0.1 M ammonium acetate, pH 4.9 (60:40). The flow-rate and run time were 1 ml/min and 10 min, respectively. The eluent was monitored at 280 nm. The method was reproducible, with good resolution between omeprazole and domperidone. The detector response was linear in the concentration range of 10–60 μg/ml for omeprazole.

Murakami *et al.* [82] developed and validated a sensitive HPLC technique to quantify omeprazole in delayed release tablets. The analysis was carried out using a RP-C_{18} column with UV–VIS detection at 280 nm. The mobile phase was diluted with phosphate buffer (pH 7.4) and acetonitrile (70:30) at a flow-rate of 1.5 ml/min. The parameters used in the validation process were linearity, range, quantification limit, accuracy, specificity, and precision. The retention time of omeprazole was about 5 min with symmetrical peaks. The linearity in the range of 10–30 μg/ml presented a correlation coefficient of 0.9995. The excipients in the formulation did not interfere with the analysis and the recovery was quantitative. Results were satisfactory and the method proved to be adequate for quality control of omeprazole delayed-release tablets.

Silva *et al.* [83] separated omeprazole and other chiral drugs on a tartardiamide-based stationary phase commercially named Kromasil CHI-TBB. The effect of temperature on the chromatographic separation of the chiral drugs using the Kromasil CHI-TBB stationary phase was determined quantitatively so as to contribute toward the design for the racemic mixtures of the named compound using chiral column. A decrease in the retention and selectivity factors was observed, when the column temperature increased. Van't Hoff plots provided the thermodynamic data. The variation of the thermodynamic parameters enthalpy and entropy are clearly negative meaning that the separation is enthalpy controlled. The chiral column (25 cm $\times$ 1 cm) used was Kromasil CHI-TBB. The column was packed with 16 μm particle diameter and 100 Å of internal pore diameter of Kromasil silica which is covalent bonded with *O*,*O*′-di(4-*tert*-butyl-benzoyl)-*N*,*N*′-diallyl-L-tartardiamide. A mobile phase of *n*-hexane–isopropanol–triethylamine–acetic acid (98:2:0.15:0.05) was used. Omeprazole (0.15 g/l) solutions were prepared using this mobile phase. The solutions and the mobile phases were filtered in a Millipore filter system (0.45 μm) and degasified in a Cole Parmer 8892 ultrasonic bath. The experiments were carried out using a single

chromatographic column in an HPLC system equipped with a Waters 1525 dual pump, a Waters 2487 dual absorbance UV–VIS detector, temperature controller, manual injector, and digital data acquisition system. The chromatograms of omeprazole and the other chiral drugs were obtained by small pulse experiments (20 μl) after a time interval necessary to the stabilization of the system at four different flow-rates (1–4 ml/min). The chromatographic experiments were performed at 25, 35, and 45 °C. Detection was carried out at 302 nm for omeprazole.

Belaz *et al.* [84] separated the enantiomers omepraozle and other proton pump inhibitors by HPLC at multimilligram scale on a polysaccharide-based chiral stationary phase using normal and polar organic conditions as mobile phase. The values of the recovery and production rate were significant for each enantiomer; better results were achieved using a solid-phase injection system. The chiroptical characterization of the compounds was performed using a polarimeter and a circular dichroism detector. The preparative HPLC system consisted of a Shimadzu liquid chromatographic-6AD pump, a Rheodyne 7725 injector fitted with a 200-μl loop or a cylindrical stainless-steel precolumn coated with Teflon for the injections for samples, and a 10-AVvp variable wavelength UV–VIS detector with a CMB SCL-10 AVvp interface. The columns were prepared at the UFSCar Laboratory [85–87]. The tris-3,5-dimethylphenylcarbamate and tris(*S*)-1-phenylethylcarbamate of amylose were coated on to APS-Neocleosil (500 Å, 7 μm, 20%, w/w) and packed into a stainless-steel 20 cm $\times$ 0.7 cm column for semipreparative chromatography (CSP 1 and 2) and into a 15 cm $\times$ 0.46-cm column for analytical separation (CPS 3 and 4, respectively). Amylose tris-(*S*)-1-phenylcarbamate coated on to APS-Hypersil (120 Å, 5 μm, 25%, w/w) packed into a stainless-steel 20 cm $\times$ 0.4 cm semipreparative column (CPS 5) was used. A Shandon HPLC packing pump was employed for column packing. The mobile phase for omeprazole was methanol at a flow-rate of 3 ml/min and detection at 302 nm on column 1 and the mobile phase was methanol at a flow-rate of 1 ml/min on column 3 were used for the semipreparative and analytical chromatographic separation of omeprazole, respectively.

Sultana *et al.* [88] developed a reversed-phase HPLC method for the simultaneous determination of omeprazole in Risek® capsules. Omeprazole and the internal standard, diazepam, were separated by Shim-pack CLC-ODS (0.4 $\times$ 25 cm, 5 μm) column. The mobile phase was methanol–water (80:20), pumped isocratically at ambient temperature. Analysis was run at a flow-rate of 1 ml/min at a detection wavelength of 302 nm. The method was specific and sensitive with a detection limit of 3.5 ng/ml at a signal-to-noise ratio of 4:1. The limit of quantification was set at 6.25 ng/ml. The calibration curve was linear over a concentration range of 6.25−1280 ng/ml. Precision and accuracy, demonstrated by within-day, between-day assay, and interoperator assays were lower than 10%.

The *in vitro* availability of omeprazole in presence of manganese, cobalt, nickel, copper, and zinc was studied by this method. Recovery of omeprazole in presence of various metals was from 41% and 74%.

Rambla-Alegre *et al.* [89] reported a chromatographic procedure that uses micellar mobile phases of sodium dodecyl sulfate and propanol buffered at pH 7 and a C_{18} column for the determination of omeprazole and its metabolites, omeprazole sulfone, and hydroxyomeprazole, in urine and serum samples. Direct injection and UV detection set at 305 nm was used. Omeprazole and its metabolites were eluted in less than 11 min with no interference by the protein band or endogenous compounds. Adequate resolution was obtained with a chemometric approach, in which the retention factor and shape of the chromatographic peaks were taken into account. The chromatographic system was equipped with a quaternary pump, thermostated autosampler tray, and column compartments, and a diode-array detector (190−700 nm). Separation was performed in a reversed phase Kromasil C_{18} column thermostated at 25 °C. The mobile Phase composition was 0.08 M sodium dodecyl sulfate, 10% propanol, 0.01 M sodium dihydrogen phosphate at pH 7. The flow-rate, injection volume, and UV wavelength were 1 ml/min, 20 μl, and 305 nm, respectively. Under these conditions, the total analysis time for omeprazole and its main metabolites was less than 11 min. The analytical parameters including linearity ($r = >0.9998$) intra- and interday precision (RSD) 06−7.9% and 0.14−4.7%, respectively) and robustness were studied in the validation of the method for the three compounds. The limit of detection and quantification were less than 6 and 25 ng/ml, respectively. Recoveries in micellar medium, plasma, and urine matrices were in the 98−102 range. The method was applied to the determination of omeprazole and its metabolites in physiological samples. Omeprazole was also analyzed in pharmaceutical formulations.

Other HPLC methods [90–115] are listed in Table 4.12.

4.2.5.3. Liquid chromatography–mass spectrometry Woolf and Matuszewski [116] described a liquid chromatography–tandem mass spectrometric method for the simultaneous determination of omeprazole and 5-hydroxyomeprazole in human plasma. Omeprazole and its 5-hydroxy-metabolite plus 2-[(4-methoxy-3-methyl-2-pyridinylmethyl)sulfinyl]-1*H*-benzimidazole as internal standard were separated from plasma by solid-phase extraction on to a Waters Oasis cartridge (60 mg bed) and elution with methanol, followed after solvent evaporation by transfer into mobile phase. For HPLC, a column (5 cm × 4.6 mm) of Zorbax XDB C_{18} silica (3 μm) was used with 35 μl sample injection, a mobile phase (1 ml/min) of 10 mM ammonium hydroxide in aqueous 21% acetonitrile adjusted to pH 8.5 with formic acid. Transfer to triple quadrupole PE Sciex API III$^+$ tandem mass spectrometer was via nebulizer at 500 °C for positive

TABLE 4.12 HPLC conditions of the methods used for the determination of omeprazole

Column	Mobile phase and [flow-rate]	Detection (nm)	Remarks	References
Waters Rad-Pak A C_{18}	1% triethylamine solution in aqueous 60% methanol adjusted to pH 7 with phosphoric acid	302	Analysis of omeprazole and its sulfone and sulfide metabolites in human plasma and urine	[90]
LiChrosorb Si 60 or Polygosil C_{18}	Methanol–aqueous ammonia–dichloromethane for omeprazole and its sulfone in plasma or acetonitrile–phosphate buffer for the hydroxy metabolite in plasma and all compounds in urine	302	Analysis of omeprazole and metabolites in plasma and urine. Drug and metabolites are extracted in CH_2Cl_2	[91]
3 cm × 4.6 mm of Spheri-5 RP-8 guard column and a column 15 cm × 4 mm of LiChrosorb RP-8 (5 or 7 μm)	Oxygenated and *N*-deoxygenated phosphate buffer solution at pH 7.6 containing 32.5% acetonitrile [1 ml/min]	d.c. mercury-drop detection or silver/silver chloride electrode	Oxygen effects in amperometric liquid chromatography detection of the drug at a mercury electrode	[92]

(*continued*)

TABLE 4.12 (*continued*)

Column	Mobile phase and [flow-rate]	Detection (nm)	Remarks	References
15 cm × 4.5 mm of Polygosil C_{18} (5 μm) and a guard column 3 cm × 4.6 mm of Spheri-5 RP-18	Acetonitrile–phosphate buffer solution (pH 7.7) from 25% of acetonitrile (maintained for 3 min) to 40% (in 1 min; maintained for 6 min) and decreased to 25% (in 5 min). [1.5 ml/min]	302	Fully automated gradient-elution LC assay of the drug and two of its metabolites	[93]
20 cm × 4.6 mm of triphenylcarbomethylcellulose immobilized on 3-aminoproyl silica	Phosphate buffer (pH 6.6)–propanol [1 ml/min]	229 and 280	Resolution of enantiomers of omeprazole and its analogs by LC on triphenylcarbamoyl cellulose-based stationary phase	[94]
15 cm × 4.6 mm of Nucleosil C_{18} (5 μm) or 10 cm × 5 mm of Novapack C_{18} (4 μm)	Acetonitrile–phosphate buffer solution (3:7 or 2:3) [1 ml/min]	280	Peak distortion in the column LC analysis of the drug dissolved in borax buffer	[95]

25 cm × 5 mm of Shimadzu Nucleosil C_8 operated at 35 °C	Acetonitrile–phosphate buffer pH 7.6 (17:33). [1 ml/min]	280	Studies on the quantitative determination of omeprazole	[96]
25 cm × 4.6 mm of Capcell Pak C_{18} SG 120 (5 μm)	Acetonitrile–0.05 M phosphate buffer pH 8.5. [0.8 ml/min]	302	Simultaneous analysis of the drug and its metabolites in plasma and urine by RPHPLC with an alkaline-resistant polymer-coated C_{18} column	[97]
NH_2, diol, CN, C_{18}, Si-60 columns thermostated at 40 °C	Carbon dioxide (3.5 grade) [1.2 ml/min] plus 60 μl/min of 1% (v/v) methanolic triethylamine mobile phase modifier	300	Packed-column supercritical fluid chromatography of the drug and related compounds	[98]
12.5 cm × 4 mm of Supersphere SI-60 (4 μm) with a guard column (1.5 cm × 3 mm) of Brownlee Aquapore Silica (7 μm)	Dichloromethane–5% ammonium hydroxide in methanol–isopropanol (191:8:1) [1.5 ml/min]	302	HPLC assay for human liver microsomal omeprazole metabolism	[99]

(*continued*)

TABLE 4.12 (*continued*)

Column	Mobile phase and [flow-rate]	Detection (nm)	Remarks	References
25 cm × 6.4 mm of YWG C_{18} (10 μm) operated at 25 °C	Methanol–water–triethylamine–phosphoric acid (3850:1650:25:4) [1 ml/min]	302	Analysis of omeprazole and its analogs by HPLC	[100]
25 cm × 4.6 mm of Capcell Pak C_{18} SG120 operated at 30 °C	Acetonitrile–0.05 M sodium phosphate buffer of pH 8.4 (13:37) [0.8 ml/min]	302	Development and preliminary application of an HPLC assay for omeprazole metabolism in human liver microsomes	[101]
15 cm × 4 mm of Resolvosil BSA-7 (5 μm)	50 mM phosphate buffer pH 7 containing 0.05–1% of propanol [1.5 ml/min]	302	Enantioselective HPLC analysis of omeprazole in human plasma	[102]
15 cm × 4.6 mm of Chiracel OJ-R (5 μm) with 1 cm × 6 mm precolumn (25−40 μm) LiChroprep PR-2 and 4 mm× 4 mm of LiChrospher 100 RP-18 (5 μm) guard column	25% aqueous acetonitrile or 50 mM sodium chlorate–acetonitrile (3:1) [0.5 ml/min] Water [0.5 ml/min]	286	Direct HPLC separation of enantiomers of omeprazole and other benzimidazole sulfoxides using CBCS phases in reversed-phase mode	[103]

15 cm × 4.6 mm of Shim Pack CLC-C_{18}) (5 μm)	Methanol–0.05 M phosphate buffer of pH 5.5 (1:1) [1 ml/min]	302	Studies on chromatographic optimization and its application in pharmacokinetics research	[104]
15 cm × 6 mm of 5 M-Shim-pack CLC-ODS operated at room temperature	Aqueous 63% methanol containing 1% triethylamine, pH adjusted to 7 with 85% phosphoric acid [1 ml/min]	302	Analysis of the drug as capsules in human plasma by RPHPLC	[105]
25 cm × 4.6 mm of Nucleosil 120-5 C_{18} at 37 °C with a precolumn 10 cm × 4.6 mm of similar material	0.1 M dipotassium hydrogen phosphate of pH 7.8−methanol (53:47) containing 40 mg/l of azide [1.2 ml/min]	302	Analysis of the drug in human plasma by HPLC	[106]
25 cm × 4.6 mm of Zorbax C-8 (5 μm)	Acetonitrile–8 mM disodium hydrogen orthophosphate buffer pH 7.5 (7:13) [1 ml/min]	302	RPHPL chromatographic assay of omeprazole in plasma	[107]

(*continued*)

TABLE 4.12 (*continued*)

Column	Mobile phase and [flow-rate]	Detection (nm)	Remarks	References
Omnipac Pax-500 fitted with a C_{18} reversed-phase guard column	0.1 M sodium phosphate–methanol–acetonitrile (3:1:1) adjusted to pH 2.3 with 85% phosphoric acid [0.7 ml/min]	254	Simple HPLC analysis of omeprazole in human plasma and gastric fluid	[108]
C_{18} column	50 mM phosphate buffer in acetonitrile (22−50% in 43 min followed by 15 min equilibration	302	Analysis of omeprazole and its metabolite in human plasma by HPLC	[109]
20 cm × 4.6 mm of Hypersil ODS 2 (5 μm)	Methanol–water–glacial acetic acid–triethylamine (120:80:1:1) [1 ml/min]	302	Analysis of omeprazole and its pharmacokinetics in human plasma by an improved HPLC	[110]
15 cm × 4.6 mm of Zorbax Eclipse XDB-C_8 (5 μm)	Phosphate buffer adjusted to pH 7 with phosphoric acid–acetonitrile (7:3) [2 ml/min]	280	Effect of various salts on stability of omeprazole as determined by HPLC	[111]

15 cm × 4 mm of ET Resolvosil-BSA-7	0.5 M phosphate buffer of pH 7.9/2% propanol [1 ml/min]	250	Direct optical resolution of racemic sulfoxide by HPLAC	[112]
15 cm × 4.6 mm of Crestpak C_{18} (5 μm) preceded by a refillable guard column packed with Perisorb RP-18 (30–40 μm)	0.05 M disodium hydrogen phosphate buffer–acetonitrile (65:35) adjusted to pH 6.5 [1 ml/min]	302	Improved HPLC analysis of omeprazole in human plasma	[113]
10 cm × 4 mm of Chiral-AGP	10 mM sodium phosphate buffer of pH 6.5 containing 10% acetonitrile	210	Omeprazole chiral separation chiral chromatography	[114]
3.5 cm × 4.6 mm of MF Ph-1 precolumn and 25 cm × 1.5 mm of Capcell Pak C_{18} UG 120 (5 μm) and 3.5 cm × 2 mm of Capcell Pak C_{18} UG 120 (5 μm)	Buffer–acetonitrile (90:10) for precolumn and buffer–acetonitrile (60:40) for the analytical column	302	Assay of omeprazole and its sulfone by semimicrocolumn LC with mixed-function precolumn in human plasma samples	[115]

chemical ionization mass spectrometery with Argon collision-induced dissociation and monitoring at m/z 214 for 5-hydroxy omeprazole, 198 for omeprazole and 147 for the internal standard. The method was validated for 10–500 ng/ml of the drug and its metabolite in plasma with ($n = 5$) an accuracy of 98.2−102.1% for the drug and 95.9−103.1% for the metabolite and precision RSD ranging from 1.8% to 4.7% for the drug and 2.8% to 6.7% for the metabolite across the calibration range.

Stenhoff *et al.* [117] determined enantiomers of omeprazole in blood plasma by normal-phase liquid chromatography and detection by atmospheric-pressure ionization tandem mass spectrometry. The enantioselective assay of omeprazole is using normal-phase liquid chromatography on a Chiralpak AD column and detection by mass spectrometry. Omeprazole is extracted by a mixture of dichloromethane and hexane and, after evaporation, redissolution and injection, separated into its enantiomers on the chiral stationary phase. Detection is made by a triple quadrupole mass spectrometer, using deuterated analogs and internal standards. The method enables determination in plasma down to 10 nmol/l and shows excellent consistency suited for pharmacokinetic studies in man.

Kanazawa *et al.* [118] performed a chiral separation of omeprazole on a chiral column with circular dichroism detection and LC/MS. A good resolution of enantiomers was obtained. The column used for the chiral separation was Chiralpak AD-RH column (4.6 mm × 150 mm) using phosphate buffer and (or ammonium acetate) acetonitrile as an eluent. After a single oral dose of omeprazole (20 mg), the plasma concentrations of the separate enantiomers of omeprazole were determined for 3.5 h after drug intake. This study is useful because of the part polymorphism plays in the therapeutic effectiveness of omeprazole and other proton pump inhibitors during the treatment of acid-related diseases. This study demonstrates the stereospecific analysis of omeprazole in human plasma as a probe drug of CYP_2C_{19} phenotyping.

Martens-Lobenhoffer *et al.* [119] used chiral HPLC-atmospheric pressure photoionization tandem mass-spectrometric method for the enantioselective quantification of omeprazole and its main metabolites in human serum. The method features solid-phase separation, normal phase chiral HPLC separation, and atmospheric pressure photoionization tandem mass spectrometry. The internal standards serve stable isotope labeled omeprazole and 5-hydroxy omeprazole. The HPLC part consists of Agilent 1100 system comprising a binary pump, an autosampler, a thermostated column component, and a diode array UV–VIS detector. The enantioselective chromatographic separation took place on a ReproSil Chiral-CA 5 μm 25 cm × 2 mm column, protected by a security guard system, equipped with a 4 mm × 2-mm silica filter insert. The analytes were detected by a Thermo Scientific TSQ Discovery Max triple quadrupole mass spectrometer, equipped with an APPI ion source with a

krypton UV-lamp. System control and data handling were carried out by the Thermo Scientific Xcalibur software. Solid-phase extraction of the samples was performed on OASIS HLB 1 ml extraction column containing 30 mg sorbet. After injection of 10 μl of the prepared samples, enantioselective chromatographic separation was achieved by HPLC normal phase gradient elution. The mobile phase A consisted of 2-propanol–acetic acid–diethylamine (100:4:1) and mobile phase B was pure hexane. At a flow-rate of 0.35 ml/min, the gradient started with a composition of 10:90 A:B, fraction of A was increased to 15% in the next 10 min and was held constantly for 1 min. Subsequently, a washing step with 25% A for 1 min was performed. After the washing step, the mobile phase composition was turned back to starting conditions. The column temperature was held constant at 20 °C. A divert valve directed the HPLC effluent without splitting to the mass spectrometer in the run-time window of 5–15.9 min, otherwise to the waste container. In the mass spectrometer detector, ions were formed by photoionization using krypton light source radiating two emission lines with energies of 10 and 10.6 eV. Vaporizer and capillary temperatures were set to 300 and 220 °C, respectively. Nitrogen served as sheath and AUX gas, with flow settings of 41 and 8 arbitrary units, respectively. Under these conditions, the analytes were ionized exclusively to $[M + H]^+$ parent ions. Prior to detection, collision induced fragmentation of the parent ions was achieved with argon serving as collision gas at a pressure of 1 mTorr. The calibration functions are linear in the range 5–750 ng/ml for the omeprazole enantiomers, and omeprazole sulfone, and 2.5–375 ng/ml for 5-hydroxyomeprazole enantiomers, respectively. Intra- and interday RSDs are 7% for omeprazole and 5-hydroxy omeprazole enantiomers and 9% for omeprazole sulfone.

Macek *et al.* [120] developed a method to quantitate omeprazole in human plasma using liquid chromatography–tandem mass spectrometry. The method is based on the protein precipitation with acetonitrile and a reversed-phase liquid chromatography performed on an octadecylsilica column (55 $\times$ 2 mm, 3 μm). The mobile phase consisted of methanol–10 mM ammonium acetate (60:40). Omeprazole and the internal standard, flunitrazepam, elute at 0.80 $\pm$ 0.1 min with a total run time 1.35 min. Quantification was through positive-ion made and selected reaction monitoring mode at m/z 346.1 $\rightarrow$ 197.9 for omeprazole and m/z 314 $\rightarrow$ 268 for flunitrazepam, respectively. The lower limit of quantification was 1.2 ng/ml using 0.25 ml of plasma and linearity was observed from 1.2 to 1200 ng/ml. The method was applied to the analysis of samples from a pharmacokinetic study.

4.2.5.4. High-performance liquid chromatography–mass spectrometry

Weidolf and Covey [121] described the application of the ionspray interface for liquid chromatography and atmospheric-pressure ionization mass spectrometry to samples obtained in a study on the metabolism of

omeprazole. In this study, [^{34}S]omeprazole was utilized for the stable isotope cluster technique. Over 40 metabolites in a sample of partially purified rat urine were resolved by gradient elution liquid chromatography with ionspray atmospheric pressure ionization mass spectrometric detection and each of them produced molecular ion 1:1 clusters (MH^+ and $[MH + 2]^+$). The chromatographic fidelity of the total-ion current was excellent. The endogenous matrix of the sample was quite low, allowing a background-substracted averaged mass spectrum of the entire total-ion current trace to produce a metabolite mass profile depicting all the molecular ion 1:1 clusters in the sample. From this mass profile, it was possible to obtain direct information concerning oxygenation and conjugation reactions of the parent compound.

Weidolf and Castagnoli [122] reported a detailed analysis of the product ion spectrum generated from the protonated molecule under electrospray ionization (ESI)-MS/MS conditions using a triple quadrupole mass spectrometer for omeprazole. Unambiguous molecular composition data of the fragment ions were obtained with the aid of regioselectively ^{14}C-, ^{34}S-, and ^{18}O-labeled analogs. Attempts have been made to provide rationale pathways for the formation of the fragment ions from four protonated omeprazole species. These results will facilitate the characterization of the complex metabolic fate of omeprazole in humans, which involve the excretion of at least 50 metabolites.

Naidong *et al.* [123] demonstrated a novel approach in 96-well solid-phase extraction by using normal phase LC/MS/MS methods with low aqueous/high organic mobile phases, which consisted of 70–95% organic solvent, 5–30% water, and small amount of volatile acid or buffer. While the commonly used solid-phase extraction elution solvents (acetonitrile and methanol) have stronger elution strength than a mobile phase on reversed-phase chromatography. Analytical methods for omeprazole and other polar compounds in biological fluids were developed and optimized.

Kanazawa *et al.* [124] determined omeprazole and its metabolites in human plasma by liquid chromatography–three-dimensional quadrupole mass spectrometry with a sonic spray ionization interface. The analytical column was YMC-Pack Pro C_{18} (5 cm $\times$ 2 mm) using acetonitrile–50 mM ammonium acetate (pH 7.25) (1:4) at a flow-rate of 0.2 ml/min. The drift voltage was 30 V. The sampling aperture was heated at 110 °C and shield temperature was 230 °C. In the mass spectrum, the molecular ions of omeprazole, hydroxyomeprazole, and omeprazole sulfone were clearly observed as base peaks. The method is sufficiently sensitive and accurate for pharmacokinetic studies of omeprazole.

Jensen *et al.* [125] investigated an HPLC/ICP-MS (inductively coupled plasma mass spectrometry) with sulfur-specific detection, as a method for obtaining metabolite profiles for omeprazole administered as a 1:1

mixture of ^{32}S- and ^{34}S-labeled material. Analysis based on the monitoring of the chromatographic eluent at either m/z 32 or 34 was not successful due to insufficient sensitivity caused by interferences from polyatomic ions. Reaction of sulfur with oxygen in the hexapole collision cell, combined with monitoring at m/z 48 (for ^{32}S) or m/z 50 (for ^{34}S), provided a facile method for metabolite profiling. Detection of m/z 48 was superior in sensitivity to detection of m/z 50.

Tolonen *et al.* [126] described a simple and efficient method for the determination of labile protons in drug metabolites using postcolumn infusion of deuterium oxide in LC/MS experiments with ESI and time-of-flight (TOF)-MS. The number of exchangeable protons in the analytes; the hydroxyl, amine, thiol, and carboxylic acid protons can easily be determined by comparing the increase in m/z values after H/D-exchange occurring online between an HPLC column and electrospray ion source. The hydroxyl metabolites and sulfur/nitrogen oxides with the same accurate mass can be distinguished. A good degree of exchange was obtained in repeatable experiments. Only a low consumption of deuterium oxide is needed in a very easy and rapidly set-up procedure. This method is applied to the study of metabolites of omeprazole in human and mouse *in vitro* samples, together with exact mass data obtained from TOF-MS experiments.

Wang *et al.* [127] developed an analytical method for the determination of omeprazole in human plasma, based on LC–MS. The analyte and the internal standard sildenafil are extracted from plasma by liquid–liquid extraction using diethyl ether–dichloromethane (60:40) and separated by reversed-phase HPLC using acetontile–methanol–10 mM ammonium acetate (37.5:37.5:25) as mobile phase. Detection is carried out by multiple reaction monitoring on a Q TRAP LC/MS/MS system. The method has a chromatographic run time of 3.5 min and is linear within the range 0.50–800 ng/ml. Intra- and interday precision expressed as RSD ranged from 0.4% to 8.5% and from 1.2% to 6.8%, respectively. Assay expressed as relative error was $<5.7\%$. The method has been applied in a bioequivalence study of two capsule formulations of omeprazole.

Frerichs *et al.* [128] developed and validated a method for the quantitation of omeprazole and hydroxyomeprazole from one 250 μl sample of human plasma using HPLC coupled to tandem mass spectrometry. The method was validated for a daily working range of 0.4–100 ng/ml, with limits of detection between 2 and 15 pg/ml. The interassay variation was less than 15% for all analytes at four control concentrations and the samples were stable for three freeze–thaw cycles under the analysis conditions and 24 h in the postpreparative analysis matrix. The method was used to analyze samples in support of clinical studies probing the activity of the cytochrome P-450 enzyme system.

Song and Naidong [129] analyzed omeprazole and 5-hydroxyomeprazole in human plasma using hydrophilic interaction chromatography with tandem mass spectrometry. Omeprazole and its metabolite 5-hydroxy omeprazole and the internal standard desoxyomeprazole were extracted from 0.05 ml of human plasma using 0.5 ml of ethyl acetate in a 96-well plate. A portion (0.1 ml) of the ethyl acetate extract was diluted with 0.4 ml of acetonitrile and 10 μl was injected onto a Betasil silica column (5 cm $\times$ 3 mm, 5 μm) and detected by atmospheric pressure ionization 3000 and 4000 with positive electrospray ionization. Mobile phase with linear gradient elution consists of acetonitrile, water, and formic acid (from 95:5:0.1 to 73.5:26.5:0.1 in 2 min). The flow-rate was 1.5 ml/min with total run time of 2.75 min. The method was validated for a low limit of quantitation at 2.5 ng/ml for both analytes. The method was also validated for specificity, reproducibility, stability, and recovery.

Hultman *et al.* [130] developed a LC/MS/MS method for the quantitative determination of esomeprazole and its two main metabolites 5-hydroxyesomeprazole and omeprazole sulfone in 25 μl human, rat, or dog plasma. The analytes and their internal standards were extracted from plasma into methyl *tert*-butyl ether–dichloromethane (3:2). After evaporation and reconstitution of the organic extract, the analytes were separated on a reversed-phase liquid chromatography column and measured by atmospheric-pressure positive ionization mass spectrometry.

Hofmann *et al.* [131] described a sensitive method for the simultaneous determination of omeprazole and its major metabolites 5-hydroxyomeprazole and omeprazole sulfone in human plasma by HPLC-electrospray mass spectrometry. Following liquid–liquid extraction HPLC separation was achieved on a Prontosil AQ, C_{18} column using a gradient with 10 mM ammonium acetate in water (pH 7.25) and acetonitrile. The mass spectrometer was operated in the selected ion monitoring mode using the respective $MH^{(+)}$ ions, m/z 346 for omeprazole, m/z 362 for 5-hydroxyomeprazole, and omeprazole sulfone and m/z 300 for the internal standard (2-[[(3,5-dimethylpyridin-2-yl)methyl]thio]-1*H*-benzimidazole-5-yl) methanol. The limit of quantification was 5 ng/ml for 5-hydroxyomeprazole and 10 ng/ml for omeprazole and omeprazole sulfone using 0.25 ml of plasma. Intra- and interassay variability was below 11% over the whole concentration range from 5 to 250 ng/ml for 5-hydroxyomeprazole and from 10 to 750 ng/ml for omeprazole and omeprazole sulfone. The method was used for the determination of pharmacokinetic parameters of esomeprazole and the two major metabolites after a single dose and under steady state conditions.

4.2.5.5. Supercritical fluid chromatography Toribio *et al.* [132] used supercritical fluid chromatography for the enantiomeric separation of omeprazole. The drug was separated at semipreparative scale on a

polysaccharide-based chiral stationary phase. A modular supercritical fluid chromatograph was adapted to operate at semipreparative scale on a Chiralpak AD (25 cm × 10 mm) column was used. The effect of two organic modifiers (ethanol and isopropanol) was studied, and different injection volumes and concentrations of omeprazole racemic mixture were evaluated to obtain high enantiomeric purities and production rates. Better results were achieved using concentration overloading instead of volume overloading. The recoveries decreased when the requirements of enantiomeric purity or the load increased, but it was possible to recover 100% of both enantiomers at an enantiomeric purity higher than 99.9% under some loading conditions, like injecting 1 and 2 ml of a solution of 3 g/l. As far as production rates are concerned, the best result for *S*-(−)-omeprazole at that purity (27.2 mg/h) was achieved with sample concentrations of 10 g/l and the injection of 2 ml, while a volume of 4 ml was better in the case of *R*-(+)-omeprazole (20.5 mg/h).

4.2.5.6. Electrophoresis McGrath *et al.* [133] used capillary zone electrophoresis to study the migration behavior of selected 1,4-benzodiazepines and metabolites over the pH range 2–12 exhibiting the ability to determine, pK_a values using this technique. The method was applied to the assay of a variety of pharmaceutical formulations which contain omeprazole, metronidazole, and 1,4-benzodiazepines and was compared with alternative analytical techniques such as reversed-phase HPLC, capillary gas chromatography, and automated differential-pulse polarography. Limit of detection of capillary zone electrophoresis and alternative techniques are compared for these molecules. The selectivity of capillary electrophoresis was demonstrated for the separation of four benzodiazepines using capillary zone electrophoresis with 20 mM citric acid + 15% methanol, and miceller electrokinetic capillary chromatography with 75 mM sodium dodecyl sulfate in 6 mM sodium tetraborate—12 mM disodium hydrogen phosphate + 5% methanol, and compared with other topical analytical techniques in terms of retention times, capacity factors, and efficiencies.

Eberle *et al.* [134] separated the enantiomers of omeprazole and structurally related drugs by capillary zone electrophoresis with bovine serum albumin as chiral selector. The separations were carried out on a fused silica column (60 cm × 50 μm, 50 cm to detector) with a buffer consisting of 100-μM-bovine serum albumin and 7% 1-propanol in 10 mM potassium phosphate pH 7.4. Electrokinetic injection was at 5–8 kV for 7 s. An applied voltage of 300 V/cm was used. Detection was at 290 nm. Detection limits were 0.04 mg/ml for the analytes studied.

Altria *et al.* [135] used a validated capillary electrophoresis method for the analysis of omeprazole among other acidic drugs and excipients. The results of validation experiments for the capillary electrophoretic

separation of water-soluble and -insoluble pharmaceutical compounds at 30 °C with 15 mM borax as run buffer and detection at 200 nm are presented. Hewlett-Packard bubble cell and Beckman instruments were used with fused-silica column (34 and 27 cm, respectively $\times 75$ mm) operated at 7 and 6.5 kV, respectively. When peak area ratios and internal standards sodium-β-naphtoxy acetate and aminobenzoic acid were used to determine 100 ppm omeprazole, the R.S.D. was 0.34−1.31% ($n = 10$). Typical detection and determination limits were 0.4 and 1.2−1.7 mg/l, respectively. Calibration graphs were linear from 50 to 150 mg/l of omeprazole.

Bonato and Paias [136] developed two sensitive and simple assay procedures based on HPLC and capillary electrophoresis for the enantioselective analysis of omeprazole in pharmaceutical formulations. Racemic omeprazole and (*S*)-omeprazole were extracted from commercially available tablets using methanol–sodium hydroxide 2.5 mol/l (90:10). Chiral HPLC separation of omeprazole was obtained on a ChiralPak AD column using hexane–ethanol (40:60) as the mobile phase and detection at 302 nm. The resolution of omeprazole enantiomers by capillary electrophoresis was carried out using 3% sulfated β-cyclodextrin in 20 mmol/l phosphate buffer, pH 4 and detection at 202 nm.

Lin and Wu [137] established a simple capillary zone electrophoresis method for the simultaneous analysis of omeprazole and lansoprazole. Untreated fused-silica capillary was operated using a phosphate buffer (50 mM, pH 9) under 20 kV and detection at 200 nm. Baseline separation was attained within 6 min. In the method validation, calibration curves were linear over a concentration range of 5–100 μM, with correlation coefficients 0.9990. RSD and relative error were all less than 5% for the intra- and interday analysis, and all recoveries were greater than 95%. The limits of detection for omeprazole and lansoprazole were 2 μM ($S/N = 3$, hydroxynamic injection 5 s). The method was applied to determine the quality of commercial capsules. Assay result fell within 94–106%.

Berzas Nevado *et al.* [138] developed a new capillary zone electrophoresis method for the separation of omeprazole enantiomers. Methyl-β-cyclodextrin was chosen as the chiral selector, and several parameters, such as cyclodextrin structure and concentration, buffer concentration, pH, and capillary temperature were investigated to optimize separation and run times. Analysis time, shorter than 8 min was found using a background electrolyte solution consisting of 40 mM phosphate buffer adjusted to pH 2.2, 30 mM β-cyclodextrin and 5 mM sodium disulfide, hydrodynamic injection, and 15 kV separation voltage. Detection limits were evaluated on the basis of baseline noise and were established 0.31 mg/l for the omeprazole enantiomers. The method was applied to pharmaceutical preparations with recoveries between 84% and 104% of the labeled contents.

Olsson *et al.* [139] developed and validated a nonaqueous capillary electrophoresis method for the enantiomeric determination of omeprazole and 5-hydroxyomeprazole. Heptakis-(2,3-di-*O*-methyl-6-*O*-sulfo)-*β*-cyclodextrin was chosen as the chiral selector in an ammonium acetate buffer acidified with formic acid in methanol. Parameters such as cyclodextrin concentration, concentration of buffer electrolyte, voltage, and temperature were studied to optimize both the enantioresolution and migration times. An experimental design was utilized for method optimization, using software Modde 5. Validation of the method showed good linearity, which was tested over a concentration range of 2.5–500 μM. The regression coefficients for *S*-omeprazole, *S*-5-hydroxyomeprazole, *R*-omeprazole, and *R*-5-hydroxyomeprazole were between 0.996 and 0.997. The limits of detection for the four enantiomers were in the range from 45 to 51 μM and the limits of quantification were between 149 and 170 M with UV detection at 301 nm.

Perez-Ruiz *et al.* [140] developed a sensitive method for the determination of omeprazole and its metabolites, hydroxylomeprazole, and omeprazole sulfone using automated solid-phase extraction and micellar electrokinetic capillary chromatography. The method involves an automated solid-phase extraction procedure and capillary electrophoresis with UV detection. Omeprazole, hydroxyomeprazole, and omeprazole sulfone could be separated by micellar electrokinetic capillary chromatography using a background electrolyte composed of 20 mM borate buffer and 30 mM sodium dodecyl sulfate, pH 9.5. The isolation of omeprazole and its metabolites from plasma was automatically accomplished with an original solid-phase extraction procedure using surface-modified styrene–divinyl benzene polymer cartridges. Good recovery data and satisfactory precision values were obtained. Responses were linear for the three analytes, from 0.08 to 2 μg/ml of plasma. Intra- and interday precision values of about 1.6% RSD ($n = 10$) and 2.5% RSD ($n = 36$), respectively, were obtained. The method is highly robust and no breakdown of the current or capillary blockages was observed during several weeks of operation. The method was applied to the determination of omeprazole in pharmaceutical preparations and for the analysis of plasma samples obtained from three volunteers who received oral doses of omeprazole.

Olsson and Blomberg [141] enantioseparated omeprazole and its metabolite 5-hydroxyomeprazole using open tubular capillary electrochromatography with immobilized avidin as chiral selector. The separation was performed with open tubular capillary electrochromatography. The protein avidin was used as the chiral selector. Avidin was immobilized by a Schiffs base type of reaction where the protein was via glutraldehyde covalently bonded to the amino-modified wall of a fused-silica capillary, 50 μm i.d. Both racemates were baseline resolved. Resolution

was 1.9 and 2.3, respectively, using ammonium acetate buffer, pH 8.5, 5% methanol, with UV detection. These values of resolution using open tubular capillary electrochromatography are higher than earlier published results regarding chiral separation of omeprazole and 5-hydroxyomeprazole on packed capillary electrochromatography. The number of theoretical plates also indicated good separation efficiency.

5. PHARMACOKINETICS AND METABOLISM

5.1. Pharmacokinetics

Regardh *et al.* [142] studied the pharmacokinetics of omeprazole in mouse, rat, dog, and man. The drug is rapidly absorbed in all species. The systemic availability is relatively high in dog and in man provided the drug is protected from acidic degradation in stomach. In man the fraction of the oral dose reaching the systemic circulation was found to increase from an average of 40.3–58.2 when the dose was raised from 10 to 40 mg, suggesting some dose-dependency in this parameter. Omeprazole distributes rapidly to extravascular sites. Omeprazole is bound to about 95% to proteins in human plasma. The drug is eliminated almost completely by metabolism and no unchanged drug has been recovered in the urine in the species studied. Two metabolites, the sulfone and sulfide of omeprazole, have been identified and quantified in human plasma.

Regardh *et al.* [143] found that about 54% of an oral dose of omeprazole, administered by young healthy subjects, is available to the systemic circulation. The distribution of the drug after an intravenous dose was consistent with localization of a major fraction of the drug in the extracellular water, with about 25% restricted to the blood. Omeprazole was rapidly cleared and possessed the characteristics of a high clearance drug; insignificant amounts of ^{14}C-omeprazole were excreted by the kidneys, though metabolites were excreted very rapidly. Six different metabolites were reported and the major one being hydroxyomeprazole.

Naesdal *et al.* [144] studied the pharmacokinetics of ^{14}C-omeprazole and its metabolites after single intravenous and oral doses of 20–40 mg, respectively, to 12 patients with chronic renal insufficiency. Blood samples for determination of total radioactivity, omeprazole, hydroxyomeprazole, sulfone, and sulfide were taken for 24 h. Urine was collected over 96 h for determination of total radioactivity and during the first 24 h for additional assay of omeprazole and metabolites. The mean systemic availability was 70% and the mean plasma $t_{1/2}$ of omeprazole was 0.6 h.

Cederberg *et al.* [145] reported that omeprazole has to be protected from exposure to the acidic gastric juice when given orally. Following a single oral dose of buffered suspension, omeprazole is rapidly absorbed

with peak plasma concentrations within 0.5 h. The volume of distribution is 0.3 l/kg corresponding to the volume of extracellular water. In contrast to the long duration antisecretory action, omeprazole is rapidly eliminated from plasma. The half-life is less than 1 h, and omeprazole is almost entirely cleared from plasma with 3–4 h. Omeprazole is completely metabolized in the liver. The two major plasma metabolites are the sulfone and hydroxyomeprazole, neither of which contributes to the antisecretory activity. About 80% of a given dose is excreted in the urine, and the remainder via the bile.

Regardh *et al.* [146] studied the pharmacokinetics of omeprazole, hydroxyomeprazole, omeprazole sulfone, and other metabolites, in eight young healthy subjects following an acute intravenous and oral dose of 10 and 20 mg of ^{14}C-labeled drug, respectively. The oral dose was given as a buffered solution. Two subjects exhibited essentially higher and more sustained plasma levels of omeprazole than the others. This was due to a higher bioavailability, lower clearance, and longer $t_{1/2}$ of omeprazole in these two subjects. Maximum concentration, 0.7–4.6 μmol/l, was reached between 10 and 25 min after oral dosing. Omeprazole was rapidly distributed to extravascular sites. Low systemic clearance of omeprazole was associated with a decreased formation rate of hydroxyomeprazole and other metabolites. Omeprazole sulfone formation seemed to be less affected. The excretion of hydroxymeprazole during the first 12 h varied between 4.6% and 15.5% of a given dose.

Andersson *et al.* [147] studied the influence of dose on the kinetics of omeprazole and two of its metabolites, hydroxyomeprazole and the sulfone. Ten healthy subjects were given omeprazole 10 and 40 mg intravenously and 10, 40, and 90 mg orally. No significant dose-related difference in parameter calculated from the intravenous experiments was detected. Following the oral solutions, there was a dose-dependent increase in the systemic availability, probably due to saturable first-pass elimination.

Sohn *et al.* [148] examined the kinetic variables of omeprazole and its two primary metabolites in plasma, 5-hydroxyomeprazole and omeprazole sulfone, and the excretion profile of its principal metabolite in urine, 5-hydroxyomeprazole, in eight extensive metabolizers and eight poor metabolizers. Each subject received a postoral dose of 20 mg of omeprazole as an enteric-coated formulation, and blood and urine samples were collected up to 24 h postdose. Omeprazole and its metabolites were measured by HPLC with UV detection. The mean omeprazole area under the concentration–time curve, elimination half-life, and apparent postoral clearance were significantly greater, longer, and lower, respectively, in the poor metabolizers than in the extensive metabolizers. The mean cumulative urinary excretion of 5-hydroxyomeprazole up to 24 h postdose was significantly less in the poor metabolizers than in the extensive metabolizers.

Landahl *et al.* [149] studied the pharmacokinetics of omeprazole and its metabolites in eight healthy elderly volunteers using ^{14}C-omeprazole. In another six healthy elderly volunteers, the pharmacokinetics of omeprazole were studied using unlabeled drug. Each volunteer received single doses of omeprazole intravenously, 20 mg, and orally, 40 mg, as solutions in a randomized crossover design. The plasma concentrations and urinary excretion of omeprazole and metabolites were followed for 24 and 96 h, respectively. The results indicate that the average metabolic capacity of omeprazole is decreased in the elderly compared with that found in earlier studies of healthy young individuals. This was reflected in an increase in bioavailability from 56% to 76%, a reduction in mean systemic clearance by approximately 50% (0.25 l/min) and a prolongation of the mean elimination half-life from 0.7 to 1 h, compared with the young.

Andersson *et al.* [150] studied the pharmacokinetics of omeprazole and its metabolites following single doses, in eight patients with liver cirrhosis. Each patient participated in two experiments in which ^{14}C-omeprazole was administered either intravenously, 20 mg, or in an oral solution, 40 mg, in a randomized crossover design. Plasma concentrations of omeprazole and two of its identified metabolites, as well as total radioactivity were followed for 24 h. Urinary excretion was followed for 96 h. The mean elimination half-life of omeprazole in the patients with cirrhosis was 2.8 h and the mean total plasma clearance was 67 ml/min (4.02 l/h); corresponding values from separate studies in young healthy volunteers were 0.7 h and 594 ml/min (35.64 l/h). Almost 80% of a given dose was excreted as urinary metabolites in both patients and young volunteers.

Okada *et al.* [151] determined omeprazole and its metabolites in human plasma as a probe for CYP_2C_{19} phenotype. The drug is metabolized in the liver to varying degree by several cytochrome P450 (CYP) isoenzymes which are further categorized into subfamilies of related polymorphic gene products. The metabolism of omeprazole is dependent on CYP_3A_4 and CYP_2C_{19}. Omeprazole is metabolized to two major metabolites, 5-hydroxyomeprazole (CYP_2C_{19}) and omeprazole sulfone (CYP_3A_4). Minor mutations in CYP_2C_{19} affect its activity in the liver and the metabolic and the pharmacokinetic profiles of omeprazole. The frequency of CYP_2C_{19} poor metabolizers in population of Asian descent has been reported to range from 10% to 20%. This study demonstrates determination of omeprazole in human plasma as a probe drug of CYP_2C_{19} phenotyping. The method allows the quantitation of omeprazole and its metabolite in human plasma after the administration of therapeutic dose of the drug. The analytical column used for LC/MS was YMC-Pack Pro C_{18} (5 cm $\times$ 2 mm) and operated at 25 °C. The mobile phase was acetonitrile–ammonium acetate at a flow-rate of 0.2 ml/min. The drift

voltage was 30 V. The sampling aperture was heated at 110 °C and shield temperature was 230 °C. The column used for chiral separation by HPLC was Chiralpak AD-RH column (15 cm × 4.6 mm) using phosphate buffer/acetonitrile as the eluent and operated at 40 °C. The flow-rate was 0.5 ml/min and detection was 302 nm.

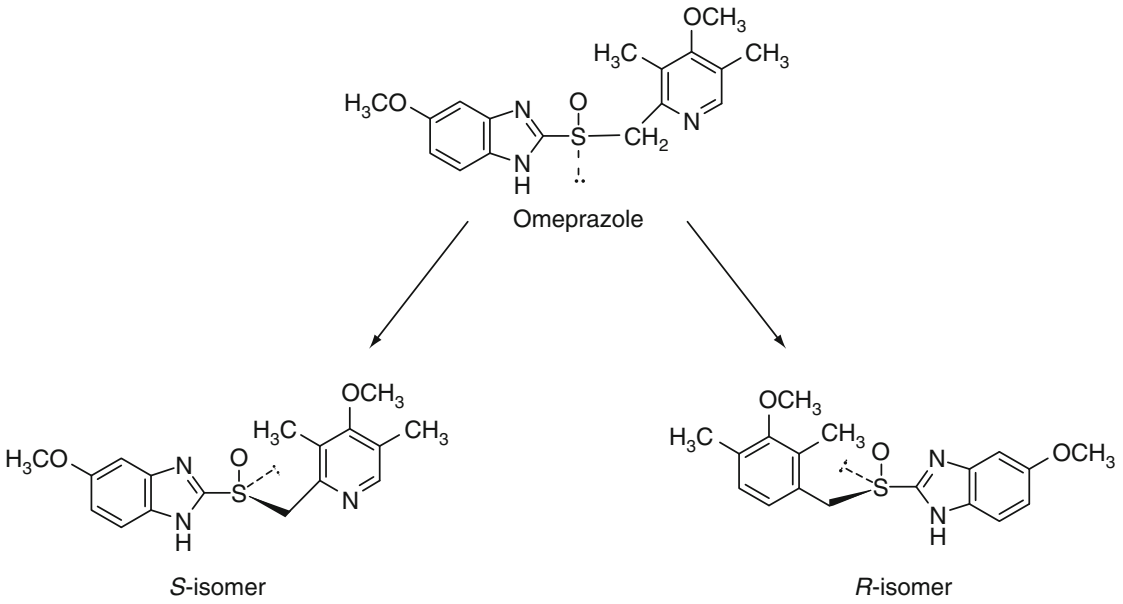
Abelo *et al.* [152] studied the stereoselective metabolism of omeprazole by human cytochrome P450 enzymes. This study demonstrates the stereoselective metabolism of the optical isomers of omeprazole in himan liver microsomes. The intrinsic clearance of the formation of the hydroxyl metabolite from *S*-omeprazole was 10-fold lower than that from *R*-omeprazole. However, the intrinsic clearance value for the sulfone and 5-*O*-desmethyl metabolites from *S*-omeprazole was higher than that from *R*-omeprazole. The sum of the intrinsic clearance of the formation of all three metabolites was 14.6 and 42.5 μl/min/mg protein for *S*- and *R*-omeprazole, respectively. This indicates that *S*-omeprazole is cleared more slowly than *R*-omeprazole *in vivo*. The stereoselective metabolism of the optical isomers is mediated primarily by cytochrome P450 (CYP)2C19, as indicated by studies using cDNA-expressed enzymes. This is the result of a considerable higher intrinsic clearance of the 5-hydroxy metabolite formation for *R*- and *S*-omeprazole. For *S*-omeprazole, CYP_2C_{19} is more important for 5-*O*-desmethyl formation than for 5-hydroxylation. Predictions of the intrinsic clearance using data from cDNA-expressed enzymes suggest that CYP_2C_{19} is responsible for 40% and 87% of the total intrinsic clearance of *S*- and *R*-omeprazole, respectively, in human liver microsome. According to experiments using cDNA-expressed enzymes, the sulfoxidation of both optical isomers is metabolized by a single isoform, CYP_3A_4. The intrinsic clearance of the sulfone formation by CYP_3A_4 is 10-fold higher for *S*-omeprazole than for *R*-omeprazole, which may contribute to their stereoselective disposition. The results of this study show that both CYP_2C_{19} and CYP_3A_4 exhibit a stereoselective metabolism of omeprazole. CYP_2C_{19} favors 5-hydroxylation of the pyridine group of *R*-omeprazole, whereas the same enzyme mainly 5-*O*-demethylates *S*-omepyrazole in the benzimidazole group. Sulfoxidation mediated by CYP_3A_4 highly favors the *S*-form.

Pique *et al.* [153] examined the pharmacokinetics of omeprazole during intravenous infusion in patients with varying degrees of liver dysfunction. Thirteen patients, five males and eight females with a mean age of 59 years with proved hepatic cirrhosis, classified according to Child-Pugh criteria as A ($n = 5$), B ($n = 4$), or C ($n = 4$). Each patient received an 80 mg bolus of omeprazole over 30 min followed by a continuous infusion of 8 mg/h for 47.5 h. Blood sample was taken frequently throughout the infusion and during the subsequent 24-h washout period

for determination of omeprazole and its metabolites. Data were evaluable for 12 patients. For omeprazole, the mean total area under the plasma concentration–time curve was 286.5 μmol h/l. Peak plasma concentration was 14.9 μmol/l and terminal elimination half-life was 4.1 h; these values were higher than those observed historically in control patient populations. Concentrations of the metabolite omeprazole sulfone were also increased, but there was a decrease in concentrations of hydroxyomeprazole. Exposure to omeprazole following intravenous administration was higher in patients with liver dysfunction than in normal population. However, in patients with severely impaired liver function, the omeprazole plasma concentration did not change by more than 100% and the drug was well tolerated.

Kita *et al.* [154] have undertaken a study to help predict the optimal dosage of omeprazole for extensive metabolizers in the anti-*H. pylori* therapy. Seven healthy Japanese subjects, classified based on the CYP_2C_{19} genotype into extensive metabolizers ($n = 4$) and poor metabolizers ($n = 3$), participated in this study. Each subject received a single oral dose of omeprazole 20, 40, and 80 mg, with at least a 1-week washout period between each dose. Plasma concentrations of omeprazole and its two metabolites were monitored for 12 h after each dose of medication. After each dose was administered, the pharmacokinetic profiles of omeprazole and its two metabolites were significantly different between extensive metabolizers and poor metabolizers. The area under the plasma concentration–time curve of omeprazole in extensive metabolziers was disproportionally increased 3.2- or 19.2-fold with dose escalation from 20 to 40 to 80 mg omeprazole, respectively. In contrast, the area under the plasma concentration–time curve of omeprazole was proportionally increased with the higher dose in poor metabolizers. The area under the plasma concentration–time curve of omeprazole after 20 mg administration to poor metabolizers was almost equal to the area under the plasma concentration–time curve in extensive metabolizers after 80 mg administration. The recommended dose of omeprazole for extensive metabolizers is a maximum of 80 mg $\times$ 2/day based on pharmacokinetic considerations.

Omeprazole is a racemate, from which the *R*- and *S*-isomers are isolated as reported by Kendall [155]. Both of these isomers convert to the same inhibitor of the H^+/K^+-ATPase and produce the same reduction in the gastric acid secretion. The *S*-isomer, esomeprazole, is metabolized more slowly and reproducibly than the *R*-isomer of omeprazole and therefore produces higher plasma concentrations for longer and, as a result, inhibits gastric acid production more effectively and for longer. Esomeprazole has the pharmacological properties of a more effective form of treatment for disorders related to gastric acid secretion.



Kumar *et al.* [156] carried out a study aiming to determine the pharmacokinetics of omeprazole in different degrees of liver cirrhosis and in patients with extrahepatic portal vein obstruction (EHPVO), compared with healthy volunteers. Ten healthy volunteers, 30 patients with cirrhosis of the liver, divided into three groups of 10 depending on severity (according to Child-Pugh classification A, B, and C) and 10 patients with EHPVO participated in this study. The subjects received an omeprazole 20 mg capsule after an overnight fast. Blood samples were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 6, 9, and 24 h after drug administration. Omeprazole level in plasma was estimated by reversed-phase HPLC. The elimination half-life was significantly increased to 2.38 ± 0.16, 3.26 ± 0.12, 3.58 ± 0.31, and 2.59 ± 0.22 h in patients with different grades of cirrhosis A, B, and C and also in patients with EHPVO, respectively, compared with 1.054 ± 0.1 h in healthy volunteers. It was concluded that the metabolism of omeprazole was significantly impaired in both liver cirrhosis and EHPVO in comparison with healthy volunteers.

Hassan-Alin *et al.* [157] investigated the pharmacokinetics of *S*-omprazole, *R*-omeprazole, and racemic omeprazole following single and repeated oral doses of 20 and 40 mg of each compound in healthy male and female subjects. Twelve subjects received 20 mg and another 12 subjects received 40 mg of *S*-omeprazole, *R*-omeprazole, and racemic omeprazole as oral solutions once daily for 5 days, separated by washout periods of at least 10 days. Blood samples were taken for analysis predose and at selected time points during a 12-h period following drug administration on study day 1 and day 5. Pharmacokinetic parameters of *S*-omeprazole, *R*-omeprazole, and racemic omeprazole and the two main metabolites, 5-hydroxyomeprazole and omeprazole sulfone, were

calculated using noncompartmental analysis. Following the 20-mg dose of each compound, values of the total area under the plasma concentration–time curve were 1.52, 0.62, and 1.04 μmol h/l for *S*-omeprazole, *R*-omeprazole, and racemic omeprazole, respectively, on day 1. Respectively, the area under the plasma concentration–time curve values on day 5 were 2.84, 0.68, and 1.63 μmol h/l. Corresponding values after the 40-mg doses were 3.88, 1.39, and 2.44 μmol h/l on day 1 and 9.32, 1.80, and 5.79 μmol h/l and day 5. Treatment with *S*-omeprazole, 20 and 40 mg, resulted in higher area under the concentration–time curve values than either *R*-omeprazole or racemic omeprazole after both single and repeated doses due to a lower metabolic rate of *S*-omeprazole than *R*-omeprazole and consequently, racemic omeprazole. *S*-Omeprazole, *R*-omeprazole, and the racemate were well tolerated.

5.2. Metabolism

Helander *et al.* [158] reported that radioactive omeprazole was given intravenously or orally to mice, and the distribution of the drug was investigated at various intervals by scintillation counting and by autoradiography. The half-life for radioactivity in the stomach was 14 h versus 30–36 h in the liver, kidneys, and blood. At 16 h after the drug was given, the radioactivity in the stomach was 10 times higher than that in the liver and kidneys, and 100 times that in the blood. Whole-body autoradiography showed sustained high levels of radioactivity only in the gastric mucosa. Light microscopic autoradiographic investigations of gastric mucosa from mice killed 1 or 16 h after the drug was given, revealed radioactivity in the parietal cells. By electron microscopy of gastric mucosa from the mouse killed 16 h after omeprazole injection, the isotope label was found mainly over the secretory surface and the tubulo-vesicles. At these locations H^+/K^+-ATPase has previously been demonstrated, and it is suggested that omeprazole or its metabolites binds to this enzyme.

Hoffmann *et al.* [159] studied the metabolic disposition of ^{14}C omeprazole in dogs, rats, and mice after the administration of pharmacologically active, single oral doses of the drug in buffer solutions (pH 9). Averages of 38% (dogs), 43% (rats), and 55% (mice) of the radiolabeled doses were excreted in the urine in 72 h. Most of the remaining dose was recovered in the feces. Omeprazole was extensively metabolized in all species studied and the metabolites were eliminated rapidly. No unchanged drug could be detected in the urine samples (less than 0.1% of dose). In each species at least 10 metabolites were detected in urine (pH 9) by gradient elution reversed-phase HPLC. Based on liquid chromatographic retention data, the metabolic patterns were very complex and exhibited some quantitative differences between species. Bile was collected from rats and from

chronic bile-fistulated dogs. Biliary excretion was a major route of elimination of omeprazole metabolites.

Hoffmann [160] identified the main urinary metabolites of omeprazole after an oral dose to rats and dogs. The structures of seven urinary metabolites of omeprazole following high oral doses to rats and dogs were determined by combining different analytical and spectroscopic techniques including derivatization and stable isotopes. Omeprazole was metabolized by aromatic hydroxylation at position 6 in the benzimidazole ring followed by glucuronidation. There was also oxidative *O*-dealkylation of both methoxy groups, and aliphatic hydroxylation of a pyridine methyl group followed by oxidation to the corresponding carboxylic acid. Due to the experimental design, implying no pH control of collected samples, all metabolites were isolated as sulfides.

Renberg *et al.* [161] identified two main urinary metabolites of ^{14}C omeprazole in humans. The excretion and metabolism of ^{14}C omeprazole given orally as a suspension was studied in 10 healthy male subjects. An average of 79% of the dose was recovered in the urine in 96 h, with most of the radioactivity (76% of dose) being eliminated in the first 24 h. Pooled urine (0–2 h) from five subjects, containing about 47% of the dose, was analyzed by reversed-phase gradient elution liquid chromatography with radioisotope detection. Omeprazole was completely metabolized to at least six metabolites. The two major metabolites were extensively purified by liquid chromatography and their structures were determined by mass spectrometry with derivatization and use of stable isotopes, ^{1}H NMR, and comparison with synthetic references. They were formed by hydroxylation of a methyl group in the pyridine ring, followed by further oxidation of the alcohol to the corresponding carboxylic acid. Both metabolites retained the sulfoxide group of omeprazole, rendering them as unstable as the parent compound at pH less than 7. They accounted for approximately 28% hydroxyomeprazole, and 23% (omeprazole acid) of the amount excreted in the 0–2-h collection interval. Based on *in vitro* studies, with the synthetic metabolites in isolated gastric glands, it is unlikely that metabolite 1 and metabolite 2 will contribute to the pharmacological effect of omeprazole in human.

Weidolf *et al.* [162] established the metabolic route of omeprazole involving glutathione through identification of end products excreted in the urine of rats after oral administration of 400 μmol/kg of a mixture of ^{3}H- and ^{14}C-omeprazole. The labeled positions enabled facile tracing of metabolites that were formed through fission of omeprazole, producing ^{3}H-pyridine and ^{14}C-benzimidazole metabolites. The structures of the metabolites were established by HPLC thromospray mass spectrometry and MS/MS. Two of the metabolites were isolated and characterized by ^{1}H NMR studies. The fact that the *N*-acetylcysteine derivative of the benzimidazole was one of the end products indicated that the initial

reaction involved glutathione. Three metabolites reflecting the fate of the pyridine moiety were identified. Their proposed formation route is *via* initial reduction to the pyridylmethylthiol compound followed by *S*-methylation and *S*-oxidation to the corresponding sulfoxide or sulfone.

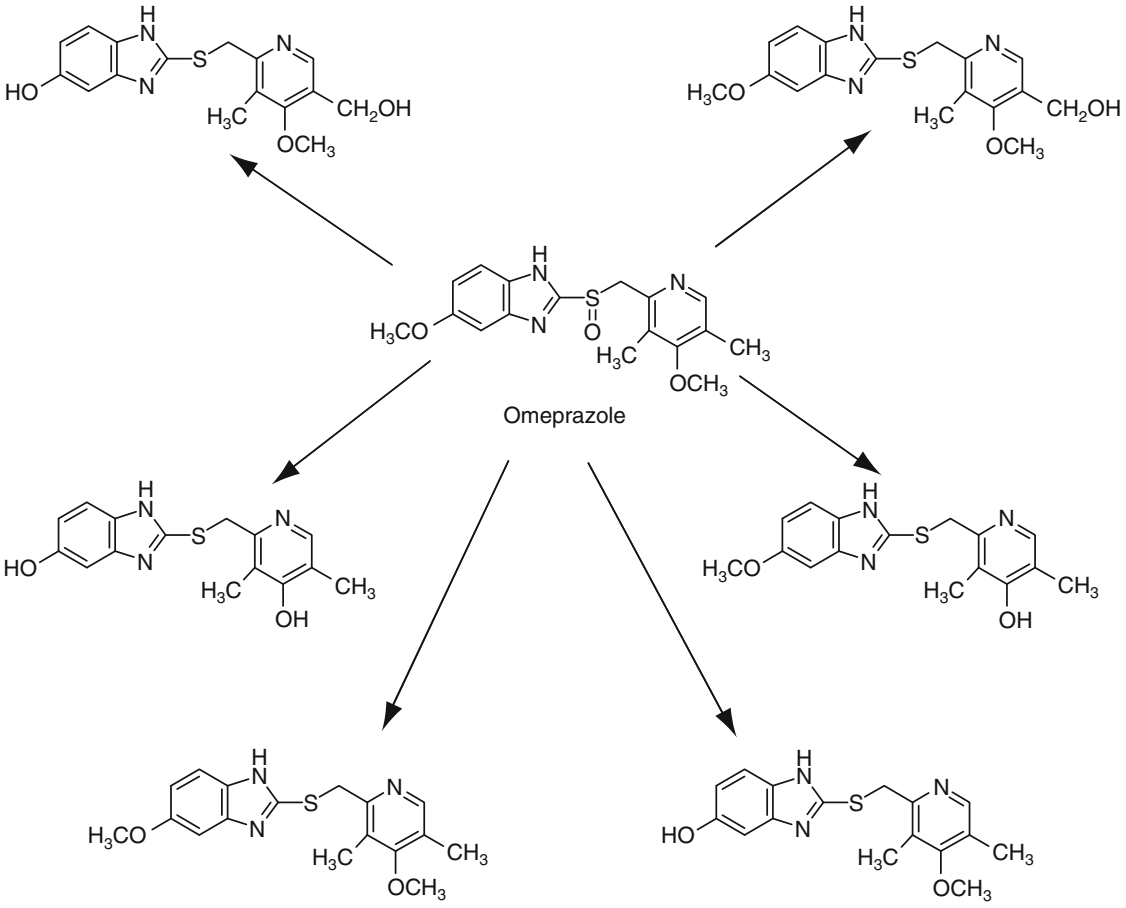
Chiba *et al.* [163] studied the oxidative metabolism of omeprazole in 14 human liver microsomes in relation to the 4′-hydroxylation capacity of *S*-mephenytoin. The formation of 5-hydroxyomeprazole and omeprazole sulfone from omeprazole exhibited a biphasic kinetic behavior, indicating that at least two distinct enzymes are involved in either of the metabolic pathways of omeprazole. These findings suggest that *S*-mephenytoin 4′-hydroxylase is an enzyme primarily responsible for the 5-hydroxylation of omeprazole and further metabolism of omeprazole sulfone, but not for the sulfoxidation of omeprazole in human liver microsomes.

Zhao and Lou [164] studied the metabolism of omeprazole to its two major metabolites, hydroxyomeprazole and omeprazole sulfone, in rat liver microsomes by a reversed-phase HPLC assay. The formation of metabolites of omeprazole depended on incubation time, substrate concentration, microsomal protein concentration, and was found to be optimal at pH 7.4. The V_{max} and K_m of omeprazole hydroxylation in the rat liver microsomal preparation were 2033 nmol/(min mg protein), and 46.8 μmol/l, respectively. The effects of seven drugs on omeprazole metabolism were tested. Mephenytoin, five benzodiazepines and pavaverine caused inhibition of omeprazole metabolism.

Meyer [165] studied the metabolic interactions of omeprazole with other drugs by cyctochrome P450 enzymes. Omeprazole was extensively metabolized by several human cytochromes P450, most prominently by mephenytoin hydroxylase (CYP_2C_{19}) and nifedipine hydroxylase (CYP_3A_4). The substrates and inhibitors of CYP_2C_{19} and CYP_3A_4 and the known genetic polymorphism of CYP_2C_{19} explain the interactions of omeprazole with carbamazepine, diazepam, phenytoin, and theophylline or caffeine.

Pearce and Lushnikova [78] incubated *C. elegans* ATCC 9245 and omeprazole and isolated putative fungal metabolites in sufficient quantities for structural elucidation. The metabolites were isolated by using semipreparative HPLC and the structures were identified by a combination of LC/MS and NMR experiments. Metabolites are shown in Scheme 4.1.

Tyrbing *et al.* [166] studied the stereoselective disposition of omeprazole and its formed 5-hydroxy metabolite in five poor metabolizers, and five extensive metabolizers of 5-mephenytoin. After a single oral dose of omeprazole (20 mg), the plasma concentrations of the separated enantiomers of the parent drug and the 5-hydroxy metabolite were determined for 10 h after drug intake. In poor metabolizers, the area under the plasma concentration versus time curve [AUC(0–8)] of (+) omeprazole was larger

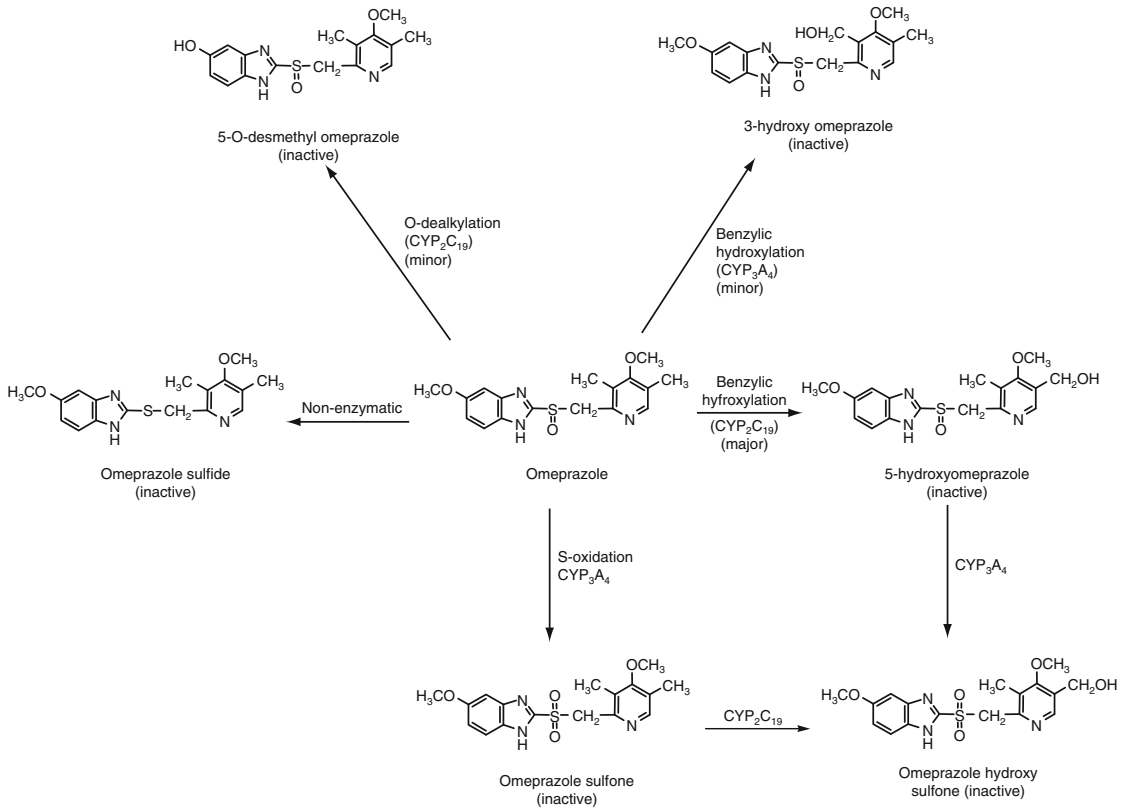


SCHEME 4.1 Biotransformation pathway of omeprazole induced by *C. elegans* ATCC 9245 [78].

and that of the 5-hydroxy metabolite of this enantiomer was smaller than the AUC (0–8) values in extensive metabolizers.

Jensen *et al.* [125] investigated an HPLC/ICP-MS with sulfur-specific detection, as a method for obtaining metabolite profiles for omeprazole administered as 1:1 mixture of ^{32}S- and ^{34}S-labeled material. Analysis based on the monitoring of the chromatographic eluent at either m/z 32 or m/z 34 was not successful due to insufficient sensitivity caused by interferences from polyatomic ions. However, reaction of sulfur with oxygen in the hexapole collision cell, combined with monitoring at m/z 48 (for ^{32}S) or m/z 50 (for ^{34}S), provided a facile method for metabolite profiling. Detection at m/z 48 was superior in sensitivity to detection of m/z 50.

Tolonen *et al.* [167] described a simple and efficient method for determination of labile protons in drug metabolites using postcolumn infusion of deuterium oxide in LC/MS experiments with ESI and TOF-MS. The number of exchangeable protons in analytes; hydroxyl, amine, thiol, and carboxylic acid protons can easily be determined by comparing the increase in m/z values after H/D-exchange occurring on line between



SCHEME 4.2 Metabolism of omeprazole [168].

an HPLC column and electrospray ion source. The hydroxyl metabolites and sulfur/nitrogen oxide with the same accurate mass can be distinguished. A good degree of exchange was obtained in repeatable experiments. Only the low consumption of deuterium oxide is needed in a very easy and rapidly set-up procedure. The method is applied in the study of metabolites of omeprazole in human and mouse *in vitro* samples, together with the exact mass data obtained from TOF-MS experiments.

Roche [168] pointed out that omeprazole and other proton pump inhibitors bind strongly to serum proteins and are extensively metabolized by the CYP450 family of enzymes. The CYP_2C_{19} isoform is important in converting parent structures to inactive metabolites, although CYP_3A_4 also plays a role in the proton pump inhibitors biotransformation. The metabolic degradation pathways for omeprazole are shown in Scheme 4.2.

6. MECHANISM OF ACTION

Lindberg *et al.* [169] proposed a mechanism of action for omeprazole, the inhibitor of the gastric H^+/K^+-ATPase, which is responsible for the gastric acid production and located in the secretory membranes of the parietal cell. Omeprazole itself is not an active inhibitor of this enzyme,

but it is transformed within the acid compartments of the parietal cell into the active inhibitor, close to the enzyme. Omeprazole **1** is transformed to the sulfenamide isomer **4**. The reaction is reversible and goes via the spiro intermediate **2** and the sulfenic acid **3**. The spiro intermediate **2** is a dihydrobenzimidazole, with a tendency to undergo aromatization to form the sulfenic acid **3** by a C–S bond cleavage. The reaction of **4** with *β*-mercaptoethanol forms the disulfide **5**. The adduct **5** reacts with molecule of *β*-mercaptoethanol in a base-catalyzed reaction to form the sulfide **8**, probably via the unstable mercaptan **7** resulting from the S–S bond cleavage during the simultaneous formation of the disulfide of *β*-mercaptoethanol. The mechanism of action is illustrated in Scheme 4.3.

Puscas *et al.* [170] suggested that omeprazole has a dual mechanism of action: H+/K+-ATPase inhibition and gastric mucosa carbonic anhydrase enzyme inhibition and that these enzymes may be functionally coupled.

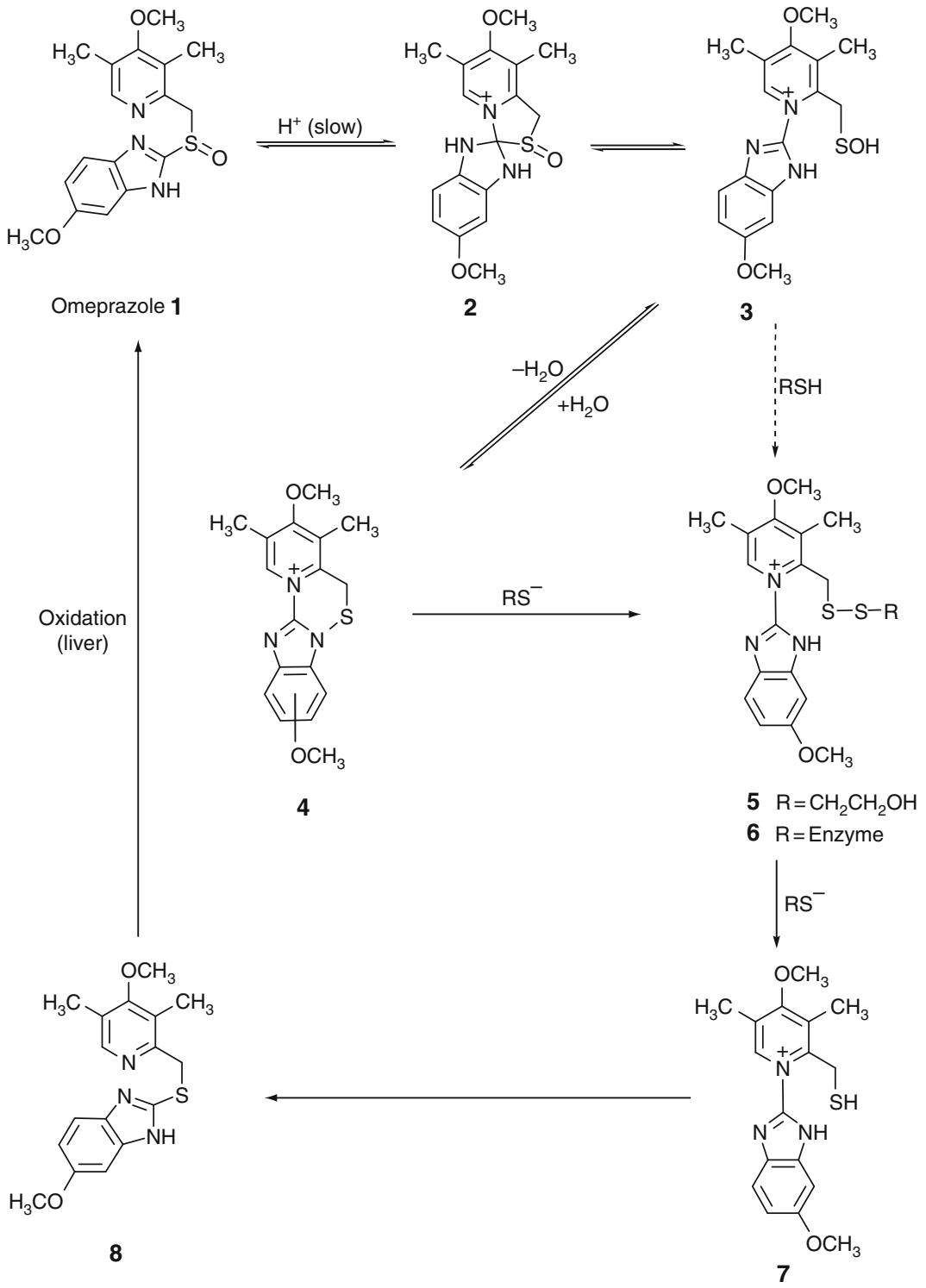
Roche [168] presented Scheme 4.4 to illustrate the activation and the reaction pathway of omeprazole.

7. STABILITY

Quercia *et al.* [171] studied the stability of omeprazole 2 mg/ml in an extemporaneously prepared oral liquid. The contents of five 20-mg omeprazole capsules were mixed with 50 ml of 8.4% sodium bicarbonate solution in a Luer-Lok syringe. Three vials of this liquid were prepared for storage at 24, 5, and −20 °C. A 3-ml sample of each was taken initially and on days 1, 2, 3, 4, 6, 8, 10, 12, 14, 18, 22, 26, and 30 and assayed by HPLC. The liquids stored at 5 and −20 °C did not change color during the study period, but the color of the liquid stored at 24 °C changed from white to brown.

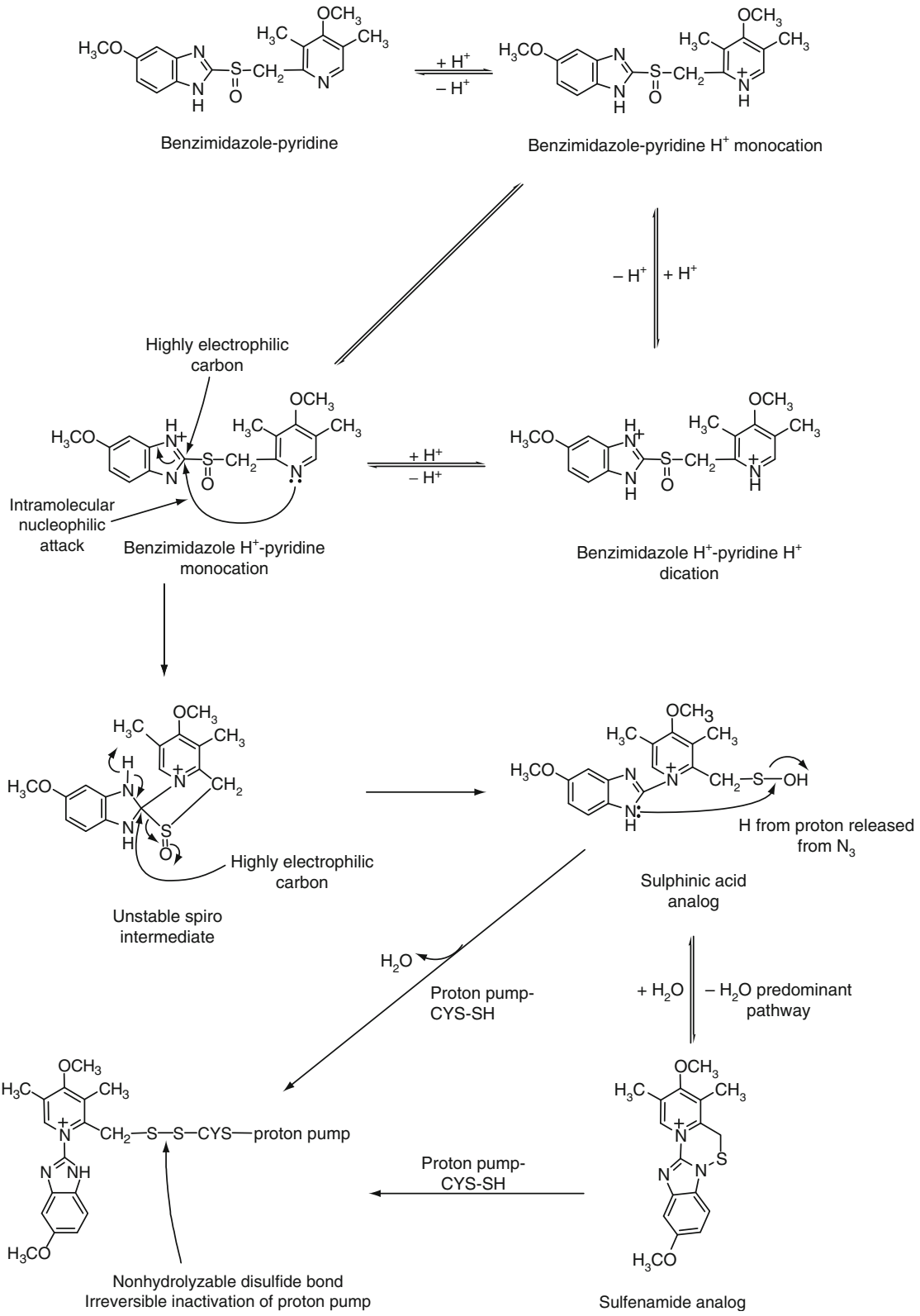
DiGiacinto *et al.* [172] determined the stability of omeprazole suspensions at ambient and refrigerated temperatures using HPLC. The contents of omeprazole capsules were suspended in separate flasks containing sodium bicarbonate 8.4% to concentrations of 3 and 2 mg/ml, respectively. The contents of each flask were drawn into six amber-colored oral syringes, with one-half of the syringes stored at 22 °C and the other half at 4 °C. Omeprazole concentrations were determined by a stability-indicating HPLC assay at baseline and at 4, 8, 12, and 24 h, and on days 4, 7, 14, 21, 30, 45, and 60 after mixing. Omeprazole was considered stable if it retained 90% of the baseline drug concentration. Omeprazole was stable for up to 14 days at 22 °C and 45 days at 4 °C.

Palummo *et al.* [173] comparatively evaluated the stability of capsules containing 20 mg of omeprazole, in enteric-coated pellets, from seven pharmaceutical laboratories on Argentine market. The stability test was



SCHEME 4.3 Mechanism of action of omeprazole [169].

performed under the conditions indicated by the international conference of harmonization: 40 °C, 75% HR, with and without light, during a 6-month period. The remaining content of omeprazole, total percentage of impurities and percentage of released active principle *in vitro*, were determined by HPLC. The organoleptic characteristics of the pellets were



SCHEME 4.4 Omeprazole activation and reaction pathway [168].

visually examined. The results obtained at 6 months indicate that, from the seven products studied, four were found to have a content of omeprazole higher than 90% of the labeled amount, in both lighting conditions

tested, and also comply with the USP 23 specifications with respect to the release *in vitro*. It is concluded that the progressive darkening of the pellets indicates, quantitatively, the level of degradation of the product and that the stability of omeprazole depends on the correct formulation and the primary container.

Choi and Kim [174] studied the stability of omeprazole tablets in human saliva. Omeprazole buccal adhesive tablet was developed and the absorption of omeprazole solutions from human oral cavity was evaluated and the physicochemical properties such as the bioadhesive forces of various omeprazole tablet formulations composed of bioadhesive polymers and alkali materials, and the stability of omeprazole tablets in human saliva were investigated. About 23% of the administered dose was absorbed from the oral cavity at 15 min after the administration of omeprazole solutions (1 mg/15 ml). A mixture of sodium alginate and hydroxypropylmethylcellulose was selected as the bioadhesive additive for the omeprazole tablet. Omeprazole tablets prepared with bioadhesive polymers alone had the bioadhesive forces suitable for buccal adhesive tablets, but the stability of omeprazole in human saliva was not satisfied. Among alkali materials, only magnesium oxide could be an alkali stabilizer for omeprazole buccal adhesive tablets due to its strong water-proofing effect. Two tablets composed of omeprazole/sodium alginate/hydroxypropylmethylcellulose/magnesium oxide (20/24/6/50, mg/tab) and (20/30/0/50, mg/tab) were suitable for omeprazole buccal adhesive tablets which could be attached to human cheeks without collapse and could be stabilized in human saliva for at least 4 h.

Yong *et al.* [175] developed an effective omeprazole buccal adhesive tablet with excellent bioadhesive force and good drug stability in human saliva. The omeprazole buccal adhesive tablets were prepared with various bioadhesive polymers, alkali materials, and croscarmellose sodium. Their physicochemical properties, such as bioadhesive force and drug stability in human saliva, were investigated. The release and bioavailability of omeprazole delivered by the buccal adhesive tablets were studied. As bioadhesive additives for omeprazole tablet, a mixture of sodium alginate and hydroxypropylmethyl cellulose was selected. The omeprazole tablets prepared with bioadhesive polymers alone had bioadhesive forces suitable for a buccal adhesive tablet, but the stability of omeprazole in human saliva was not satisfactory. Magnesium oxide is an alkali stabilizer for omeprazole buccal adhesive tablets. Croscarmellose sodium enhanced the release of omeprazole from the tablets but it decreased the bioadhesive forces and stability of omeprazole tablets in human saliva.

Leitner and Zollner [176] compared pH stabilities of the intravenous formulations of omeprazole. The solutions prepared according to the official instructions were exposed to four different light conditions. Both manufacturers state reference solutions for acceptable discoloration.

Those were prepared according to the European Pharmacopeia. Discoloration of solutions was evaluated as criterion for the stability of the proton pump inhibitors prodrugs. Optical alterations under different light conditions were compared and documented photographically with standardized illumination. Intensity and spectral composition of light as well as temperature had only minor influence on discoloration as measure of degradation of the prodrugs. Both formulations for injection fulfill the specifications for pH stability within the stated time frames mentioned in the summaries of product characteristics. After 1 h omeprazole injectable formulation showed a substantial optical discoloration. After 6 h these changes were more intense than the reference solution β-1,3-glucanase 5 (BG5) allows.

Moschwitzer *et al.* [177] developed an intravenously injectable chemically stable aqueous omeprazole formulation using nanosuspension technology. The feasibility of omeprazole stabilization using the DissoCubes technology and the optimal production parameters for a stable, highly concentrated omeprazole nanosuspension were studied. The HPLC analysis has proved the predominance of the nanosuspension produced by high-pressure homogenization in comparison to an aqueous solution. After 1 month of production, no discoloration or drug loss was recognizable when the nanosuspension was produced at 0 °C. It can be stated that the production of nanosuspensions by high-pressure homogenization is suitable for preventing degradation of labile drugs.

Riedel and Leopold [178] developed two reversed-phase HPLC methods to investigate the degradation of omeprazole in organic polymer solutions and aqueous dispersions of enteric-coating polymers (Eudragit L-100, S-100, CAP, HP-55, HPMCAS-HF, -LF, and shellac). The overall goal of the study was to determine the influence of the polymer structure on the degradation of omeprazole, whether the acid structure of the enteric-coating polymers caused an instability of the drug. It was investigated whether a difference in omeprazole degradation could be detected between organic polymer solutions and aqueous dispersions. pK_a values of the polymers and pH values of the aqueous dispersions were determined to see whether there was a correlation with the extent of degradation of omeprazole induced by enteric polymers. As the polymers containing phthalate moieties are very susceptible to hydrolysis, the influence of free phthalic acid on omeprazole stability was investigated. The degradation kinetics of omeprazole in organic polymer solutions were determined. Omeprazole degradation is more pronounced in aqueous polymer dispersions than in organic polymer solutions. The influence of organic polymer solutions on the stability of omeprazole depends on the amount of acidic groups in the polymeric structure.

Burnett and Balkin [179] investigated the stability and viscosity of preparations of a commercially available, flavored, immediate-release

powder for oral suspension (omeprazole–sodium bicarbonate) during refrigerator and room temperature storage. Omeprazole–sodium bicarbonate 20-mg packets were suspended to initial omeprazole concentrations of 0.6 and 2 mg/ml, and omeprazole–sodium bicarbonate 40-mg packets were suspended to initial omeprazole concentrations of 1.2, 2, 3, and 4 mg/ml. Suspensions were stored at 4 °C in darkness (refrigerated) or 22–25 °C (room temperature) in light for 1 week. A third set of suspensions was stored refrigerated for 1 month. Omeprazole's stability was quantified after 0, 6, 12, 24, 48, and 168 h in 1-week samples and after 0, 7, 14, 21, and 28 days in 1-month samples using high-pressure liquid chromatography. Viscosities of refrigerated suspensions were measured after 0, 1, and 7 days. Refrigerated suspension retained 98% and 96% of their initial omeprazole concentrations after 1 week and 1 month, respectively. Stability at room temperature suspensions was concentration dependent. After 1 week, the 0.6- and 1.2-mg/ml suspensions retained 87.2% and 93.1% of their respective initial omeprazole concentrations, whereas the 2-, 3-, and 4-mg/ml suspensions retained 97% of their initial omeprazole concentrations.

8. REVIEW

Bosch *et al.* [180] reviewed the analytical methodologies that have been used for the determination of omeprazole. The drug has been determined in formulation and in biological fluids by a variety of methods such as spectrophotometry, HPLC with UV detection, and liquid chromatography coupled with tandem mass spectrometry. The overview includes most relevant analytical methodologies used for the determination of the drug since the origin.

ACKNOWLEDGMENT

The author thanks Mr. Tanvir A. Butt, Pharmaceutical Chemistry Department, College of Pharmacy, King Saud University, for his secretarial assistance in preparing this manuscript.

REFERENCES

[1] S. Sweetman (Ed.), Martindale, The Complete Drug Reference, Pharmaceutical Press, Electronic version 2007.
[2] M.J. O'Neil (Ed.), The Merck Index, 14th ed., Merck & Co., Inc., NJ, USA, 2006, p. 1179.
[3] A.C. Moffat, M.D. Osselton, B. Widdop (Eds.), Clarke's Analysis of Drugs and Poisons, third ed., Vol. 2, Pharmaceutical Press, London, 2004, pp. 1366–1368.
[4] J.H. Block, J.M. Beale Jr. (Eds.), Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, 11th ed., Lippincott Williams & Wilkins, 2004, p. 954.

[5] P. Richardson, C.J. Hawkey, W.A. Stack, Drugs 56 (1998) 307–335.
[6] H.D. Langtry, M.I. Wilde, Drugs 56 (1998) 447–486.
[7] R.R. Berardi, L.S. Welage, Am. J. Health-Syst. Pharm. 55 (1998) 2289–2298.
[8] G.J.E. Brown, N.D. Yeomans, Drug Safety 21 (1999) 503–512.
[9] B.L. Erstad, Ann. Pharmacother. 35 (2001) 730–740.
[10] M. Robinson, J. Horn, Drugs 63 (2003) 2739–2754.
[11] R. Dekel, C. Morse, R. Fass, Drugs 64 (2004) 277–295.
[12] A.E. Brandstrom, B.R. Lamm, U.S. Patent 4,620,008. (Aktiebolaget Hassle), October 28, 1986.
[13] C. Slemon, B. Macel 1995, US Patent 5,470,983. (Torcan Chemical Ltd., Canada) November 28, 1995.
[14] H. Cotton, T. Elebring, M. Larsson, L. Li, H. Sorensen, S. von Unge, Tetrahedron: Asymmetry 11 (2000) 3819–3825.
[15] Aktiebolag Haessle. Jpn, Kokai Tokkyo Koho JP 62-72666 (Refs. 24 and 25. In: Rao *et al.*; Ref. 20).
[16] J.E. Baldwin, R.M. Adlington, N.P. Crouch, US Patent 6043371, 2000..
[17] S.P. Singh, S.M.J. Mukarram, D.G. Kulkami, M. Purohit, U.S. Patent 6,245,913 B1 (Wockhardt Europe Limited), June 12, 2001.
[18] X.L. Liu, Shanxi Med. Univ. J. 33 (2002) 330–332.
[19] http://www.stn-international.de/archive/presentations/online_information03/psspa.pdf.
[20] G.W. Rao, W.X. Hu, Z.Y. Yang, Chin. J. Synth. Chem. 10 (2002) 297–301.
[21] H. Ohishi, Y. In, T. Ishida, M. Inoue, F. Sato, M. Okitsu, Acta Cryst. C45 (1989) 1921–1923.
[22] R.M. Claramunt, C. Lopes, I. Alkorta, J. Elguero, R. Yang, S. Schulman, Magn. Reson. Chem. 42 (2004) 712–714.
[23] R.M. Claramunt, C. Lopes, J. Elguero, Arkivoc V (2006) 5–11.
[24] British Pharmacopoeia 2005, Vol. 2, The Stationary Office, London, 2005, pp. 1457–1460.
[25] The United States Pharmacopeia, USP 31, Vol. 3, The United States Pharmacopeial Convention, Rockville, MD, 2008, pp. 2850–2853.
[26] S.N. Dhumal, P.M. Dikshit, I.I. Ubharay, B.M. Mascarenhas, C.D. Gaitonde, Indian Drugs 28 (1991) 565–567.
[27] C.S.P. Sastry, P.Y. Naidu, S.S.N. Murthy, Indian J. Pharm. Sci. 59 (1997) 124–127.
[28] N. Ozaltin, A. Kocer, J. Pharm. Biomed. Anal. 16 (1997) 337–342.
[29] M. Tuncel, D. Dogrukol-Ak, Pharmazie 52 (1997) 73–74.
[30] A. Karlsson, S. Hermansson, Chromatographia 44 (1997) 10–18.
[31] N.M. El-Kousy, L.I. Bebawy, J. AOAC Int. 82 (1999) 599–606.
[32] A.A. Wahbi, O. Abdel Razak, A.A. Gazy, H. Mahgoub, M.S. Moneeb, J. Pharm. Biomed. Anal. 30 (2002) 1133–1142.
[33] K. Karljikovic-Rajic, D. Novovic, V. Marinkovic, D. Agbaba, J. Pharm. Biomed. Anal. 32 (2003) 1019–1027.
[34] F. Salama, N. El-Abasawy, S.A.A. Razeq, M.M.F. Ismail, M.M. Fouad, J. Pharm. Biomed. Anal. 33 (2003) 411–421.
[35] A. Riedel, C.S. Leopold, Pharmazie 60 (2005) 126–130.
[36] R. Yang, S.G. Schulman, P.J. Zavala, Anal. Chim. Acta 481 (2003) 155–164.
[37] C.S.P. Sastry, P.Y. Naidu, S.S.N. Murty, Indian Drugs 33 (1996) 607–610.
[38] C.S.P. Sastry, P.Y. Naidu, S.S.N. Murty, Talanta 44 (1997) 1211–1217.
[39] A.H. Zhang, F. Wang, X.J. Chen, L.K. Wu, Yaowu Fenxi-Zazhi 16 (1996) 194–195.
[40] S. Pinzauti, P. Gratteri, S. Furlanetto, P. Mura, E. Dreassi, R. Phan-Tan-Luu, J. Pharm. Biomed. Anal. 14 (1996) 881–889.
[41] A. Radi, J. Pharm. Biomed. Anal. 31 (2003) 1007–1012.
[42] A.M. Qaisi, M.F. Tutunji, L.F. Tutunji, J. Pharm. Sci. 95 (2005) 384391.

[43] Jin-Long Yan, J. Appl. Sci. 6 (2006) 1625–1627.
[44] S. McClean, E. O'Kane, V.N. Ramachandran, W.F. Smyth, Anal. Chim. Acta 292 (1994) 81–89.
[45] J.R. Ames, P. Kovacic, J. Electroanal. Chem. 343 (1992) 443–450.
[46] N. Ozaltin, A. Temizer, Electroanalysis 6 (1994) 799–803.
[47] D. Dogrukol-Ak, M. Tuncel, Pharmazie 50 (1995) 701–702.
[48] H. Knoth, H. Oelschlager, J. Volke, J. Ludvik, Pharmazie 52 (1997) 686–691.
[49] H. Oelschlaeger, H. Knoth, Pharmazie 53 (1998) 242–244.
[50] F. Belal, N. El-Enany, M. Rizk, J. Food Drug Anal. 12 (2004) 102–109.
[51] N. El-Enany, F. Belal, M. Risk, J. Biochem. Biophys. Methods 70 (2008) 889–896.
[52] E.X. Cao, Y.H. Zeng, Fenxi Kexue Xuebao 17 (2001) 110–113.
[53] S. Mangalan, R.B. Patel, B.K. Chakravarthy, J. Planar. Chromatogr. Modern TLC 4 (1991) 492–493.
[54] S. Ray, P. Kumar-De, Indian Drugs 31 (1994) 543–547.
[55] D. Dogrukol-Ak, Z. Tunalier, M. Tuncel, Pharmazie 53 (1998) 272–273.
[56] D. Agbaba, D. Novovic, K. Karljikovic-Rajic, V. Marinkovic, J. Planar. Chromatogr. Modern TLC 17 (2004) 169–172.
[57] B.A. Persson, P.O. Lagerstrom, I. Grundevik, Scand. J. Gastroenterol. Suppl. 108 (1985) 71–77.
[58] M.A. Amantea, P.K. Narang, J. Chromatogr. 426 (1988) 216–222.
[59] S.H. Shim, S.J. Bok, K.I. Kwon, Arch. Pharm. Res. 17 (1994) 458–461.
[60] K. Balmer, B.A. Persson, P.O. Lagerstroem, J. Chromatogr. 660 (1994) 269–273.
[61] W.K. Kang, D.S. Kim, K.I. Kwon, Arch. Pharm. Res. 22 (1999) 86–88.
[62] M. Motevalian, G. Saeedi, F. Keyhanfar, L. Tayebi, M. Mahmoudian, Pharm. Pharmacol. Commun. 5 (1999) 265–268.
[63] G. Garcia-Encina, R. Farran, S. Puig, L. Martinez, J. Pharm. Biomed. Anal. 21 (1999) 371–382.
[64] D. Castro, A.M. Moreno, S. Torrado, J.L. Lastres, J. Pharm. Biomed. Anal. 21 (1999) 291–298.
[65] B.A. Persson, S. Andersson, J. Chromatogr. 906 (2001) 195–203.
[66] Q.B. Cass, A.L.G. Degani, N. Cassiano, J. Liq. Chromatogr. Relat. Technol. 23 (2000) 1029–1038.
[67] G.W. Sluggett, J.D. Stong, J.H. Adams, Z. Zhao, J. Pharm. Biomed. Anal. 25 (2001) 357–361.
[68] M.C. Dubuc, C. Hamel, M.S. Caubet, J.L. Brazier, J. Liq. Chromatogr. Relat. Technol. 24 (2001) 1161–1169.
[69] H.M. Gonzalez, E.M. Romero, T.de J. Chavez, A.A. Peregrina, V. Quezada, C. Hoyo-Vadillo, J. Chromatogr. 780 (2002) 459–465.
[70] F.C. Cheng, Y.F. Ho, L.C. Hung, C.F. Chen, T.H. Tsai, J. Chromatogr. 949 (2002) 35–42.
[71] Q.B. Cass, V.V. Lima, R.V. Oliveira, N.M. Cassiano, A.L. Degani, J. Pedrazzoli, J. Chromatogr. B 798 (2003) 275–281.
[72] A. Schubert, A.L. Werle, C.A. Schmidt, C. Codevilla, L. Bajerski, R. Chiappa, et al., J. AOAC Int. 86 (2003) 501–504.
[73] R.M. Orlando, P.S. Bonato, J. Chromatogr. B 795 (2003) 227–235.
[74] N.L. Rezk, K.C. Brown, A.D. Kashuba, J. Chromatogr. B 844 (2006) 314–321.
[75] M. Shimizu, T. Uno, T. Niioka, N. Yaui-Furukori, T. Takahata, K. Sugawara, J. Chromatogr. B 832 (2006) 241–248.
[76] A. Zarghi, S.M. Foroutan, A. Shafaati, A. Khoddam, Arzneimittelforschung 56 (2006) 382–386.
[77] H. Jia, W. Li, K. Zhao, J. Chromatogr. B. 837 (2006) 112–115.
[78] G.M. Pearce, M.V. Lushnikova, J. Mol. Catal. B: Enzym. 41 (2006) 87–91.

[79] Z.A. El-Sherif, A.O. Mohamed, M.G. El-Bardicy, M.F. El-Tarras, Chem. Pharm. Bull. 54 (2006) 814–818.
[80] R. Linden, A.L. Ziulkoski, M. Wingert, P. Tonello, A.A. Souto, J. Braz. Chem. Soc. 18 (2007) 733–740.
[81] L. Sivasubramanian, V. Anilkumar, Indian J. Pharm. Sci. 69 (2007) 674–676.
[82] F.S. Murakami, A.P. Cruz, R.N. Pereira, B.R. Valente, M.A.S. Silva, J. Liq. Chromatogr. Relat. Technol. 30 (2007) 113–121.
[83] I.J. de Silva Jr., J.P. Sartor, P.C.P. Rosa, V. de Veredas, A.G. Barreto Jr., C.C. Santana, J. Chromatogr. 1162 (2007) 97–102.
[84] K.R.A. Belaz, M. Coimbra, J.C. Barreiro, C.A. Montanari, Q.B. Cass, J. Pharm. Biomed. Anal. 47 (2008) 81–87.
[85] S.A. Matlin, M.E. Tiritan, Q.B. Cass, D.R. Boyd, Chirality 8 (1996) 147–152.
[86] M.E. Tiritan, Q.B. Cass, A. Del Alamo, S.A. Matlin, S.J. Grieb, Chirality 10 (1998) 573–577.
[87] S.A. Matlin, M.E. Tiritan, A.J. Crawford, Q.B. Cass, D.R. Boyd, Chirality 6 (1994) 135–140.
[88] N. Sultana, M. Saeed Arayne, F. Hussain, A. Shakeel, J. Saudi Chem. Soc. 10 (2006) 141–148.
[89] M. Rambla-Alegre, J. Esteve-Romero, S. Garda-Broch, Anal. Chim Acta 633 (2009) 250–256.
[90] G.W. Mihaly, P.J. Prichard, R.A. Smallwood, N.D. Yeomans, W.J. Louis, J. Chromatogr. 278 (1983) 311–319.
[91] P.O. Lagerstrom, B.A. Persson, J. Chromatogr. 309 (1984) 347–356.
[92] B. Persson, S. Wendsjo, J. Chromatogr. 321 (1985) 375–384.
[93] I. Grundevik, G. Jerndal, K. Balmer, B.A. Persson, J. Pharm. Biomed. Anal. 4 (1986) 389–398.
[94] P. Erlandsson, R. Isaksson, P. Lorentzon, P. Lindberg, J. Chromatogr. 532 (1990) 305–319.
[95] T. Arvidsson, E. Collijn, A.M. Tivert, L. Rosen, J. Chromatogr. 586 (1991) 271–276.
[96] Z. He, Yaowu Fenxi Zazhi 11 (1991) 276–278.
[97] K. Kobayashi, K. Chiba, D.R. Sohn, Y. Kato, T. Ishizaki, J. Chromtogr. 579 (1992) 299–305.
[98] O. Gyllenhaal, J. Vessman, J. Chromatogr. 628 (1993) 275–281.
[99] T. Andersson, P.O. Lagerstrom, J.O. Miners, M.E. Veronese, L. Weidolf, D.J. Birkett, J. Chromatogr. 619 (1993) 291–297.
[100] S. Luo, T. Li, Yaowu Fenxi Zazhi 13 (1993) 263–264.
[101] K. Kobayashi, K. Chiba, M. Tani, Y. Kuroiwa, T. Ishizaki, J. Pharm. Biomed. Anal. 12 (1994) 839–844.
[102] A.M. Cairns, R.H.Y. Chiou, J.D. Rogers, J.L. Demertriades, J. Chromatogr. 666 (1995) 323–328.
[103] M. Tanaka, H. Yamazaki, H. Hakushi, Chirality 7 (1995) 612–615.
[104] X.P. Xu, C.Z. Dai, T. Li, Fenxi Ceshi Xuebao 16 (1997) 48–53.
[105] X.Y. Xu, J.H. Lu, M.L. Wang, C. Xu, R.L. Wang, L.Y. He, Yaowu Fenxi Zazhi 17 (1997) 169–171.
[106] J. Macek, P. Ptacek, J. Klima, J. Chromatogr. B. 689 (1997) 239–243.
[107] S. Gangadhar, G.S.R. Kumar, M.N.V.S. Rao, Indian Drugs 34 (1997) 99–101.
[108] P.K.F. Yeung, R. Little, Y. Jiang, S.J. Buckley, P.T. Pollak, H. Kapoor, et al., J. Pharm. Biomed. Anal. 17 (1998) 1393–1398.
[109] P.N.V. Tata, S.L. Bramer, Anal. Lett. 32 (1999) 2285–2295.
[110] L. Ding, J. Yang, H.L. Yan, Z.X. Zhang, D.K. An, Yaowu Fenxi Zazhi 19 (1999) 300–303.
[111] A. Ekpe, T. Jacobsen, Drug Dev. Ind. Pharm. 25 (1999) 1057–1065.
[112] Anonymous, Macherey-Nagel Applicaton Note A-1054, 2000, p. 2.

[113] K.H. Yuen, W.P. Choy, H.Y. Tan, J.W. Wong, S.P. Yap, J. Pharm. Biomed. Anal. 24 (2001) 715–719.
[114] Anonymous, ChromTech. Application Note, 2001, p. 1.
[115] D.S. Yim, J.E. Jeong, J.Y. Park, J. Chromatogr. B. 754 (2001) 487–493.
[116] E.J. Woolf, B.K. Matuszewski, J. Chromatogr. 828 (1998) 229–238.
[117] H. Stenhoff, A. Blomqvist, P.O. Lagerstrom, J. Chromatogr. B. 734 (1999) 191–201.
[118] H. Kanazawa, A. Okada, M. Higaki, H. Yokota, F. Mashige, K. Nakahara, J. Pharm. Biomed. Anal. 30 (2003) 1817–1824.
[119] J. Martens-Lopenhoffer, I. Reiche, U. Troger, K. Monkemuller, P. Malfertheiner, S.M. Bode-Boger, J. Chromatogr. B. 857 (2007) 301–307; Corrigendrum to this article, J. Chromatogr. B., 859(2007) 289.
[120] J. Macek, J. Klima, P. Ptacek, J. Chromatogr. B. 852 (2007) 282–287.
[121] L. Weidolf, T.R. Covey, Rapid Commun. Mass Spectrom. 6 (1992) 192–196.
[122] L. Weidolf, N. Castagnoli Jr., Rapid Commun. Mass Spectrom. 15 (2001) 283–290.
[123] W. Naidong, W.Z. Shou, T. Addison, S. Maleki, X. Jiang, Rapid Commun. Mass Spectrom. 16 (2002) 1965–1975.
[124] H. Kanazawa, A. Okada, Y. Matsushima, H. Yokota, S. Okubo, F. Mashige, et al., J. Chromatogr. 949 (2002) 1–9.
[125] B.P. Jensen, C. Smith, I.D. Wilson, L. Weidolf, Rapid Commun. Mass Spectrom. 18 (2004) 181–183.
[126] A. Tolonen, M. Turpeinen, J. Uusitalo, O. Pelkonen, Eur. J. Pharm. Sci. 25 (2005) 155–162.
[127] J. Wang, Y. Wang, J.P. Fawcett, Y. Wang, J. Gu, J. Pharm. Biomed. Anal. 39 (2005) 631–635.
[128] V.A. Frerichs, C. Zaranek, C.E. Haas, J. Chromatogr. B. 824 (2005) 71–80.
[129] Q. Song, W. Naidong, J. Chromatogr. B 830 (2006) 135–142.
[130] I. Hultman, H. Stenhoff, M. Liljeblad, J. Chromatogr. B 848 (2006) 317–322.
[131] U. Hofmann, M. Schwab, G. Treiber, U. Klotz, J. Chromatogr. B 831 (2006) 85–90.
[132] L. Toribio, C. Alonso, M.J. del Nozal, J.L. Bernal, M.T. Martin, J. Chromatogr. 1137 (2006) 30–35.
[133] G. McGrath, S. McClean, O. O'Kane, W.F. Smyth, F. Tagliaro, J. Chromatogr. 735 (1996) 237–247.
[134] D. Eberle, R.P. Hummel, R. Kuhn, J. Chromatogr. 759 (1997) 185–192.
[135] K.D. Altria, S.M. Bryant, T.A. Hadgett, J. Pharm. Biomed. Anal. 15 (1997) 1091–1101.
[136] P.S. Bonato, F.O. Paias, J. Braz. Chem. Soc. 15 (2004) 318–323.
[137] Y.H. Lin, S.M. Wu, LC.GC Europe 18 (2005) 164–167.
[138] J.J. Berzas Nevado, G. Castaneda Penalvo, R.M. Rodriguez Dorado, Anal. Chim. Acta 533 (2005) 127–133.
[139] J. Olsson, F. Stegander, N. Marlin, H. Wan, L.G. Blomberg, J. Chromatogr. 1129 (2006) 291–295.
[140] T. Perez-Ruiz, C. Martinez-Lozano, A. Sanz, E. Bravo, R. Galera, J. Pharm. Biomed. Anal. 42 (2006) 100–106.
[141] J. Olsson, L.G. Blomberg, J. Chromatogr. B 875 (2008) 329–332.
[142] C.G. Regardh, M. Gabrielsson, J.K. Hoffman, I. Lofberg, I. Skanberg, Scand. J. Gastroenterol. 108 (Suppl.) (1985) 79–94.
[143] C.G. Regardh, Scand. J. Gastroenterol. 118 (Suppl.) (1986) 99–104.
[144] J. Naesdal, T. Andersson, G. Bodemar, R. Larsson, C.G. Regardh, I. Skanberg, et al., Clin. Pharmacol. Ther. 40 (1986) 344–351.
[145] C. Cederberg, T. Andersson, I. Skanberg, Scan. J. Gastroenterol. 166 (Suppl.) (1989) 33–40.
[146] C.G. Regardh, T. Andersson, P.O. Lagerstrom, P. Lundborg, I. Skanberg, Ther. Drug Monit. 12 (1990) 163–172.

[147] T. Andersson, C. Cederberg, C.G. Regardh, I. Skanberg, Eur. J. Clin. Pharmacol. 39 (1990) 195–197.
[148] D.R. Sohn, K. Kobayashi, K. Chiba, K.H. Lee, S.G. Shin, T. Ishizaki, J. Pharmacol. Exp. Ther. 262 (1992) 1195–1202.
[149] S. Landahl, T. Andersson, M. Larsson, B. Lernfeldt, P. Lundborg, C.G. Regardh, et al., Clin. Pharmacokinet. 23 (1992) 469–476.
[150] T. Andersson, R. Olsson, C.G. Regardh, I. Skanberg, Clin. Pharmacokinet. 24 (1993) 71–78.
[151] A. Okada, H. Kanazawa, F. Mashige, S. Okubo, H. Yokota, Anal. Sci. 17 (Suppl.) (2001) i871.
[152] A. Abelo, T.B. Andersson, M. Antonsson, A.K. Naudot, I. Skanberg, L. Weidolf, Drug Metab. Dispos. 28 (2000) 966–972.
[153] J.M. Pique, F. Feu, G. de Prada, K. Rohss, G. Hasselgren, Clin. Pharmacokinet. 41 (2002) 999–1004.
[154] T. Kita, T. Sakaeda, N. Aoyama, T. Sakai, Y. Kawahara, M. Kasuga, et al., Biol. Pharm. Bull. 25 (2002) 923–927.
[155] M.J. Kendall, Aliment Pharmacol. Ther. 17 (Suppl. 1) (2003) 1–4.
[156] R. Kumar, Y.K. Chawla, S.K. Garg, P.K. Dixit, S.K. Satapathy, R.K. Dhiman, et al., Methods Find. Exp. Clin. Pharmacol. 25 (2003) 625–630.
[157] M. Hassan-Alin, T. Andersson, M. Niazi, K. Rohss, Eur. J. Clin. Pharmacol. 60 (2005) 779–784.
[158] H.F. Helander, C.H. Ramsay, C.G. Regardh, Scand. J. Gastroenterol. 108 (Suppl.) (1985) 95–104.
[159] K.J. Hoffmann, L. Renberg, S.G. Olovson, Drug Metab. Dispos. 14 (1986) 336–340.
[160] K.J. Hoffmann, Drug Metab. Dispos. 14 (1986) 341–348.
[161] L. Renberg, R. Simonsson, K.J. Hoffmann, Drug Metab. Dispos. 17 (1989) 69–76.
[162] L. Weidolf, K.E. Karlsson, I. Nilsson, Drug Metab. Dispos. 20 (1992) 262–267.
[163] K. Chiba, K. Kobayashi, K. Manabe, M. Tani, T. Kamataki, T. Ishizaki, J. Pharmacol. Exp. Ther. 266 (1993) 52–59.
[164] L. Zhao, Y.Q. Lou, Acta Pharm. Sin. 30 (1995) 248–253.
[165] U.A. Meyer, Eur. J. Gastroenterol. Hepatol. 1 (Suppl.) (1996) S21–S25.
[166] G. Tyrbing, Y. Bottiger, J. Widen, L. Bertilsson, Clin. Pharmacol. Ther. 62 (1997) 129–137.
[167] A. Tolonen, M. Turpeinen, J. Uusitalo, O. Pelkonen, Eur. J. Pharm. Sci. 25 (2005) 155–162.
[168] V.F. Roche, Am. J. Pharm. Educ. 70 (2006) 1–11.
[169] P. Lindberg, P. Nordberg, T. Alminger, A. Brandstrom, B. Wallmark, J. Med. Chem. 29 (1986) 1327–1329.
[170] I. Puscas, M. Coltau, M. Baican, G. Domuta, J. Pharmacol. Exp. Ther. 290 (1999) 530–534.
[171] R.A. Quercia, C. Fan, X. Liu, M.S. Chow, Am. J. Health Syst. Pharm. 54 (1997) 1833–1836.
[172] J.L. DiGiacinto, K.M. Olsen, K.L. Bergman, E.B. Hoie, Ann. Pharmacother. 34 (2000) 600–605.
[173] M. Palummo, A. Cingolani, L. Dall, M.G. Volonte, Bull. Chim. Farm. 139 (2000) 124–128.
[174] H.G. Choi, C.K. Kim, J. Contr. Rel. 68 (2000) 397–404.
[175] C.S. Yong, J.H. Jung, J.D. Rhee, C.K. Kim, H.G. Choi, Drug Dev. Ind. Pharm. 27 (2001) 447–455.
[176] A. Leitner, P. Zollner, Wein Med. Wochenschr 152 (2002) 568–573.
[177] J. Moschwitzer, G. Achleitner, H. Pomper, R.H. Muller, Eur. J. Pharm. Biopharm. 58 (2004) 615–619.

[178] A. Reidel, C.S. Leopold, Drug Dev. Ind. Pharm. 31 (2005) 151–160.
[179] J.E. Burnett, E.R. Balkin, Am. J. Health Syst. Pharm. 63 (2006) 2240–2247.
[180] M. Espinosa Bosch, A.J. Ruiz Sanchez, F. Sanchez Rojas, C. Bosch Ojeda, J. Pharm. Biomed Anal. 44 (2007) 831–844.