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Journal of Pharmaceutical and Biomedical Analysis
25 (2001) 357–361

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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# Omeprazole determination using HPLC with coulometric detection

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Received 25 April 2000; received in revised form 18 August 2000; accepted 24 August 2000

**Abstract**

A sensitive high performance liquid chromatography (HPLC) method for the determination of omeprazole and three related benzimidazoles is reported. Coulometric detection was carried out at +800 mV using a porous carbon electrode. The linear range is 0.01–10 μg/ml. The method has a high degree of precision; the relative standard deviation 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 +1105 mV. The response factors for the four compounds are similar indicating that the oxidative process does not involve the sulfur moiety exclusively. The data are most consistent with oxidation primarily of the benzimidazole groups. The method was applied successfully to the determination of omeprazole in a paste formulation. 

*Keywords:* Omeprazole; Electrochemistry; Coulometric detection; Cyclic voltammetry; High performance liquid chromatography

## 1. Introduction

Omeprazole is the most significant of the substituted benzimidazole sulfoxides that function as proton pump inhibitors in the treatment of gastric ulcers. Formulated as Prilosec© (or Losec©), it is the active component in the world's top-selling pharmaceutical product [1]. Several HPLC methods employing UV [2–12] or mass spectrometric [13] detection have been reported for the determination of omeprazole and its metabolites in various biological fluids. HPLC employing electrochemical detection has been reported in which bulk omeprazole was detected indirectly via interference with the reduction of residual oxygen in the mobile phase and directly, in thoroughly deoxygenated mobile phases, at −1.2 V [14]. Omeprazole has also been determined by capillary electrophoresis [15], spectrophotometry [16], polarography [17–20], voltammetry [21] and thin-layer chromatography [22–24]. However, these methods lack the sensitivity required for quantitation of omeprazole in biological fluids or its low level impurities and degradates in bulk drug substances and formulated products. HPLC employ-

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0731-7085/01/$ - see front matter 
PII: S0731-7085(00)00519-7

ing coulometric detection is potentially more selective and sensitive than UV detection and may be applicable to determination of low levels of omeprazole and corresponding impurities, degradates and metabolites. We report here a novel HPLC method for direct determination of omeprazole and three related benzimidazoles (Fig. 1) using coulometric detection in the oxidation mode. In addition to being metabolites, the compounds **II** and **III** (Fig. 1) are acid and oxidative degradates, respectively. The method was applied to the determination of omeprazole in a paste formulation.

## 2. Experimental

### 2.1. Instrumentation

The liquid chromatograph consisted of a Shimadzu SCL-10A (Kyoto, Japan) system controller, two Shimadzu LC-10AD pumps, a Shimadzu SIL-10A autoinjector, a MetaTherm (Metachem Technologies, Torrance, CA) column heater, a Shimadzu CR501 integrator, and an ESA (Chelmsford, MA) Coulochem II detector equipped with a model 5020 guard cell (located between the pump and the injection valve) and a model 5010 analytical cell containing a porous graphite electrode. The reference electrode was solid-state palladium. The HPLC column was a Luna (Phenomenex, Torrance, CA) C8 (100 Å, 5 μm, 250 × 4.6 mm i.d.).

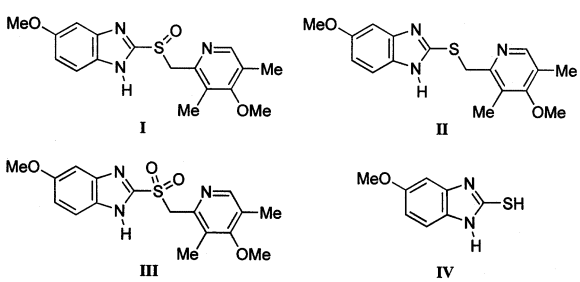


Fig. 1. Structures of omeprazole (**I**) and related sulfide (**II**), sulfone (**III**) and thiolbenzimidazole (**IV**).

### 2.2. Materials

HPLC-grade acetonitrile, tetrahydrofuran (THF), water, sodium phosphate monobasic monohydrate, sodium phosphate dibasic anhydrous, sodium phosphate tribasic dodecahydrate, 85% phosphoric acid, and 50% w/w sodium hydroxide solution were used as received from Fisher Scientific (Fair Lawn, NJ). Omeprazole and related benzimidazoles were obtained from Merck Research Laboratories (Rahway, NJ).

### 2.3. HPLC method

The mobile phase consisted of 36% (v/v) acetonitrile in 0.01 M phosphate buffer (pH 7.6) pumped at 1.0 ml/min. The column was thermostated at 35°C. The injection volume was 20 μl and the run time was 30 min. Coulometric detection was performed at + 800 mV (100 μA full scale) and the guard cell potential was + 900 mV.

### 2.4. Standard solutions

An omeprazole stock standard solution was prepared by weighing ~25 mg of omeprazole reference standard and transferring it to a 250 ml volumetric flask. Approximately 200 ml THF was added to the flask to dissolve the solid material. Dilution to the mark with 35% (v/v) acetonitrile in 0.005 M phosphate buffer (pH 11.5) afforded a 0.1 mg/ml solution. Subsequent dilutions were carried out by pipetting a known volume of stock solution into the appropriate volumetric flask and dilution to volume with the 35% (v/v) acetonitrile in 0.005 M phosphate buffer (pH 11.5) solution. A similar procedure was followed for the preparation of standard solutions of the three related benzimidazoles.

### 2.5. Paste formulation assay

The contents of three syringes were expelled into a beaker and mixed well with a spatula. Approximately 0.4 g of the paste was weighed into a 250 ml volumetric flask and 200 ml THF was added. The mixture was sonicated for ~10 min until the paste was completely dispersed. The

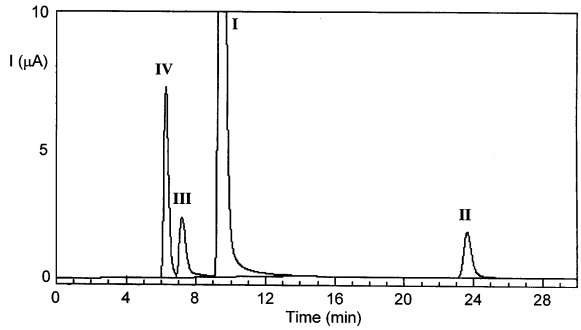


Fig. 2. Representative chromatogram of omeprazole (**I**) and related benzimidazoles (**II**–**IV**).

solution was diluted to the mark with 35% (v/v) acetonitrile in 0.005 M phosphate buffer (pH 11.5). A 2.00 ml aliquot of the stock sample solution was pipetted into a 250 ml volumetric flask and diluted to volume with the 35% (v/v) acetonitrile in phosphate buffer (pH 11.5) to afford an injection solution containing $\sim 4.7$ μg/ml omeprazole. The samples were filtered through a 0.45 μm nylon syringe filter, transferred to HPLC vials and injected in duplicate. A single 5 μg/ml standard solution was used for quantitation.

### 2.6. Hydrodynamic voltammetry

The hydrodynamic voltammogram was recorded in a point-by-point manner by recording the omeprazole peak area, obtained via the HPLC method above, as a function of detector cell potential. The guard cell potential was fixed at + 1100 mV.

### 2.7. Cyclic voltammetry

The Coulochem II detector was equipped with a model 5040 analytical cell containing a glassy carbon or a gold disk electrode and a 1 mg/ml solution of omeprazole in 36% (v/v) acetonitrile in 0.01 M phosphate buffer (pH 7.6). The scan rate was 10 mV/s over the range + 200 to + 1400 mV. The output was recorded on a Hewlett-Packard 7040A X-Y recorder.

## 3. Results and discussion

It is well established that omeprazole is stable at high pH but degrades under acidic conditions to afford the corresponding sulfide and other products [17,25–28]. Thus, it is necessary to use slightly basic mobile phases in HPLC methods for its determination. The use of an aqueous acetonitrile mobile phase adjusted to pH 7.6 with phosphate buffer in conjunction with a C8 Luna column afforded sufficient stability and resolution of omeprazole and the three related benzimidazoles (Fig. 1) while allowing an acceptable column life. The sample diluent was at a higher pH (11.5) to maximize the stability of the samples prior to and during assay. A representative chromatogram is shown in Fig. 2.

Omeprazole and related benzimidazoles have been detected electrochemically at negative potentials [14,17–21,29]; however, the methods are complicated by the reduction of residual dissolved oxygen at potentials more negative than − 400 mV. In basic aqueous solutions the reduction of oxygen proceeds via reactions 1 and 2 leading ultimately to the formation of hydroxide ion.

$$O_2 + 2e^- + H_2O \rightarrow HO_2^- + OH^- \quad (1)$$

$$HO_2^- + 2e^- + H_2O \rightarrow 3OH^- \quad (2)$$

Persson and Wendsjö took advantage of this apparent complication by using the oxygen reduction as an indirect probe for the determination of omeprazole [14]. The use of positive potentials for coulometric detection allows the direct detection of analytes without the requirement of rigorous deoxygenation of the mobile phase. A hydrodynamic voltammogram was generated to assess the optimum potential for oxidative coulometric detection of omeprazole (Fig. 3). Using a potential in the plateau region ( + 1100 mV) would produce the maximum instrument response, but + 800 mV was selected as a compromise between sensitivity, background noise and cell life.

The HPLC method is sensitive, linear, precise, and robust. The limit of quantitation (LOQ), the injection solution concentration at which the signal-to-noise ratio is $\sim 10$, for omeprazole is 6 ng/ml (0.12 ng on column). The LOQ's for com-

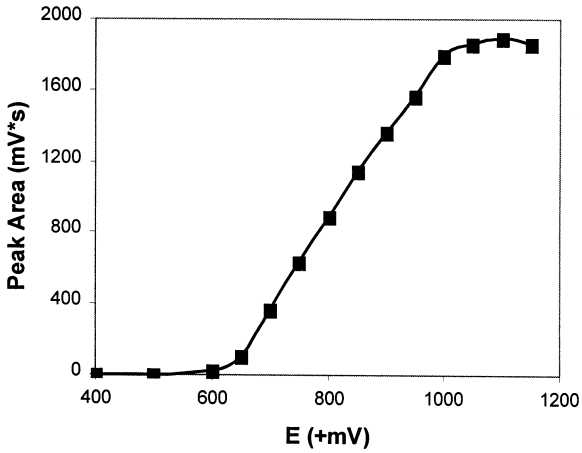


Fig. 3. Hydrodynamic voltammogram for omeprazole.

Table 1
Summary of precision data for omeprazole determination ($n = 4$)

| Omeprazole conc. (μg/ml) | Mean peak area (μV*s) | %RSD |
|---|---|---|
| 10.56 | 4 729 585 | 0.92 |
| 5.28 | 2 517 320 | 0.48 |
| 1.06 | 537 458 | 0.68 |
| 0.528 | 266 188 | 0.06 |
| 0.106 | 52 152 | 0.92 |
| 0.053 | 26 130 | 0.73 |
| 0.011 | 4416 | 4.66 |

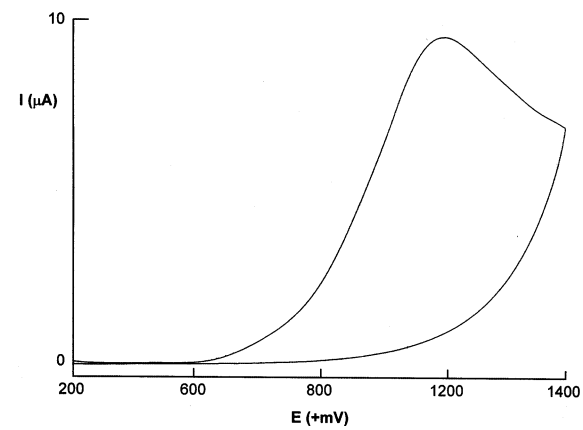


Fig. 4. Cyclic voltammogram for omeprazole.

pounds **II**, **III** and **IV** (Fig. 1) are 30, 11 and 4 ng/ml, respectively. The coulometric response of omeprazole is linear between 0.01 and 10 μg/ml while the responses of the three related benzimidazoles are linear in the range 0.02–2 μg/ml. In each case the correlation coefficient $r^2$ was $>$ 0.999. Downward curvature of the plots occurs at higher concentrations due to overload of the electrode despite its large surface area. The method shows excellent precision with injection RSD's below 1% between 0.05 and 10 μg/ml (see Table 1). No loss in signal response attributable to electrode fouling was observed after numerous injections.

The relative response factors (RRF) for the benzimidazoles were determined from the slopes of the peak area versus concentration plots. After correction for molecular weights, compounds **II, III** and **IV** (Fig. 1) have very similar response factors relative to omeprazole (RRF = 0.75, 0.69, 0.78, respectively) suggesting that they all undergo similar oxidation pathways in the electrode. The possible sites of oxidation include the sulfur moiety, the benzimidazole group, and/or the substituted pyridine ring. The fact that the benzimidazolethiol has a similar coulometric response to the other three compounds indicates that oxidation does not occur mainly on the pyridine ring. The data indicate that oxidation does not occur primarily at the sulfur moiety either since the oxidation of the sulfone **III** occurs about as readily as the other three compounds. If oxidation were localized on the sulfur groups, the response factors would be expected to decrease in order **III** $\sim$ **IV** $>$ **I** $>$ **III** (Fig. 1). Thus, it is likely that oxidation occurs mainly on the benzimidazole but the relative response factors are insensitive to the relative inductive ($\sigma_I$) and resonance ($\sigma_R$) effects of the sulfur groups [30]. However, further investigations are necessary to confirm the proposed pathway.

The cyclic voltammogram recorded for omeprazole (Fig. 4) using a glassy carbon electrode shows an oxidative wave with a peak potential at + 1105 mV in good agreement with the peak potential obtained from the hydrodynamic voltammogram. A similar cyclic voltammogram was obtained under the same conditions using a gold disk electrode ($E_p = +1125$ mV). These

Table 2
Omeprazole determination in a 37.1% w/w paste formulation

| Sample | Omeprazole %w/w | % Label claim |
|---|---|---|
| 1 | 38.0 | 102.3% |
| 2 | 36.3 | 98.0% |
| 3 | 37.9 | 102.1% |
| 4 | 37.5 | 101.0% |
| 5 | 37.1 | 100.1% |
| Average | 37.4 | 100.7% |
| RSD | 1.8% | |

voltammograms are typical of irreversible oxidations. It is likely that the radical cation generated from oxidation of omeprazole at the electrode undergoes rapid decomposition (e.g. rearrangement [17,26,27,29], fragmentation, or addition of water [31]) before it can be reduced back to neutral.

The results from the determination of omeprazole in a 37% w/w paste formulation are listed in Table 2. The placebo did not present any interferences with omeprazole or the other benzimidazoles. The method is precise (RSD = 1.8%, $n = 5$) and accurate (avg. assay 100.7% of label claim).

## 4. Conclusions

A novel, sensitive analytical method for determination of omeprazole has been developed. The LOQ for omeprazole (6 ng/ml, 0.12 ng) is lower than the most sensitive HPLC method reported by Gangadhar and coworkers [9] (LOQ = 0.25 ng). The method is applicable to an omeprazole paste formulation and is likely suitable for omeprazole determination in other formulations and biological fluids.

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