# Analysis of Omeprazole and Lansoprazole in Capsules by Capillary Zone Electrophoresis

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**A simple capillary zone electrophoresis method is discussed for the simultaneous analysis of omeprazole and lansoprazole.**



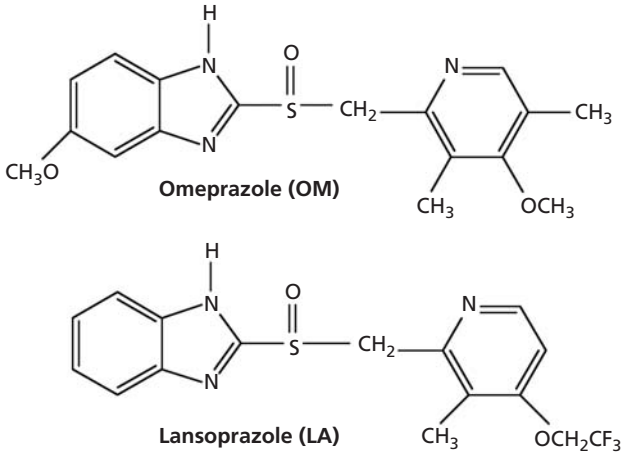
A simple capillary zone electrophoresis method (CZE) was established for simultaneous analysis of omeprazole (OM) and lansoprazole (LA). Untreated fused-silica capillary was operated using a phosphate buffer (50 mM, pH 9.0) under 20 kV and detection at 200 nm. Baseline separation was attained within 6 min. In method validation, calibration curves were linear over a concentration range of 5 to 100 µM, with correlation coefficients 0.9990. Relative standard deviation (RSD) and relative error (RE) were all less than 5% for the intra-day and inter-day analysis, and all recoveries were greater than 95%. The limits of detection for both omeprazole and lansoprazole were 2.0 µM (S/N = 3, hydrodynamic injection 5 s). This method was applied to determine the quality of commercial capsules. Assay results fell within 94–106%.

OM and LA (Figure 1) are substituted benzimidazole sulphoxides that inhibit gastric acid secretion by interacting with ($H^+/K^+$)-ATPase, which is a gastric proton pump in the parietal cell. They are used in the treatment of acid-related disorders such as gastric ulcer, duodenal ulcer and reflux oesophagitis, and are also effective in controlling acidity in Zollinger-Ellison syndrome patients who do not satisfactorily respond to histamine $H_2$-receptor antagonist.[1]

In 2003, the FDA approved OM, one of the top-selling pharmaceuticals all over the world, to be an over-the-counter drug.[2] It is necessary to establish a selective method for quality control of OM and LA in pharmaceuticals.

Some methods, including high performance liquid chromatography (HPLC),[2–12] spectrophotometry,[13–14,22] polarography,[15–16] voltammetry,[17] and capillary electrophoresis (CE),[18–20] have been reported, but only the polarographic method mentioned above provides a simultaneous analysis of both OM and LA.[16] USP XXV refers only to the HPLC method for OM analysis,[21] not its pharmaceutical form and makes no mention at all about LA. CE is now firmly established as a viable option for the analysis of pharmaceuticals. In reviewing the CE methods, Eberle et al. used bovine serum albumin for their chiral resolution,[18] Tivesten et al. tried non-aqueous CE using *N*-methylformamide,[19] and Altria et al. applied borate buffer for the assay of OM.[20] In this study, a simple CZE method using a phosphate buffer was developed for the simultaneous determination of both OM and LA. Optimization of parameters and validation of this method were investigated. Application of determining quantities of OM and LA in capsules was also demonstrated.

**Figure 1:** Structures of omeprazole and lansoprazole.



## Materials and Methods

**Materials:** All chemicals used were of analytical grade. OM, LA (Sigma, St. Louis, Missouri, USA), $Na_2HPO_4$, methanol (Merck, Darmstadt, Germany), and 4-aminopyridine (Acros, New Jersey, USA) as internal standard (IS), were used without further treatment. Milli-Q water (Millipore, Bedford, Massachusetts, USA) was used for the preparation of buffer and related aqueous solutions. Losec® capsules (20 mg of OM/cap) (AstraZeneca AB, Sweden) and Takepron® capsules (30 mg of LA/cap) (Sato Yakuhin Kogyo Co., Japan) were used for the applications.

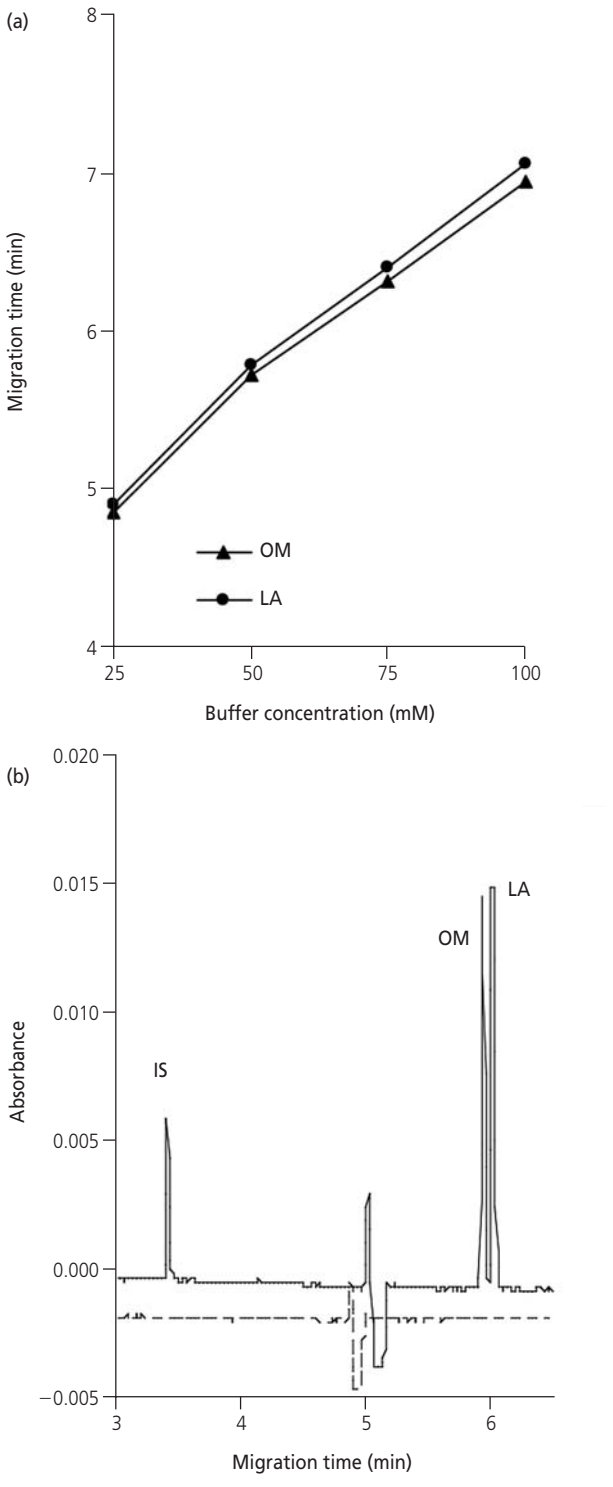
**CE system:** A Beckman P/ACE System 2200 (Fullerton, California, USA) equipped with a filter UV detector and a liquid-cooling device was used. CZE was performed in an uncoated fused-silica capillary (Polymicro Technologies, Phoenix, Arizona, USA) of 50 µm i.d. and 50 cm effective length (total length = 57 cm) and detected at 200 nm. Samples were loaded by pressure injection (50 mbar for 5 s) and separated using phosphate buffer (50 mM, pH 9.0). Before start-up, the capillary was preconditioned with water for 10 min, 0.1 N HCl for 10 min, water for 5 min, 0.1 N NaOH solution and water for 10 min, and with running buffer for 5 min in regular sequences. Between runs, the capillary was rinsed with running buffer for 5 min. Electrophoresis was performed at 25 °C and 20 kV. The current gradually increased to about 65 µA during the first 15 s after voltage application. All operations and electropherograms were computer-controlled using GOLD version software.

**Reference and sample solutions**: Stock solutions of OM and LA of 1 mM were prepared in methanol/$H_2O$ (1:1, v/v) and suitably diluted as reference solutions. Sample solutions were prepared as follows: 10 capsules of Losec® and Takepron® were weighed, respectively. Both of the accurately weighed amounts of granule were transferred to a 10 mL volumetric flask and dissolved in methanol/water (1:1, v/v) for 10 min with the aid of sonication. An aliquot of the resulting extract was centrifuged at 1000 g for 10 min. The supernatant was transferred, diluted and added to the IS, then subjected to CZE analysis.

## Results and Discussion

**Concentrations and pHs of phosphate buffer:** Effects of phosphate buffer concentrations on the migration of OM and LA are shown in Figure 2(a). CE separation of the analytes in phosphate buffer (pH 9.0) in the concentration range 50–100 mM can reach baseline resolution. As a result of the current generated, the problem of heat generation/dissipation must be taken into consideration. When the concentration of phosphate buffer is greater than 100 mM, the current is higher than 100 µA. To prevent the generation of too much Joule heat and with regard to the separation time, 50 mM of phosphate buffer was selected. Figure 2(b) is the typical electropherogram of OM and LA with IS. Both analytes possess the same charges; OM has smaller molecular weight and migrates earlier than LA. We also compared resolutions

**Figure 2:** (a) Effects of phosphate buffer concentration on the migration of OM and LA, each at 100 µM. (b) Typical electropherogram of OM and LA (solid line), and blank (dotted line) in 50 mM phosphate buffer. Other CE conditions: phosphate buffer (pH 9); applied voltage, 20 kV; uncoated fused-silica capillary, 50 cm (effective length) × 50 µm i.d.; sample size, 5 s by pressure; wavelength, 200 nm.

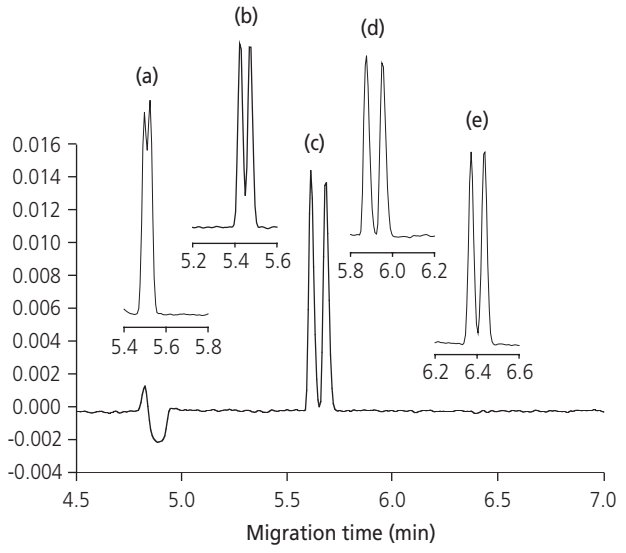


between phosphate, Tris and borate buffers, and found the best resolution using phosphate buffer (data not shown). Meanwhile, borate buffer could reach baseline separation only after adding sodium dodecyl sulphate.

OM and LA have pKa values of 3–4 (pyridinium) and 8–9 (benzimidazole).[13,18,19] Thus, to obtain separation selectivity, buffer with a high or low pH is required. It has also been reported that the analytes are not stable at a pH of less than [5,7,19,22], which was confirmed in our laboratory. We found that when the buffer pH is lower than 7, there will be many tiny peaks in the electropherograms, which might be degraded products of OM and LA (data not shown). Therefore, phosphate buffers (50 mM) with higher pHs (8.0, 8.5, 9.0, 9.5 and 10.0) were studied, as shown in Figure 3. The results indicate that baseline resolution was achieved when pH ⩾ 9.0. To shorten the separation time, the pH was set at pH 9.0. The stability of analytes in this condition was examined for 12 h and did not show any degraded products.

**Analytical voltage:** Both electroosmotic and electrophoretic velocities are directly proportional to field strength,[23] so using higher voltage results in the shorter separation time. It also yields the higher efficiencies because diffusion is the most important feature contributing to band broadening. The limiting factor here is Joule heat and current generated. Five different voltages (10, 15, 20, 25 and 30 kV) were studied. The optimum voltage was set at 20 kV, which affords the shortest migration time and acceptable current.

**Figure 3:** Electropherograms of buffer pH on the migration of OM and LA, each at 100 µM: (a) pH 8.0, (b) pH 8.5, (c) pH 9.0, (d) pH 9.5 and (e) pH 10.0. See Figure 2 for other CE conditions. The migration order is OM followed by LA.




**Method validation:** To evaluate the quantitative applicability of this method, five different concentrations of OM and LA (5–100 µM) were analysed using 4-aminopyridine (200 µM) as an IS. Linearity between the normalized peak-area ratios (Y) of the related analyte to the IS and the concentration ($X$, µM) of analyte was investigated. The regression equations of intra- and inter-day analysis were calculated from the assay values of prepared standards triplicates on a single day (n = 3) and on 5 consecutive days (n = 5). As shown in Table 1, the results of the linear regression equations indicate that high linearity ($r \geqslant 0.999$) between $Y$ and $X$ was attained over the range studied. At wavelength 200 nm, detection limits (S/N = 3, injection 5 s) and quantification limit (S/N = 9, injection 5 s) were 2 µM and 5 µM, respectively, for each analyte. For greater precision and accuracy in evaluation, the RSD and RE of the method, based on statistical determination (n = 3) of each analyte at 10, 40 and 70 µM, were studied. The results are shown in Table 2. All RSDs and REs were less than 5.0%. Recoveries of extraction from the capsules using three spiked levels (20, 40, 60 µM) were also studied and shown as Table 3. They were greater than 94%.

**Applications:** Application of the method to the assay of OM and LA in capsules was studied. The results of percentage of claimed content were 94.95–100.62% for Losec® and 101.40–105.01% for Takepron®, as shown in Table 4. All of the analytical values fell within the labelled amount of 90–110%.

## Conclusion

A simple and selective CZE method has been established for the assay of OM and LA in capsules. Compared with the HPLC method for OM reported in USP XXV[21] and the polarographic method,[16] our CE method provides a more efficient assay for quality control of OM and LA in capsules.

## Acknowledgement

We gratefully acknowledge the support of the National Science Council of Taiwan (NSC93-2113-M-037-016) in funding this work.

**Table 1:** Regression analysis for the determination of OM and LA.

| Range (5–100 µM) | Regression equation | Coefficient of correlation (r) |
|---|---|---|
| **Intra-day*** | | |
| OM | Y = (0.0164 ± 0.0001)X + (0.0302 ± 0.0131) | 0.9990 |
| LA | Y = (0.0162 ± 0.0002)X + (0.0112 ± 0.0176) | 0.9995 |
| **Inter-day*** | | |
| OM | Y = (0.0162 ± 0.0005)X + (0.0174 ± 0.0171) | 0.9993 |
| LA | Y = (0.0160 ± 0.0007)X + (0.0062 ± 0.0207) | 0.9993 |

*The regression equations of intra-day analyses were calculated from the assay values of prepared standards on a single day (n = 3), and those of inter-day analyses were calculated from the assay values of prepared standards on five different days (n = 5).

**Table 2:** Precision and accuracy for the determination of OM and LA.

| Concentration known (μM) | | Concentration found (μM) | RSD (%) | RE[a] (%) |
|---|---|---|---|---|
| **Intra-day analysis (n = 3)** | | | | |
| OM | 10 | 9.67 ± 0.32 | 3.34 | −3.23 |
| | 40 | 40.24 ± 1.10 | 2.74 | 0.59 |
| | 70 | 72.18 ± 2.08 | 2.88 | 3.12 |
| LA | 10 | 10.01 ± 0.22 | 2.25 | 0.12 |
| | 40 | 39.64 ± 1.50 | 3.79 | −0.90 |
| | 70 | 71.78 ± 1.74 | 2.43 | 2.54 |
| **Inter-day analysis (n = 5)** | | | | |
| OM | 10 | 9.86 ± 0.39 | 3.98 | −1.43 |
| | 40 | 40.46 ± 1.39 | 3.43 | 1.16 |
| | 70 | 70.48 ± 2.00 | 2.84 | 0.68 |
| LA | 10 | 10.04 ± 0.49 | 4.86 | 0.39 |
| | 40 | 39.99 ± 1.38 | 3.46 | −0.01 |
| | 70 | 71.00 ± 1.85 | 2.61 | 1.43 |

[a]RE (% relative error) = (concentration found – concentration known) × 100/ (concentration known)

**Table 3:** Recoveries of OM and LA added to the commercial formulation

| Concentration spiked (μM) | Concentration[a] found (μM) | Recovery (%) |
|---|---|---|
| **Omeprazole** | | |
| – | 20.68 ± 1.32 | – |
| 20 | 40.74 ± 0.53 | 100.33 |
| 40 | 60.71 ± 0.42 | 100.08 |
| 60 | 78.11 ± 2.82 | 95.71 |
| **Lansoprazole** | | |
| – | 19.31±0.53 | – |
| 20 | 38.14 ± 1.30 | 94.13 |
| 40 | 57.98 ± 1.05 | 96.67 |
| 60 | 78.81 ± 1.44 | 99.16 |

[a]Mean ± SD (n = 3)

**Table 4:** Assay results of OM and LA in capsules obtained from commercial sources.

| Sample | Amount found[c] (mg) | Percentage of claimed content (%) |
|---|---|---|
| **OM[a]** | | |
| 1 | 19.00 ± 1.38 | 94.95 |
| 2 | 19.97 ± 0.24 | 99.83 |
| 3 | 20.13 ± 0.85 | 100.62 |
| 4 | 19.05 ± 0.55 | 95.25 |
| | Mean | 97.66 |
| | S.D. | 2.58 |
| **LA[b]** | | |
| 1 | 30.42 ± 0.46 | 101.40 |
| 2 | 31.53 ± 0.47 | 105.01 |
| 3 | 31.11 ± 0.77 | 103.69 |
| 4 | 30.80 ± 0.60 | 102.67 |
| | Mean | 103.21 |
| | S.D. | 1.35 |

[a]Labelled amount of OM in each capsule is 20 mg.
[b]Labelled amount of LA in each capsule is 30 mg.
[c]Mean ± SD (n = 3)

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