High Sensitivity Concurrent Determination of Omeprazole and its Two Metabolites in Human Plasma by Column Switching High Performance Liquid Chromatography

Omeprazole is a proton pump inhibitor (PPI) that acts on the gastric mucosa to suppress gastric acid secretion and is being used in the treatment of various gastrointestinal diseases, including gastric and duodenal ulcers. Omeprazole is metabolized in the liver and two main detectable metabolites are omeprazole sulfone and 5-hydroxyomeprazole. At present, concurrent determination of omeprazole and its metabolites is performed in studies of Helicobacter pylori, which is thought to contribute to the onset of gastric and duodenal ulcers. Here, using a TSKgel ODS-100Z, 5µm column, the plasma levels of omeprazole and its metabolites were concurrently determined.

Related article: J Chromatogr B, 832: 241-8, 2006.

Table 1 shows the preprocessing methods for plasma samples. By alkalizing plasma, substances are stabilized and single extraction is performed using an organic solvent. Table 2 summarizes the HPLC conditions for the present study. Figure 1a shows a chromatogram for the standard sample, while Figure 1b shows a chromatogram obtained at four hours after administering 40mg of omeprazole to a healthy individual. Using a TSKgel ODS-100Z, 5µm column, the chromatograms revealed favorable selectivity without tailing.

Figure 1a. Chromatogram for standard sample

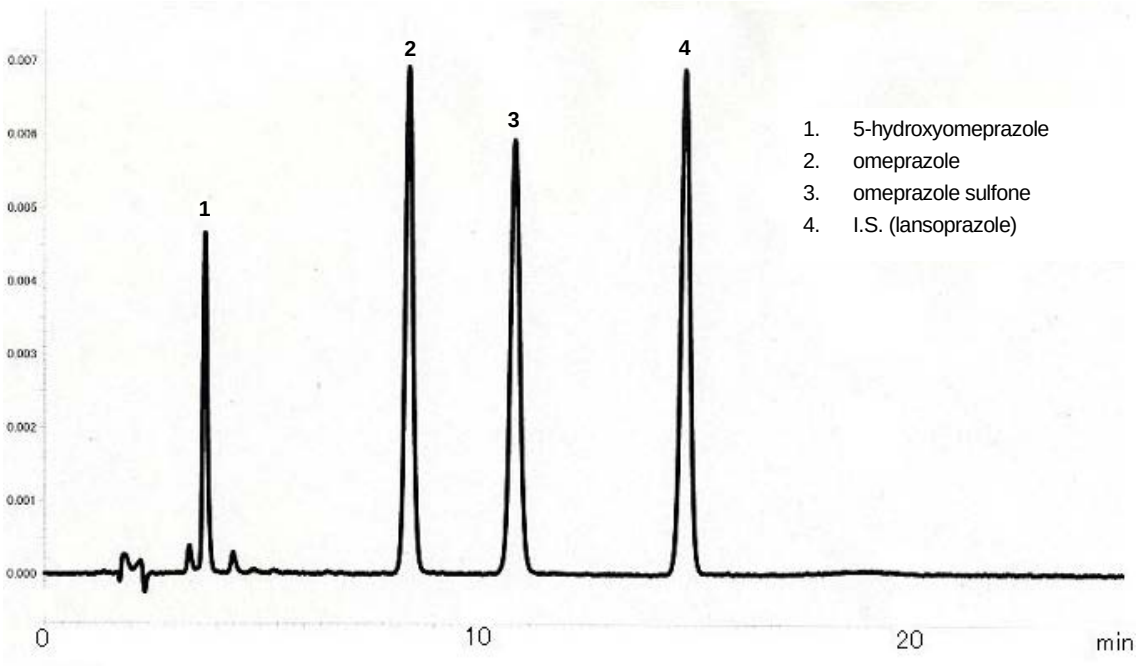


Figure 1b. Chromatogram obtained four hours after omeprazole administration

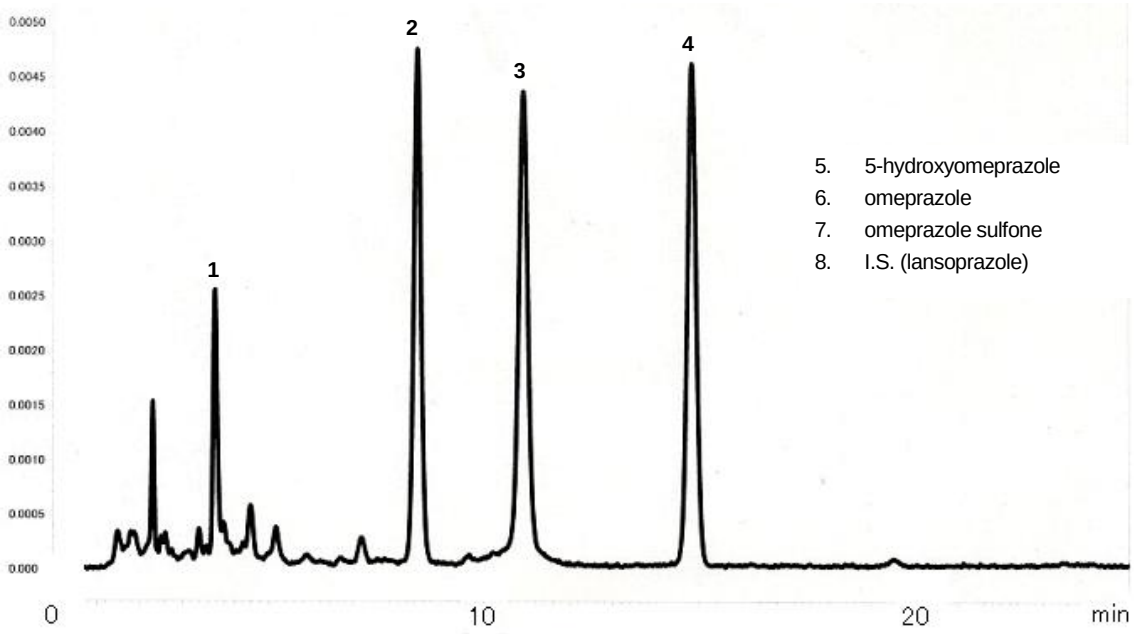


Table 1 Sample preprocessing methods

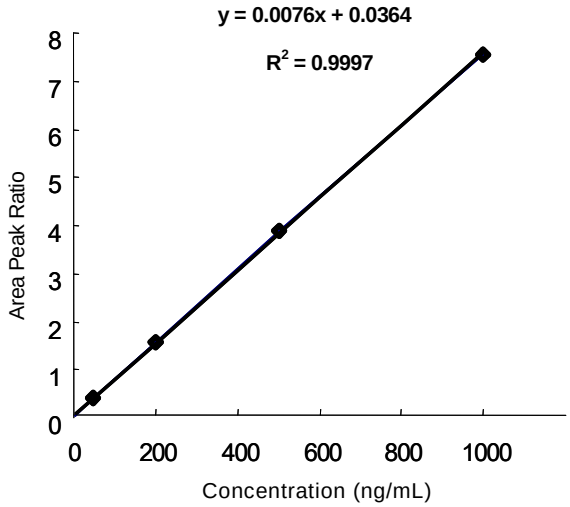
1.	Place 1mL of plasma sample in sterile 10mL tube.
2.	Add 20μL of internal standard substance, lansoprazole (concentration: 100ng/mL), and stir.
3.	Add 100μL of pH 9 phosphate buffer solution to alkalize plasma.
4.	Add 4mL of diethyl ether-dichloroethane mixture (60:40, v/v), stir and centrifuge at 3500rpm for 10 minutes.
5.	Collect supernatant of organic solvent phase and evaporate to dryness at 50°C.
6.	Dissolve again using methanol/mobile phase mixture to prepare sample.

Table 2. Analysis methods

Column:	TSKgel ODS-100Z, 5μm, 4.6mm ID × 15cm
Mobile phase:	pH 6.0 phosphate buffer/acetonitrile/methanol=65/30/5
Flow rate:	0-16 min; 1.0mL/min, 17-25 min; 1.5mL/min
Detection:	UV@302nm
Temperature:	40°C
Injection vol.:	10μL

Figure 2 shows a calibration curve produced after adding human plasma to omeprazole. The calibration curve exhibited favorable linearity with a correlation coefficient of ≥0.999 at a concentration range of 50-1000ng/mL, and the results were the same for the metabolites.

Figure 2. Omeprazole calibration curve



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