PHARMACOKINETIC PROFILE AND INCURRED ESOMEPRAZOLE SAMPLE STABILITY IN PLASMA USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY - PHOTODIODE ARRAY

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ABSTRACT

Objective: Esomeprazole (ESO) is one of the proton-pump inhibitors and is used to treat gastroesophageal reflux disease [1,2]. ESO as PPI inhibits hydrogen-potassium adenosine triphosphatase in gastric parietal cells and thus blocks gastric acid secretion [1,2]. ESO is the first single optical isomer PPI, derived from omeprazole, which provides better acid control than other racemic PPI and has favorable pharmacokinetic profile compared to omeprazole [3].

Methods: Samples were analyzed using high-performance liquid chromatography with a C18 column with detection at 300 nm using a photodiode array detector. Lansoprazole was used as an internal standard.

Results: The ESO pharmacokinetics profile in the plasma samples yielded the values of Cmax, 704.57–1425.85 ng/mL; tmax is 2.25 h; and AUC0-∞ is 2444 ng.h/mL. ISS testing of plasma samples values were 6.50%, 5.73%, and 4.57% on first Cmax concentration; 3.55%, 4.84%, and 3.68% on 2nd Cmax concentration; and 4.04%, 4.80%, and 4.98% on elimination phase concentration.

Conclusion: ISS testing results of plasma samples from six healthy subjects who were administered doses of 40 mg of ESO stored for 28 days showed that it fulfilled the acceptance criteria (<20%) of the 2011 EMEA Bioanalytical Guidelines with a %diff value in all incurred samples of 6.5%.

Keywords: Esomeprazole, Lansoprazole, Plasma, Incurred sample, High-performance liquid chromatography, Photodiode array.

INTRODUCTION

Esomeprazole (ESO) is a proton-pump inhibitor (PPI) that is suggested for the reduction of symptoms in patients with gastroesophageal reflux disease [1,2]. ESO as PPI inhibits hydrogen-potassium adenosine triphosphatase in gastric parietal cells and thus blocks gastric acid secretion [1,2]. ESO is the first single optical isomer PPI, derived from omeprazole, which provides better acid control than other racemic PPI and has favorable pharmacokinetic profile compared to omeprazole [3].

Method validation includes a long-term stability parameter; however, long-term in vitro stability tests do not represent the in vivo stability of a drug compound. Therefore, incurred sample stability (ISS) testing is required for clinical samples containing the analyte, which involves a reanalysis of actual clinical samples over a period of time for determining whether the analyte is stable and whether the analytical concentration is reproducible [4-6].

ESO has high sensitivity to heat and acidic medium [7,8]; therefore, it is formulated in delayed-release tablets and capsules for oral administration [8]. The issue of ESO stability should be a concern because it is sensitive to acidic pH, heat, and moisture and is also easily oxidized [7,9,10], all of which leads to poor long-term storage results on samples [7].

ESO is a highly variable drug (HVD), with coefficient of variation (CV)% of the pharmacokinetic parameters > 50% [11]. The bioequivalence study regarding HVDs is schematically recommended using about 30 subjects to meet the requirements of the European Medicines Agency (EMA) and Food and Drug Administration [12]. Therefore, a longer time span of bioequivalence study is needed, and the storage time for samples is increased as well.

The Global Contract Research Organizations (CROs) Council for Bioanalysis recommends that ISS tests should not be routinely conducted but rather performed on a case-by-case basis when certain analytical stability issues are suspected in incurred samples [13]. Due to the known instability of ESO, an ISS analysis was performed using plasma samples in this study to be used in future bioequivalence tests.

MATERIALS AND METHODS

Materials

Chemicals and reagents

Nexium® 40 mg tablet was purchased from PT AstraZeneca Indonesia (Jakarta, Indonesia). ESO magnesium trihydrate was purchased from Dr. Reddy’s Laboratories Ltd. (Hyderabad, India); lansoprazole, which was used as an internal standard, was purchased from Sigma-Aldrich Pvt. Ltd. (Singapore). The chromatography mobile phases contained chromatographic grade methanol, sodium dihydrogen phosphate, disodium hydrogen phosphate, and acetonitrile, which were purchased from Merck KgaA (Darmstadt, Germany). Reagents such as dichloromethane, o-phosphoric acid, and sodium hydroxide were obtained from Merck KgaA (Darmstadt, Germany). Aquabidest was obtained from PT Ikapharmindo Putramas (Jakarta, Indonesia).

Calibration standards and quality controls (QC)

Stocksolutions of ESO and lansoprazole were prepared at concentrations of 1.0 mg/mL in methanol. Calibration curves were prepared by spiking with an appropriate volume of methanol for producing various concentrations of 5, 25, 70, 200, 500, 800, 1200, and 1500 ng/mL. QC samples were prepared at low middle, and high ESO concentrations of 15, 725, and 1125 ng/mL, respectively.
Methods

Verification and validation

This study validated a method using high-performance liquid chromatography (HPLC) with a photodiode array detector set at a wavelength of 300 nm. Separation was conducted on a C18 column (Waters, Sunfire™ 5 μm; 250 mm x 4.6 mm). The analysis is used an isocratic separation with acetonitrile-phosphate buffer pH 7.6 (40:60% v/v), a column temperature of 40°C, and a flow rate of 1.00 mL/min for 10 min. The method had been previously optimized and fully validated in this laboratory [14].

Verification and partial validation were performed on the method. System suitability tests were conducted using a solution containing ESO magnesium trihydrate 50 μg/mL and lansoprazole 50 μg/mL. 20 μL of the solution was injected onto the column, and the retention time, peak area, n value, and tailing factor were determined. Precision (CV %) was determined from six repeat injections. Partial validation comprised intra-run accuracy, precision, recovery, and the linearity of the calibration curve and was determined using the criteria from the Bioanalytical guidelines (2011).

Sampling

The test articles used were plasma samples obtained from six selected healthy subjects who had been administered 40 mg of ESO magnesium (Nexium®). This study was approved by the Medical Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia (0036/UN2.F1/ETIK/2018), and the subjects signed an informed consent form before participation. Blood samples were collected 12 times from 6 healthy subjects 30 min before drug administration (pre-dose) and 0.5, 1, 1.5, 2.5, 3, 4, 6, 8, and 10 h following the administration of 40 mg of ESO magnesium. Blood was collected by a trained phlebotomist using Venipuncture Technique and collected in 5 mL anticoagulant tubes. The blood collected then was centrifuged to extract the plasma, using 11 Rcf for 20 min. The plasma obtained was then transferred to a new container.

ESO samples were prepared from the plasma using liquid–liquid extraction. A 500 μL aliquot of plasma was placed in a sample tube, and 25 μL of 50 μg/mL lansoprazole was added. The samples were vortexed for 10 s and 5 mL of dichloromethane added before shaking on a vortex for 3 min. The sample was then centrifuged at 1149 Rcf for 15 min, and 4 mL of the supernatant was transferred to a new container.

The supernatant is then evaporated under a stream of nitrogen gas at 40°C and the residue dissolved in chromatography buffer and shaken by vortex for 2 min. After 30 s, 20 μL was analyzed using HPLC.

Pharmacokinetic analysis was performed by calculating the mean $C_{max}$, $t_{max}$, AUC0–τ, and AUC0–∞ of the subjects.

ISS testing was performed on subjects’ plasma stored at −80°C on days 7, 14, and 28 after collection, and samples were processed and analyzed as described previously. ISS was analyzed at two concentrations in the $C_{max}$ phase and one concentration in the elimination phase for each subject.

RESULTS AND DISCUSSION

System suitability

System suitability tests were conducted for determining the reproducibility and suitability of the selected methods. CV% passed the required criteria (CV ≤2%) and the results are presented in Table 1 with a representative chromatogram in Fig. 1.

Calibration curve linearity

Linearity was r=0.99 and accuracy was (% diff) ±20% for the lower limit of quantitation (LLOQ) and 5±15% for other concentrations. The linear equation for the calibration curve was y=0.0018x+0.0017, with x being ESO magnesium concentration (μg/mL) and y being the peak area (μV/s)
area ratio between ESO and the lansoprazole internal standard. The calibration curve met the accuracy requirements with %diff ≤ ±20% for LLOQ and ≤ ±15% for other concentrations. The results are presented in Table 2 and the calibration curve is shown in Fig. 2.

Accuracy, precision, and recovery
Accuracy is a measure of how close the determined concentration of the analyte is to the actual concentration in the sample, which is described by the parameter %diff. Precision is the relative similarity of repeated measurements, which is described by the coefficient of variation (CV%). For determining the values of these parameters, plasma ESO was analyzed at several concentrations, i.e., LLOQ, QC low, QC medium, and QC high, with five replicates at each concentration. Accuracy and precision requirements were ±15% for %diff and CV% in QC samples and ±20% for LLOQ samples. The recovery test was performed by comparing peak areas between extracted and unextracted samples. There were no defined requirements regarding recovery as long as the results were precise and reproducible. The accuracy and precision results are presented in Table 3 and the recovery results are shown in Table 4.

Pharmacokinetic profiles of subjects’ plasma
ESO concentrations were plotted to produce a pharmacokinetic profile for each subject to determine their pharmacokinetic parameters, namely, the maximum concentration in plasma (C_{max}), the maximum time (t_{max}), t_{1/2}, AUC_{0-\infty} and AUC_{0-t}. The values of the determined pharmacokinetic parameters for each subject are presented in Table 5, with graphs as plotted in Fig. 3.
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Table 3: Intraday accuracy and precision

| Concentration (ng/mL) | ESO | IS | PAR | Measurement concentration (ng/mL) | Mean (ng/mL)±SD | CV (%) | %diff |
|-----------------------|-----|----|-----|----------------------------------|----------------|--------|-------|
| LLOQ                  | 3094| 237940| 0.0130 | 4.89 | 4.88±0.07 | 1.37 | -2.30 |
| 5.00                  | 3290| 253562 | 0.0130 | 4.85 | 4.85±0.07 | 1.29 | -2.92 |
| 7.00                  | 3065| 238765 | 0.0128 | 4.73 | 4.73±0.07 | 1.28 | -5.42 |
| 10.00                 | 3078| 236743 | 0.0130 | 4.88 | 4.88±0.07 | 1.33 | -2.33 |
| 11.00                 | 3109| 240726 | 0.0129 | 4.80 | 4.80±0.07 | 1.31 | -3.95 |
| QCL                   | 5343| 230798 | 0.0232 | 14.41 | 14.41±0.40 | 2.71 | -3.95 |
| 15.00                 | 5370| 226480 | 0.0237 | 14.93 | 14.93±0.40 | 2.71 | -0.44 |
| 17.50                 | 5678| 233749 | 0.0243 | 15.48 | 15.48±0.40 | 2.71 | -3.19 |
| 20.00                 | 5340| 225369 | 0.0237 | 14.92 | 14.92±0.40 | 2.71 | -0.54 |
| QCM                   | 5374| 229821 | 0.0234 | 14.63 | 14.63±0.40 | 2.71 | -2.49 |
| 750.00                | 171112| 221656 | 0.7720 | 717.19 | 717.19±22.06 | 3.15 | -4.37 |
| 1125.00               | 269448| 230160 | 1.1707 | 1091.40 | 1091.40±270.92 | 3.15 | -2.99 |
| Mean (ng/mL)±SD       | 2271.11| 221792 | 1.1626 | 1083.79 | 1083.79±16.20 | 1.94 | -3.66 |

CV: Coefficient of variation, ESO: Esomeprazole, SD: Standard deviation, PAR: Peak area ratio, LLOQ: Lower limit of quantitation, QCL: Quality control low, QCM: Quality control medium, QCH: Quality control high

Table 4: Recovery tests results

| Concentration (ng/mL) | ESO | IS | Unextracted area (µV/s) | Recovery (%) | Mean | SD | CV (%) |
|-----------------------|-----|----|------------------------|--------------|------|----|--------|
| QCL                   | 6598| 287347 | 5643 | 220798 | 85.53 | 86.76 | 6.21 |
| 15.00                 | 6570| 305247 | 5778 | 223749 | 87.95 | 95.98 | 10.27 |
| QCM                   | 174494| 253290 | 171112| 221656 | 90.94 | 99.31 | 9.37 |
| 750.00                | 173890| 261748 | 173114| 221656 | 90.94 | 99.31 | 9.37 |
| QCH                   | 291768| 287908 | 268448 | 230160 | 92.35 | 100.30 | 9.02 |
| 1125.00               | 295822| 241156 | 262606 | 231324 | 88.77 | 99.31 | 9.37 |
| Mean±SD               | 293824| 282644 | 264853 | 231854 | 90.14 | 100.30 | 9.02 |

CV: Coefficient of variation, ESO: Esomeprazole, SD: Standard deviation, QCL: Quality control low, QCM: Quality control medium, QCH: Quality control high

Table 5: Individual subjects' pharmacokinetic parameters

| Subject No. | Cmax (ng/mL) | tmax (h) | t1/2 (h) | AUC0−t (ng.h/mL) | AUC0−∞ (ng.h/mL) | AUC0−∞/AUC0−t (%) |
|-------------|--------------|----------|----------|-----------------|-----------------|--------------------|
| E1          | 136.34       | 3        | 2.93     | 3616.29         | 3616.29         | 100                |
| E2          | 1131.25      | 2        | 1.69     | 1927.51         | 1927.51         | 100                |
| E3          | 1356.26      | 2        | 1.62     | 2835.11         | 2835.11         | 100                |
| E4          | 1425.85      | 2.5      | 1.85     | 2271.11         | 2271.11         | 100                |
| E5          | 1068.11      | 2        | 1.84     | 2325.35         | 2325.35         | 100                |
| E6          | 704.57       | 2        | 1.89     | 1689.27         | 1689.27         | 100                |
| Mean±SD    | 1174.06±270.92 | 2.25±0.42 | 1.94±0.50 | 2444.10±693.92 | 2444.10±693.92 | 100                |
| CV (%)      | 23.08        | 18.59    | 25.73    | 28.39           | 28.39           | 100                |

CV: Coefficient of variation, SD: Standard deviation

According to the EMEA Bioanalytical Guidelines, 2011, incurred stability samples should include two concentrations in the Cmax phase and one concentration in the elimination phase in each healthy subjects' plasma. Since tmax varies between subjects, the ISS testing time point also varies. In the subject of E1, tmax was at the eighth sampling time and so the ISS samples were at the sixth and seventh time points (i.e., elimination phase). In the subject E4, tmax was at the sixth sampling point, so the ISS samples were at the fifth, sixth, and seventh time points (elimination phase). In the remaining subjects, tmax was at the seventh and eighth time points (i.e., at or close to t1/2). The ISS of ESO in plasma, therefore, meets the requirements up to 28 days with the highest %diff from the average ISS sample being 100% in all study subjects.

The ISS of ESO in plasma, therefore, meets the requirements up to 28 days with the highest %diff from the average ISS sample being 6.50%.

CONCLUSION

The pharmacokinetics profiles of ESO in the plasma of six healthy subjects exhibited a Cmax range between 704.57 and 1425.85 ng/mL with an average of 1174.16 ng/mL and a mean tmax of 2.25 h after a single dosage of a 40 mg enteric-coated ESO magnesium tablet. The mean AUC0−t. was 2444.10 ng.h/mL with the value of AUC0−∞ being 100% in all study subjects.

The ISS of ESO in plasma, therefore, meets the requirements up to 28 days with the highest %diff from the average ISS sample being 6.50%.
Fig. 4: Incurred sample stability trends for each subject

Table 6: Mean ISS results

| ISS sample | %diff |
|------------|-------|
| Day 7      |       |
| 1          | 6.50  |
| 2          | 5.73  |
| 3          | 4.57  |
| Day 14     |       |
| 1          | 3.55  |
| 2          | 4.84  |
| 3          | 3.68  |
| Day 28     |       |
| 1          | 4.04  |
| 2          | 4.80  |
| 3          | 4.98  |

1=1st $C_{\text{max}}$ concentration; 2=2nd $C_{\text{max}}$ concentration; 3=Elimination phase concentration. ISS: Incurred sample stability.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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