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Research Article

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. It is sensitive to low pH, heat, moisture, and oxidation, which often means that ESO in clinical samples is degraded at the time of storage, affecting analysis results. This study aimed to analyze the *in vivo* stability of ESO in subjects' plasma samples by testing the incurred sample stability (ISS) of ESO in plasma following 7, 14, and 28 days of storage at two concentrations close to C_{max} and one concentration in the elimination phase.

Methods: Samples were analyzed using high-performance liquid chromatography with a C_{18} 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 C_{max} 704.57–1425.85 ng/mL; t_{max} is 2.25 h; and AUC_{0-t} is 2444 ng.h/mL. ISS testing of plasma samples values were 6.50%, 5.73%, and 4.57% on first C_{max} concentration; 3.55%, 4.84%, and 3.68% on 2nd C_{max} 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.

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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 >30% [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)

Stock solutions 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 C_{18} column (Waters, Sunfire™ 5 µm; 250 mm×4.6 mm). The analysis 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, 1.75, 2, 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}, $t_{1/2}$, AUC_{0-t}, and $AUC_{0-\infty}$ 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 ≤±15% for other concentrations. The linear equation for the calibration curve was y=0.0018x+0.0017, with x being ESO magnesium concentration (ng/mL) and y being the peak

Table 1: System suitability test results

No	Area (µV/s)		Retention time (min)		Tf		n		Resolution
	ESO	IS	ESO	IS	ESO	IS	ESO	IS	
1	2357381	539861	5.494	7.931	0.80799	0.76666	4511.66	3384.75	5.141017
2	2285480	526162	5.474	7.916	0.81503	0.76009	4944.48	3377.22	5.216312
3	2289826	529335	5.465	7.888	0.81707	0.76597	4513.43	3236.96	5.211082
4	2280802	529837	5.478	7.902	0.82394	0.77218	5199.16	3271.09	5.259361
5	2286938	527111	5.471	7.891	0.81744	0.77098	5245.28	3338.61	5.250374
Mean±SD	2300085.4±32194.96	530461.2±5470.86	5.4764±0.01	7.9056±0.02	0.81629±0.01	0.76717±0.00	4882.8±356.88	3561.72±65.36	5.295629±0.05
CV (%)	1.40	1.03	0.20	0.23	0.70	0.62	7.31	1.97	0.89

Tf: Tailing factor, CV: Coefficient of variation, ESO: Esomeprazole, SD: Standard deviation

Table 2: Calibration curve concentrations

Concentration (ng/mL)	Area (µV/s)		PAR	Measurement concentration (ng/mL)	%diff
	Esomeprazole	IS			
0.00	0	130422	0.0000	0	0.00
5.00	1582	130394	0.0121	5.83	16.50
20.00	5237	135500	0.0386	20.60	2.99
75.00	16576	132418	0.1252	68.81	−8.26
200.00	45930	132582	0.3464	192.06	−3.97
500.00	124540	133669	0.9317	518.13	3.63
800.00	189132	139330	1.3574	755.31	−5.59
1200.00	315734	134666	2.3446	1305.26	8.77
1500.00	335618	130218	2.5774	1434.94	−4.34
Slope (b)	**Intercept (a)**	**r**	**R^2**		
0.0018	0.0017	0.9966	0.9931		

PAR: Peak area ratio

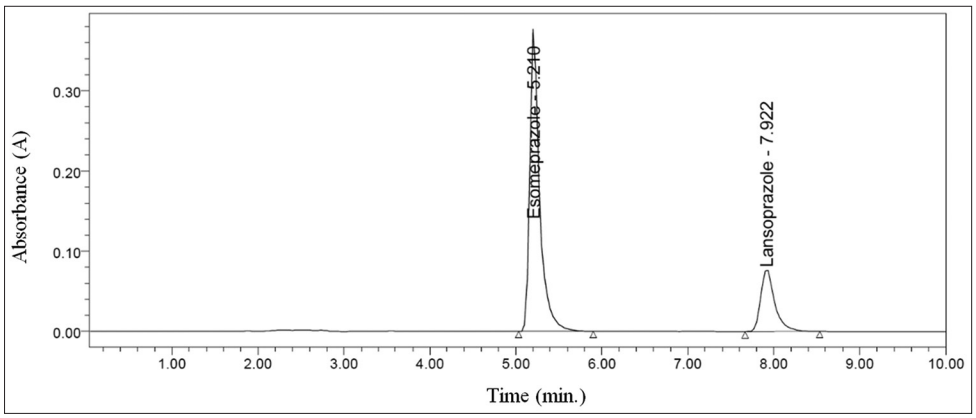

Fig. 1: Representative chromatogram for system suitability tests

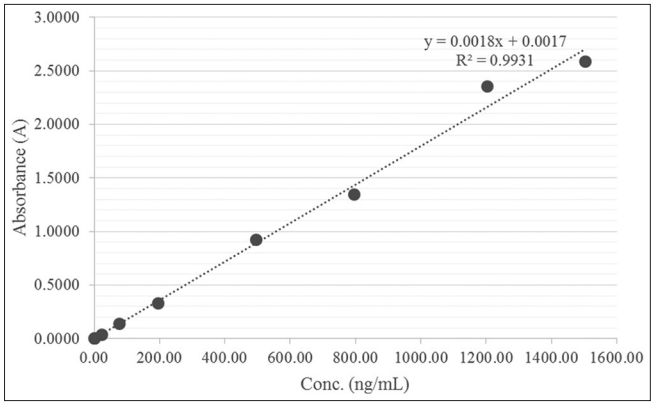

Fig. 2: Calibration curve

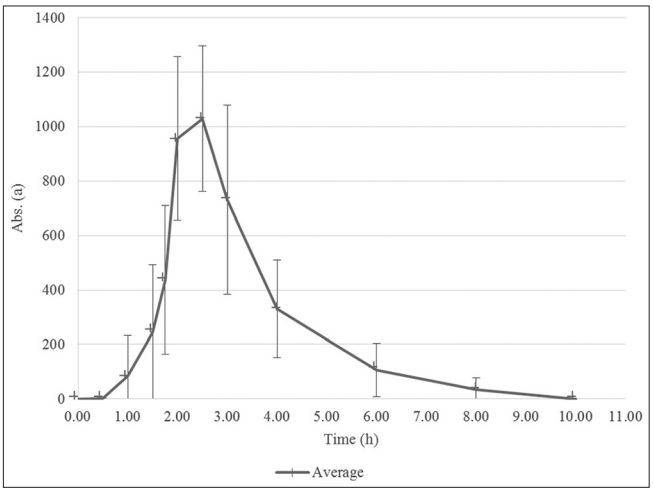

Fig. 3: Averaged pharmacokinetic profile of six subjects

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 that 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-t}, and $AUC_{0-\infty}$. The values of the determined pharmacokinetic parameters for each subject are presented in Table 5, with graphs as plotted in Fig. 3.

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	4.83±0.07	1.37	−2.30
5.00	3290	253662	0.0130	4.85			−2.92
	3065	238765	0.0128	4.73			−5.42
	3078	236743	0.0130	4.88			−2.33
	3109	240726	0.0129	4.80			−3.95
QCL	5343	230798	0.0232	14.41	14.87±0.40	2.71	−3.95
15.00	5370	226480	0.0237	14.93			−0.44
	5678	233749	0.0243	15.48			3.19
	5340	225369	0.0237	14.92			−0.54
	5374	229821	0.0234	14.63			−2.49
QCM	171112	221656	0.7720	717.19	701.38±22.06	3.15	−4.37
750.00	173114	220363	0.7856	729.97			−2.67
	166318	222166	0.7486	695.27			−7.30
	161112	221656	0.7269	674.85			−10.02
	168885	227430	0.7426	689.61			−8.05
QCH	269448	230160	1.1707	1091.40	1079.95±16.20	1.50	−2.99
1125.00	262606	231324	1.1352	1058.11			−5.95
	265698	225714	1.1771	1097.45			−2.45
	265654	231648	1.1468	1068.97			−4.98
	257853	221792	1.1626	1083.79			−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)	Extracted area (µV/s)		Unextracted area (µV/s)		Recovery (%)	Mean	SD	CV (%)
	ESO	IS	ESO	IS				
QCL	6598	287347	5643	220798	85.53	86.76	5.72	6.21
15.00	6570	305247	5778	223749	87.95			
	6151	281050	5340	215369	86.82			
QCM	174494	253290	171112	221656	98.06	99.31		
750.00	173890	261748	173114	220363	99.55			
	163682	309794	164177	299434	100.30			
QCH	291768	287908	269448	230160	92.35	90.42		
1125.00	295822	241156	262606	231324	88.77			
	293824	282644	264853	231854	90.14			

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.	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (ng.h/mL)	$AUC_{0-\infty}$ (ng.h/mL)	$AUC_{0-t}/AUC_{0-\infty}$ (%)
E1	1363.34	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	1.65	2271.11	2271.11	100
E5	1063.11	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	

CV: Coefficient of variation, SD: Standard deviation

According to the EMEA Bioanalytical Guidelines, 2011, incurred stability samples should include two concentrations in the C_{max} phase and one concentration in the elimination phase in each healthy subjects' plasma. Since t_{max} varies between subjects, the ISS testing time point also varies. In the subject of E1, t_{max} was at the eighth sampling time and so the ISS samples were at the seventh and eighth time points (i.e., at or close to t_{max}) and the 10th time point (i.e., elimination phase). In the subject E4, t_{max} was at the seventh sampling point, so the ISS samples were at the sixth and seventh time points (around t_{max}) and the 10th time point (elimination phase). In the remaining subjects, t_{max} was at the sixth sampling point, so the ISS samples were at the fifth, sixth, and ninth time points.

ISS

Testing was performed on the 7th, 14th, and 28th days of plasma storage and was counted from the day the pharmacokinetic profiles were created. The mean %diff of the ISS tests is shown in Table 6 and the concentrations trends in Fig. 4.

CONCLUSION

The pharmacokinetics profiles of ESO in the plasma of six healthy subjects exhibited a C_{max} range between 704.57 and 1425.85 ng/mL with an average of 1174.16 ng/mL and a mean t_{max} of 2.25 h after a single dosage of a 40 mg enteric-coated ESO magnesium tablet. The mean AUC_{0-t} was 2444.10 ng.h/mL with the value of $AUC_{0-t}/AUC_{0-\infty}$ 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%.

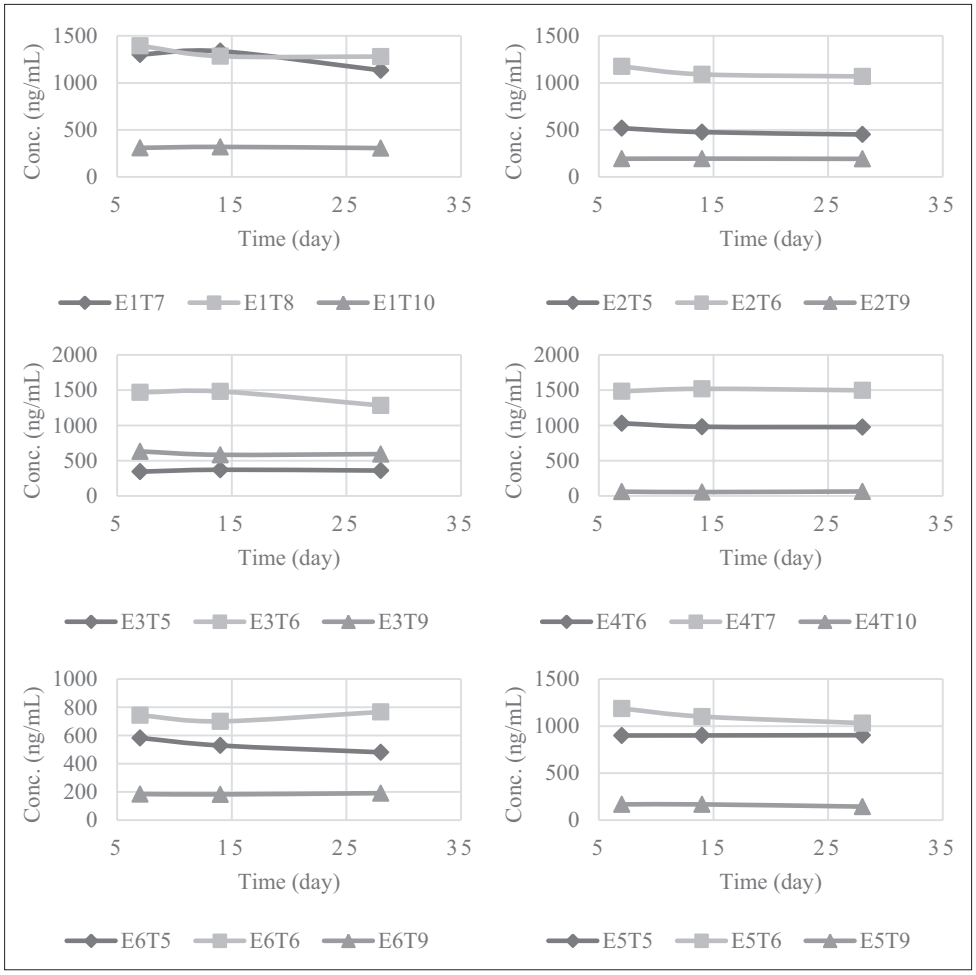

Fig. 4: Incurred sample stability trends for each subject

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_{max} concentration; 2=2nd C_{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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