Pharmacokinetic Study and Incurred Sample Stability of Esomeprazole in Dried Blood Spot Sample Using High Performance Liquid Chromatography-Photodiode Array

Yahdiana Harahap[1,*], Anja Tamabri[1], Vicha[1], Herman Suryadi[1], Sunarsih[2], and Callista Andinie Mulyadi[1]

[1]*Faculty of Pharmacy, Universitas Indonesia, Depok 16424, West Java, Indonesia*

[2]*Dea Medika Clinic, Jl. Bina Marga No. 7, Gunung Putri, Bogor 16961, West Java, Indonesia*

* ***Corresponding author:***

email: yahdiana03@yahoo.com

Received: January 7, 2019
Accepted: August 15, 2019

DOI: 10.22146/ijc.42404

Abstract: *In the past years, Esomeprazole (EMP) was analyzed in human plasma samples, which still has stability issues; thus, the new biosampling technique known as Dried Blood Spot (DBS) might solve the issue. This research aims to evaluate the incurred sample stability of esomeprazole in dried blood spot using high performance liquid chromatography-photodiode array with lansoprazole as an internal standard. The analytical separation was performed on a C-18 column (Waters, Sunfire™ 5 µm; 250 × 4.6 mm) at 40 °C. The mobile phase used was acetonitrile–phosphate buffer pH 7.6 (40:60% v/v) with a flow rate of 1.00 mL/min; and was detected at 300 nm. The analyte was extracted from dried blood spot by methanol. Incurred sample stability was evaluated from 6 healthy subjects on day 0, 7, 14, and 28, respectively. This method was linear in the range concentration of 70–1400 ng/mL with r > 0.98. Pharmacokinetic study shows that the average of AUC_{0-t} of EMP in the DBS sample was 1765.41 ngh/mL. The highest percent difference value of esomeprazole's incurred samples stability on day 7, 14, and 28 from 6 healthy subjects were 9.81%. This result fulfilled the acceptance criteria, which is the percent difference should not be greater than 20%, and 67% of total samples have to fulfill the criteria. The incurred sample stability result showed that esomeprazole was stable in the DBS sample at least until 28 days with the highest value of percent difference is 9.81%.*

Keywords: *dried blood spot; esomeprazole; lansoprazole; HPLC; incurred sample*

■ INTRODUCTION

Esomeprazole (EMP) is one of the proton pump inhibitors indicated for gastroesophageal reflux. Until now, the analysis of EMP was carried out in plasma samples, which has several disadvantages [1]. New biosampling method known as Dried Blood Spot (DBS) has several advantages, including easy and non-invasive sample collection; harmless and is easily transported; the price of DBS paper is also relatively cheap, easy to handle and store; analytes in the absorbed matrix on DBS paper are generally more stable; the process of collecting DBS samples minimizes the risk of infection; and less blood volume is needed compared to analysis in liquid or plasma blood [2-5]. Previous pharmacokinetic (PK) studies of EMP was done using human plasma sample [6]. The stability of EMP in human plasma is still an issue from the latest PK study of EMP [7]. Omeprazole's Red Blood Count (RBC) to plasma partitioning value is 5.25. Analysis of drugs with high RBC to plasma partitioning value (> 2) is better performed using the whole blood rather than in plasma or serum because it will generate a more sensitive and accurate result for *in vivo* pharmacokinetic studies [8]. With the advantages of the DBS technique and usage of the whole blood, as mentioned above, this method might solve the stability issue of EMP in the bioanalytical study.

Besides being unstable in an acidic media, EMP is also unstable against the light, moisture, and oxidation [8]. The Global CRO Council suggests evaluating the incurred sample stability if there is an instability issue in the analyte [9]. Incurred sample stability needs to be

done because the use of calibration standards and quality control (QC) samples when validating *in vitro* cannot describe the condition of *in vivo* sample stability due to metabolic processes in the body [10]. To conduct the incurred stability in the DBS sample, this study used 6 healthy subjects who had administered 40 mg EMP film-coated tablet. The pharmacokinetic parameters should be determined on the day the blood was collected (day 0), and the incurred stability was determined on 7, 14, and 28 days. This study had obtained ethical clearance from the Ethical Committee of Medical Faculty Universitas Indonesia No. 0036/UN2.F1/ETIK/2018.

■ EXPERIMENTAL SECTION

Materials

Esomeprazole (Esteve Quimica), Lansoprazole (Sigma-Aldrich), methanol HPLC grade, methanol, phosphoric acid, sodium hydroxide, formic acid, disodium hydrogen phosphate, sodium dihydrogen phosphate, acetonitrile, and dichloromethane were purchased from Merck (Darmstadt, Germany). The other materials used were Aqua pro injection was purchased from Ikapharmindo (Jakarta, Indonesia), and Perkin Elmer 226 papers were purchased Perkin Elmer (Waltham, Massachusetts, USA).

Instrumentation

The instruments used were HPLC equipped with pump (Shimadzu, LC-20AD), auto-sampler (Shimadzu, SIL-20A), column C-18 (Waters, Sunfire™; 5µm, 250 × 4.6 mm), photodiode array detector (Waters 2996), and data processor (Dell), pH meter (Eutech pH 510), analytical scales (Acculab), filter paper (Whatman), degasser (Elmasonic S60H), centrifuge (Digisystem DSC-300SCD), freezer (Biomedical Labtech Deep Freezer), vortex (Maxi Mix II), evaporator (TurboVap LV), micropipette Eppendorf (Socorex), blue tip, yellow tip, and glasses equipment.

Procedure

Chromatography system

The analytical separation used C-18 column (Waters, Sunfire™ 5µm; 250 × 4.6 mm) and acetonitrile – phosphate buffer pH 7.6 (40:60) as mobile phase with a flow rate of 1.0 mL/min isocratically. The column temperature was set at 40 °C, and the injection volume was 20 µL. Detection was performed with Photodiode Array (PDA) with a wavelength of 300 nm [11].

Optimization of esomeprazole in dried blood spot sample preparation

Whole blood samples containing EMP were spotted on DBS paper and dried. The DBS paper was cut in accordance with the spot size, extracted using methanol, shaken with vortex, sonicated, and centrifuged. The supernatant was evaporated under a nitrogen gas flow at 40 °C for 15 min, and the residue was reconstituted using 100 µL of solvent. Then it was centrifuged for 5 min in the autosampler vial, and 20 µL of the aliquot was injected into the HPLC. The optimized parameters were blood volume spotting, duration of drying, extraction method, the volume of extracting solution, time of vortex shaking, sonication time, centrifugation time, evaporating treatment, and solvent type for reconstitution of residue.

Method validation of esomeprazole analysis in dried blood spot

This study referred to the European Medicines Agency (EMEA) Guideline for Bioanalytical Method Validation 2011 and the Food and Drug Administration (FDA) Guidance for Bioanalytical Method Validation 2013 [12-13]. Validation parameters were selectivity, carry over, lower limit of quantification (LLOQ), the linearity of calibration curve, accuracy, precision, recovery, dilution integrity, and stability.

Blood sampling on healthy volunteers

This study was approved (no.: 0036UN2/F1/ETIK/2018) by the Ethics Committee of Faculty of Medicine, Universitas Indonesia. Prior to the study, all 6 subjects (age: 20–55 years old, BMI: 18.5–24.9 kg/m^2) had been declared healthy based on the results of medical check-ups and had signed the informed consent. One day before blood sampling, healthy volunteers were quarantined and fasted for 8 h. About 150 µL blood was collected at 30 min before drug administration (pre-dose); 0.5, 1, 1.5, 1.75, 2, 2.5, 3, 4, 6, 8, and 10 h after

administration of 40 mg of esomeprazole tablet. Blood collection was done through the fingertip (finger prick) with a sterile lancet needle. The blood that had been collected was then stored in a 0.5 mL vacutainer and spotted on DBS paper as much as 30 µL to be dried for approximately 2.5 h. Blood sampling was conducted in the Faculty of Pharmacy, Universitas Indonesia, Depok.

Pharmacokinetic study and incurred sample stability

Pharmacokinetic parameters were obtained based on the first-order equation of the rate of elimination, and the area under curve (AUC) was calculated by using the trapezoidal rule. Incurred sample stability was observed on DBS samples that represent both the high and low concentration range of results obtained, thereby analysis samples around the C_{max} and elimination phase. This test was done by storing the DBS sample that contains the analyte of the healthy subject for a certain time in a zip lock bag at room temperature. On day 0, 7, 14, and 28 at each selected sample concentration, the DBS paper was extracted and analyzed. The acceptance criteria from EMEA 2011 is the percent difference between the initial concentration and the concentration measured during the repeat analysis should not be greater than 20% of their mean for at least 67% of the repeats.

■ RESULTS AND DISCUSSION

Optimization of Esomeprazole in Dried Blood Spot Sample Preparation

Optimization of DBS sample preparation was carried out to afford the optimum extraction methods of esomeprazole from the DBS sample, thus could improve the analytical method sensitivity and recovery.

The purpose of blood volume spotting optimization is to obtain the maximum peak area with the smallest blood volume. These parameters relate to the application of methods on the subject. For the convenience of the subject, the blood volume was taken as small as possible. Optimized blood volume was 20, 25, and 30 µL. The largest area was obtained at 30 µL of blood spotted.

The purpose of drying time optimization is to obtain the fastest drying time with the largest area obtained. Drying time may affect recovery, so optimization is necessary [14]. The optimized drying time was 2, 2.5, and 3.5 h. The result showed that the largest area was obtained at 2.5 h of drying time.

The volume of methanol as an extracting solvent greatly affects the number of analytes that can be extracted from the DBS sample. This is related to methanol saturation by analytes and DBS samples, which are completely submerged during the extraction process by methanol. The purpose of methanol volume optimization is to obtain an efficient methanol volume with the largest area. The volume of methanol optimized is 500, 1000, and 1500 µL. From the optimization results, a volume of 500 µL of methanol shows a large and constant area compared to the volume of 1000 and 1500 µL. The increasing volume of methanol requires longer evaporation time.

Vortex time needs to be optimized to get efficient time. If the vortex time is too fast, the extracting solvent will not penetrate completely into the DBS paper. However, if the vortex time is too long, the organic solvents will form emulsions. In this study, the optimized vortex time is 1, 3, and 5 min. The result showed that the largest area is obtained at the time of vortex for 3 and 5 min. Therefore, vortex for 3 min is the most efficient time.

Sonication is performed to improve the efficiency of extraction. Sonication is the agitation process of particles in a solution using ultrasonic sound waves [15]. In this study, the optimization of sonication time is 5, 15, and 25 min. Based on the results of the experiment, the largest area was obtained when the sonication was performed for 15 min. If sonication time is too short, the analyte will not completely be dissolved from the DBS paper. Whereas if the sonication time is too long, there will be problems in analyte stability due to the heat generated from the sonication process.

The centrifugation step is important to ensure the DBS paper is completely submerged by the extracting solvent and the impurities from the biological matrix and paper fibers that are removed from the DBS paper settle so that a clearer supernatant is obtained [16]. Clearer extraction results are expected to reduce the number of impurities and extend the life of the columns. In this study, the optimization of the centrifugation time

was 1, 3, and 5 min. The result showed that the largest area was obtained on centrifugation for 1 min. The longer centrifugation time allows the precipitation of the analytes in the sample together with the protein precipitated by methanol.

Evaporation of the sample is performed to get a larger sample concentration so that the sensitivity of the method will increase. Optimization was carried out in 2 types of treatment, the extraction result was directly injected into HPLC, and the extraction results were evaporated under a nitrogen gas flow for 15 min at 40 °C then reconstituted using 100 µL mobile phase. The larger and constant areas were obtained in the treatment, which was continued by evaporation.

The optimized type of reconstituting solvent is methanol and the mobile phase. The consideration of solvent type optimization for reconstitution is related to esomeprazole solubility. Based on the results, the larger area was obtained from the reconstitution using a mobile phase that is acetonitrile-buffer phosphate pH 7.6 (40:60) compared to methanol. Sampled extract chromatogram with optimum preparation method can be seen in Fig. 1.

Method Validation of Esomeprazole Analysis in Dried Blood Spot

Lower Limit of Quantification (LLOQ) and calibration curve

The LLOQ of this method was 70 ng/mL, with the coefficient of variation (CV) value of 10.60% and % diff between –17.14 to 8.88%. The calibration curve was linear, with the correlation coefficient $r > 0.98$ in the concentration range from 70 to 1400 ng/mL.

Selectivity

Selectivity test was performed by analyzing esomeprazole at LLOQ concentration of 70 ng/mL and blood blank from 6 different sources every 2 replicas. The study results showed no interference in the retention time of the analyte and the internal standard.

Carry-over

The Carry-over test was performed by analyzing esomeprazole at the concentrations of the upper limit of quantification (ULOQ), blank, and LLOQ, respectively. The results of the carry-over test in this study showed that the interference value of the LLOQ esomeprazole area was obtained by 12.69 and 13.50%, while the interference value of the internal standard area was 0.60 and 0.58% so that the results meet the requirement.

Accuracy, precision, and recovery

The accuracy, precision, and recovery tests were performed by analyzing the analyte at concentrations lower limit of quantification (LLOQ, 70 ng/mL), quality control low (QCL, 200 ng/mL), quality control middle (QCM, 700 ng/mL), and quality control high (QCH, 1000 ng/mL). There are 2 types of accuracy and precision tests performed, namely within-run and between-run. In testing the accuracy and precision within-run, there were 5 replicas analyzed in the one continue analysis time. Meanwhile, the test of accuracy and precision between-run is done by doing 3 times analysis of each 5 replicas at each concentration within a period of at least 2 different days. The results of the test are shown in Table 1.

Dilution integrity

Dilution integrity tests are performed to ensure that dilution does not affect the accuracy and precision of measurement results. Dilution integrity test was done by making a test solution at a concentration above ULOQ, which was 2000 ng/mL, and diluting the solution with whole blood so that a concentration of 1000 and 500 ng/mL were obtained. The result obtained shown in Table 2.

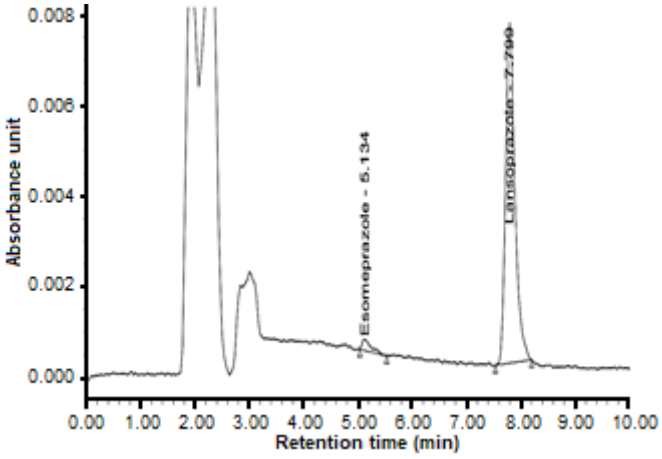

Fig 1. Chromatogram of esomeprazole at LLOQ (Lower Limit of Quantification)

Table 1. Within-run and between-run accuracy and precision

Actual Conc. (ng/mL)	Within-Run			Between-Run		
	Measured Conc. (Mean ± SD; ng/mL)	CV (%)	Bias (%)	Measured Conc. (Mean ± SD; ng/mL)	CV (%)	Bias (%)
70.0	68.49 ± 4.70	6.86	5.41	68.49 ± 3.86	5.64	8.66
200.0	208.93 ± 23.08	11.04	8.07	201.05 ± 9.16	4.55	6.55
700.0	708.27 ± 31.95	4.51	3.49	729.35 ± 19.95	2.74	5.06
1000.0	908.16 ± 41.76	4.60	9.18	989.37 ± 74.34	7.51	7.35

Table 2. Dilution integrity data

Dilution Factor	Actual Conc. (ng/mL)	Measured Conc. (Mean ± SD; ng/mL)	CV	% Bias
1 ×	2000.00	1977.73 ± 48.59	2.46	2.32
1/2 ×	1000.00	1018.42 ± 67.63	6.64	5.92
1/4 ×	500.00	497.39 ± 35.18	7.07	5.71

Stability

The stability test is performed to ensure that every step is taken, and storage conditions during the preparation do not affect the concentration of the analyte. In this study, the stability test carried out was the stock solution stability test, short-term stability, long-term stability, and autosampler stability.

The results of the stock solution stability test showed that esomeprazole was stable for 24 h at room temperature and protected from light storage and stable until day 14 at a storage temperature of -80 °C and protected from light. Whereas, the results of the stock solution stability test showed that the stock solution of lansoprazole was stable for 24 h at room temperature and protected from light storage, and stable for 14 days at -80 °C storage protected from light.

Short-term stability test results showed that the analyte contained in the DBS sample was stable at room temperature for 24 h. Whereas, the results of the long-term stability test showed that the analytes contained in the DBS sample were stable for 28 days with room temperature protected from light storage.

An autosampler stability test is carried out to ensure the injection process in autosampler does not affect the concentration of analytes within a certain period. This test needs to be done because often, the sample must be left in the autosampler for a certain duration before being injected into HPLC. The results of the autosampler stability test showed that the sample in the autosampler could be left for 24 h before being injected into HPLC.

Pharmacokinetic Study

The pharmacokinetic study is essential to determine the C_{max} and elimination phase of esomeprazole in the DBS sample. The validated method was applied to the pharmacokinetic study in healthy subjects following an oral route of administration of 40 mg film-coated tablet of esomeprazole. From the results of sample analysis, data on the concentration of esomeprazole in blood per unit of time were obtained. This data was then plotted into the time vs. concentration curve of 6 subjects and can be seen in Fig. 2. From the data, several pharmacokinetic parameters

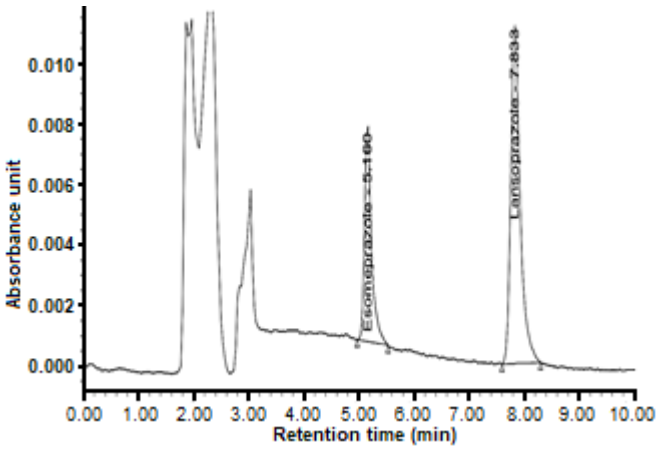

Fig 2. Chromatogram of ULOQ

obtained, including a maximum concentration in DBS sample (C_{max}), time to reach maximum concentration in DBS (t_{max}), half-life ($t_{1/2}$), and Area Under Curve (AUC). Based on the pharmacokinetic profile data, it can be seen that C_{max} obtained from all subjects ranged from 795.14–1135.73 ng/mL with an average of 916.65 ng/mL. The coefficient of variation of C_{max} from the six subjects was 14.59%. Time to reach maximum concentration in the six subjects ranged from 2–3 h with an average of 2.25 h and a coefficient of variation of 18.59%. There was a decrease in AUC value in the DBS sample when compared with plasma. Esomeprazole's AUC_{0-t} in the plasma sample was 3064.09 ngh/mL, while the mean AUC_{0-t} in DBS was 1765.41 ngh/mL. Sample concentration in DBS compared to the concentration in plasma are as follows: (1) analytes in DBS were not perfectly attracted during extraction, due to semi-polar analyte retained on polar DBS paper, and it was showed by recovery obtained only 67.13%; (2) blood samples for DBS are taken in peripheral blood vessels whose drug levels are lower than in the vein; (3) the blood volume for DBS samples is much smaller than plasma samples, so the concentration of analytes will also be smaller [17]. Besides that, ratio $AUC_{0-t}/AUC_{0-\infty}$ obtained in this study was 100% for all subjects as shown in Table 3, and its fulfilled EMEA criteria, which only requires greater than 80% [17-18].

Incurred Sample Stability

According to the EMEA 2011, samples taken for the incurred sample stability (ISS) test are samples whose blood-collection points that are in approximately C_{max} and the elimination phase. Because the t_{max} obtained varies between subjects, the point in time that ISS also varies. The highest percent difference (% difference) value of esomeprazole's incurred samples stability on day 7, 14, and 28 days as shown in Table 4 from subject 1 to subject 6 were 9.81% as shown in Table 5. This result fulfilled the acceptance criteria of the validation method based on the EMEA Bioanalytical Guideline 2011, which is the % difference should not be greater than 20%, and 67% of total samples have to fulfill the criteria. So, the incurred sample stability result showed that esomeprazole was stable in the DBS sample at least until 28 days. Some factors can support this stability in the DBS sample, such as the analyte is in a solid/dry state, so that collisions between particles can be minimized, which can further maintain the stability of esomeprazole compared to liquid samples such as plasma [19-20].

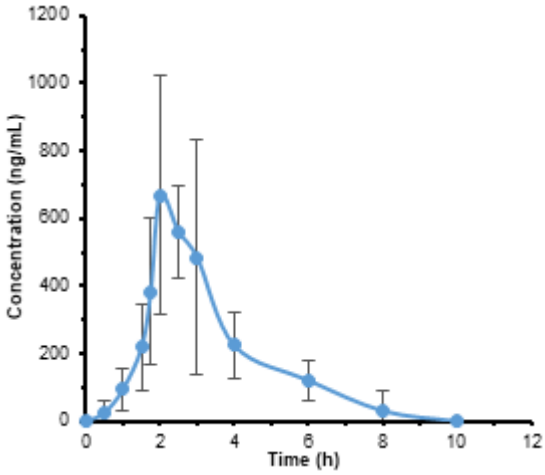

Fig 3. Mean concentration of esomeprazole in DBS sample from 6 healthy subjects after administration of 40 mg esomeprazole enteric coated tablet

Table 3. Summary of pharmacokinetic parameters of esomeprazole in DBS sample

No. Subject	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (ngh/mL)	$AUC_{0-\infty}$ (ngh/mL)	$AUC_{0-t}/AUC_{0-\infty}$ (%)
1	1135.73	3	2.93	2675.5	2675.5	100
2	931.79	2	1.69	1466.6	1466.6	100
3	837.97	2	1.62	1494.39	1494.39	100
4	800.33	2.5	1.65	1831.43	1831.43	100
5	795.14	2	1.84	1581.92	1581.92	100
6	998.93	2	1.89	1542.63	1542.63	100
Mean	917	2	2	1765	1765	100
SD	133.78	0.42	0.50	464.47	464.47	0
CV	14.59	18.59	25.73	26.31	26.31	0

Table 4. ISS data of esomeprazole in DBS sample of subject 1

Time (h)	Area (μV.s)		PAR	Conc. Measured (ng/mL)	% diff
	Analyte	IS			
Day 7					
2.50	7362	198307	0.0371	582.49	0.04
3.00	13562	198672	0.0683	1107.65	-2.50
6.00	3268	206340	0.0158	223.51	0.51
Day 14					
2.50	6037	180675	0.0334	583.64	0.24
3.00	11205	175400	0.0639	1139.17	0.30
6.00	2758	207315	0.0133	216.98	-2.45
Day 28					
2.50	4993	143893	0.0347	527.81	-9.81
3.00	8448	125922	0.0671	1088.90	-4.21
6.00	2794	167170	0.0167	216.24	-2.80

Table 5. Mean ISS data of Esomeprazole in DBS sample from all 6 subjects

Day 7	
ISS Point	% diff
1	-0.84
2	-1.43
3	-0.08
Day 14	
1	-1.88
2	-0.48
3	-0.92
Day 28	
1	-2.88
2	-5.46
3	-5.32

■ CONCLUSION

Based on the incurred sample stability test results, esomeprazole was stable in DBS sample at least until 28 days, because it fulfilled the acceptance criteria of EMEA Bioanalytical Method Validation Guideline 2011, in which the percent difference of the initial analysis and the repeat analysis was not greater than 20% on all incurred sample stability samples.

■ ACKNOWLEDGMENTS

Directorate of Research and Community Engagement, Universitas Indonesia, for financial support with Grant No. 1241/UN2.R3.1/HKP.05.00/2018.

■ REFERENCES

[1] Food and Drug Administration, 2014, *NEXIUM (esomeprazole magnesium) delayed-release capsules, for oral use*, Reference ID: 3675799, Food and Drug Administration, U.S. Department of Health and Human Services, USA.

[2] Xu, Y., Fang, W., Zeng, W., Leijen, S., and Woolf, E.J., 2012, Evaluation of dried blood spot (DBS) technology versus plasma analysis for the determination of MK-1775 by HILIC-MS/MS in support of clinical studies, *Anal. Bioanal. Chem.*, 404 (10), 3037–3048.

[3] Li, W., and Lee, M.S., 2014, *Dried Blood Spots: Applications and Techniques*, John Wiley & Sons, Inc., Hoboken, New York.

[4] Zakaria, R., Allen, K.J., Koplin, J.J., Roche, P., and Greaves, R.F., 2016, Advantages and challenges of dried blood spot analysis by mass spectrometry across the total testing process, *EJIFCC*, 27 (4), 288–317.

[5] Sharma, A., Jaiswal, S., Shukla, M., and Lal, J., 2014, Dried blood spots: Concepts, present status, and future perspectives in bioanalysis, *Drug Test. Anal.*, 6 (5), 399–414.

[6] Hasselgren, G., Hassan-Alin, M., Andersson, T., Claar-Nilsson, C., and Röhss, K., 2001, Pharmacokinetic study of esomeprazol in the elderly, *Clin. Pharmacokinet.*, 40 (2), 145–150.

[7] Harahap, Y., Baskara, A.E., and Harmita, 2017, Method validation of esomeprazol analysis in human plasma using high performance liquid chromatography–photodiode array, *J. Young Pharm.*, 9 (Suppl. 1), s24–s28.

[8] Wen, Z., Huang, Y., Behler, N., Bambal, R., Bhoopathy, S., and Owen, A., 2010, *Determination of red blood cell partitioning and whole blood to plasma ratio using human, rat, and mouse blood: Methods, model-compounds, and specific differences*, Absorption Systems, Exton, Pennsylvania, USA.

[9] Reddy, P., Sait, S., Vasudevmurthy, G., Vishwanath, B., Prasad, V., and Reddy, S.J., 2011, Stability indicating simultaneous estimation of assay method for naproxen and esomeprazole in pharmaceutical formulations by RP-HPLC, *Der Pharma Chemica*, 3 (6), 553–564.

[10] Lowes, S., LeLacheur, R., Shoup, R., Garofolo, F., Dumont, I., Martinez, S., Zimmer, J., Caturla, M.C., Couerbe, P., Awaiye, K., Fatmi, S., Farmen, R., Sheldon, C., Bower, J., Fiscella, M., Fast, D., Cape, S., Hulse, J., Kamerud, J., Zhang, T., Pasas-Farmer, S., Garofolo, W., Moussallie, M., Rocci, M., Allinson, J., Gouty, D., Buonarati, M., Boudreau, N., Pellerin, B., Lin, J., Xu, A., Hayes, R., Bouhajib, M., Stipancic, M., Nicholson, R., Nehls, C., Warren, M., Karnik, S., Houghton, R., Stovold, C., Reuschel, S., Cojocaru, L., Marcelletti, J., Fang, X., Smith, I., and Watson, A., 2014, Recommendations on incurred sample stability (ISS) by GCC, *Bioanalysis*, 6 (18), 2385–2390.

[11] Es'haghi, Z., 2011, "Photodiode array detection in clinical applications: Quantitative analyte assay advantages, limitations, and disadvantages" in *Photodiodes—Communications, Bio-Sensings, Measurements and High-Energy Physics*, Chapter 9, Eds. Shi, J.W., InTechOpen, New York.

[12] Committee for Medicinal Products for Human Use (CHMP), 2011, *Guideline on bioanalytical method validation*, European Medicines Agency London, UK.

[13] United States Food and Drug Administration, 2013, *Guidance for industry. Bioanalytical method validation*, U.S. Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER), and Center for Veterinary Medicine (CVM), USA.

[14] Koster, R.A., Greijdanus, B., Alffenaar, J.W., and Touw, D.J., 2015, Dried blood spot analysis of creatinine with LC-MS/MS in addition to immunosuppressants analysis, *Anal. Bioanal. Chem.*, 407 (6), 1585–1594.

[15] Hielscher Ultrasound Technology, 2018, *Probe-type sonication vs. ultrasonic bath: An efficiency comparison*, https://www.hielscher.com/probe-type-sonication-vs-ultrasonic-bath-an-efficiency-comparison.html, accessed on 7 December 2018.

[16] Lad, R., 2010, Validation of individual quantitative methods for determination of cytochrome P450 probe substrates in human dried blood spots with KCKT–MS/MS, *Bioanalysis*, 2 (11), 1849–1861.

[17] Wilhelm, A.J., den Burger, J.C., and Swart, E.L., 2014, Therapeutic drug monitoring by dried blood spot: Progress to date and future directions, *Clin. Pharmacokinet.*, 53 (11), 961–973.

[18] Committee for Medicinal Products for Human Use (CHMP), 2010, *Guideline on the investigation of bioequivalence (Rev. 1)*, European Medicines Agency, London, United Kingdom.

[19] Déglon, J., Thomas, A., Mangin, P., and Staub, C., 2011, Direct analysis of dried blood spots coupled with mass spectrometry: Concepts and biomedical applications, *Anal. Bioanal. Chem.*, 402 (8), 2485–2498.

[20] Chunduri, R.H.B., and Dannana, G.S., 2016, Development and validation of a high throughput UPLC–MS/MS method for simultaneous quantification of esomeprazol, rabeprazole, and levosulpiride in human plasma, *J. Pharm. Anal.*, 6 (3), 190–198.