Original Article

A New LC/MS/MS Method for the Analysis of Phyllanthin in Rat Plasma and its Application on Comparative Bioavailability of Phyllanthin in Different Formulations after Oral Administration in Rats

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ABSTRACT

Introduction: A simple, short UPLC/MS/MS method for quantitation of phyllanthin in rat plasma in less than 2 minutes have been developed and fully validated. The validated method was used to investigate the pharmacokinetic properties of phyllanthin in PA extract and phospholipid complex of PA extract in rat. **Methods:** The separation was carried out on Acquity C_{18} (50 x 2.1 mm; 1.7 μm), with a mobile phase of 10 mM aqueous amonium acetate and acetonitrile (10:90; v/v), at a flow rate of 0.2 mL/min. Felodipin was used as internal standard. Phyllanthin is extracted from a small volume of rat plasma (100 μl) by means of liquid-liquid extraction method with tert butyl methyl ether. Electrospray ionization (ESI) mass spectrometry was applied in positive mode at capillary voltage of 4000 V for both phyllanthin and IS, cone voltage of 24 V for phyllanthin and 20 V for IS, desolvation temperature of 360°C, cone gas flow of 25 L/h, collision energy of 12 V for phyllanthin and 10 V for IS. Multiple reaction monitoring (MRM) was used to monitor the transitions at m/z (Q1/Q3) 436.41/355.36 for phyllanthin and 384.20/352.18 for IS. **Results:** The linear calibration curve of phyllanthin was obtained over the concentration range of 0.5 – 100 ng/mL. The intra- and inter-day precisions were less than 7.08 % and the accuracies were within ± 7.55%. The Cmax values of phyllanthin from two different preparations in rat plasma after oral administration of 2.0 mg/kg were 11.44 and 31.44 ng/ml, and the AUC values were 18.07 and 41.43 h.ng/ml, respectively. **Conclusion:** A simple, short UPLC/MS/MS method for quantitation of phyllanthin in rat plasma in less than 2 minutes have been developed and fully validated. The bioavailability of phyllanthin from the phospholipid complex of PA extract in rat plasma was significantly improved compared with that of raw PA extract after oral administration.

Key words: Phyllanthin, Pharmacokinetics, Plasma, Quantitation, Phospholipid, LC-MS/MS.

HIGHLIGHT

• A rapid and selective ultra performance liquid chromatography (UPLC) with detector MS/MS to quantify phyllanthin in rat plasma in 1.5 minutes is described.

• Phyllanthin is extracted from a small volume of rat plasma (100 μl) by means of liquid-liquid extraction method.

• The validated method was successfully applied to pharmacokinetic studies of oral administration of different phyllanthin formulations to rats.

• Phyllanthin from complex with phospholipid showed significantly higher AUC, C_{max} and a longer half-life than that from raw *Phyllanthus amarus* extract indicating higher bioavailability of the phyllanthin- phospholipid complex.

INTRODUCTION

Phyllanthin is the crucial biologically active lignan obtained from various *Phyllanthus* species, such as *P. amarus*, *P. urinaria*, and *P. maderaspatensis*. A number of animal studies have shown that phyllanthin possesses hepatoprotective activity against carbon tetrachloride, galactosamine and ethanol treatment.[1-3] It is also revealed that phyllanthin was effective in treating diabetes and various chemical and virus induced liver abnormalities, including hepatitis[4], anti-fibrotic[5] and anti-inflammatory.[6] Furthermore, phyllanthin is known to possess immunomodulatory[7], nephroprotective[8], and anticancer[9] properties. Pharmacokinetic study of phyllanthin after oral administration to rats showed that phyllathin can be absorbable via GI tract[10,11], however, the bioavailability of phyllanthin was only 0.62% in rat[12]. Because the content of phyllanthin in herb or herb extract is very low, and this herbal drug was used as a total extract, not a single pure compound of phyllanthin, it is important to quantitate such a low level of phyllanthin in the blood. There are several methods for the quatitative analysis of phyllanthin from rat plasma such as HPLC–fluorescence detection[10,12], HPLC with PDA detection method[11,12], and however, these methods

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showed high LOQ and the analysis time is long (up to 30 minutes).[10,11] In our study, we try to develop a new, simple and rapid LC/MS/MS method for the analysis of phyllanthin in rat plasma and reduce the LOQ of phyllanthin to detect even trace level of phyllanthin in rat plasma.

The pharmacokinetic study has demonstrated that phyllanthin has the malabsorption through intestine, which induced the oral administration with high dose to reach a therapeutic level.[13] To resolve this problem, some scientists try to develop several dilivery systems such as formulating self-microemulsifying drug delivery systems (SMEDDS) containing phyllanthin[12] or mixed micellar lipid formulation of phyllanthin and piperine.[14] Up-to-date the complexing technique of herbal drug molecules with nutritional phospholipids has emerged as a potential carrier system for enhancing the bioavailability of plant extracts/actives with poor absorption.[15] This technique has been applied to improve the bioavailability of silybin[17], curcumin[18], ginsenosides[19], however, up to now, there are no reports about whether or not the phospholipid complex can improve the bioavailability of phyllanthin. In this study, we evaluate pharmacokinetic features of phospholipid complex of standardized *P. amarus* extract (PAE) compare with the standardized *P. amarus* extract on rats after oral administration. The objective of the present study was to develop a simple and reliable LC-MS/MS method for evaluation the pharmacokinetics of the standardized *P. amarus* extract and its phospholipid complex in rats.

EXPERIMENTAL

Materials and reagents

The whole plant of *P. amarus* was obtained from the medicine market in Ha Noi city, Vietnam in February 2016. The plants were identified by Dr. Hoang Viet Dung of Faculty of Medicinal Materials, Vietnam Military Medical University, and the voucher specimen (*P. amarus*) was deposited at the Institute of Biomedicine and Pharmacy, Vietnam Military Medical University.

The USP reference standard of phyllanthin and felodipin was purchased from Sigma Aldrich (St. Louis, MO, USA). All other materials and chemicals used were of either pharmaceutical or analytical grade. Phospholipon 85G was purchased from Lipoid, Switzerland.

Preparation of the P. amarus extract

The *P. amarus* extract was prepared according to the method published[20]. The aerial parts of *P. amarus* were allowed to dry under shade. The dried materials (200 g) was ground and extracted in 80% ethanol at the ratio of 1:30 (w/v) at room temperature for 72 h. The extraction was repeated two times on the residue. The total resultant filtrate was collected and filtered through Whatman No.1 filter paper (Whatman, England). The excess solvent was evaporated under reduced pressure using rotary evaporator (Buchi R210, Switzerland) at 55°C to dryness to obtain extract of *P. amarus* (9.28 g). The contents of phyllanthin in the extract was quantitatively determined by high performance liquid chromatography with fluorescence detection, which was a modified method of a previously published method.[21] The content of phyllanthin in the extract was quantitatively measured to be 9.12%. Dry extract of *P. amarus* was amorphous solid with brown color and pungent odor.

Formulation of the phospholipid complex of PAE (PAE-PC)

The phospholipid complex was formulated with PAE and phospholipon 85 G at a molar ratio of 1:1. The mixture of PAE and phospholipid was refluxed in a 100 mL round bottom flask with 20 mL of dichloromethane for 2 h. The reaction was carried out under the temperature not exceeding 40°C. The resultant solution was evaporated and 10 ml of n-hexane was added to it with continuous stirring. The drug–phospholipid complex was precipitated and the precipitate was filtered and dried under vacuum to remove traces of solvents. The drug–phospholipid complexes were kept in an amber glass bottle and stored at room temperature.

Pharmacokinetic study

UPLC - MS/MS conditions

An ACQUITY UPLC H-Class coupled with Waters Xevo TQD system and ACQUITY UPLC Sampler Manager (Waters, Milford, MA, USA) were used for analysis. Masslynx version 4.1 (Waters, Milford, MA, USA) was used for data acquisition and processing. Acquity C_{18} (50 x 2.1 mm; 1.7 μm) was used as the stationary phase. The isocratic elution was accomplished with a aqueous 10 mM ammonium acetate and acetonitrile (10:90; v/v) at a flow rate of 0.2 mL/min. The column temperature was 40°C. Injection volume was 5 μL. Autosampler was set at 4°C. Electrospray ionization (ESI) mass spectrometry was applied in positive mode at capillary voltage of 4000 V for both phyllanthin and IS, cone voltage of 24 V for phyllanthin and 20 V for IS, desolvation temperature of 360°C, cone gas flow of 25L/h, collision energy of 12 V for phyllanthin and 10 V for IS. Multiple reaction monitoring (MRM) in positive ion mode was used to monitor the transitions at m/z (Q1/Q3) 436.41/355.36 for phyllanthin and 384.20/352.18 for IS.

Preparation of calibration standards, quality control and internal standard

The standard stock solution of phyllanthin was prepared in acetonitrile at a concentration of 1 mg.mL^{-1}. Then, this stock solution of phyllanthin was diluted with acetonitrile to obtain fresh standard working solutions at the following concentration levels: 100 and 10 μg.mL^{-1}. Internal standard was prepared at the concentration of 500 μg/mL as IS stock solution. All solutions were stored at 4 °C before analysis.

Calibration standard (CS) samples and quality control (QC) samples were prepared by diluting corresponding standard working solutions with the blank plasma of rats. Then concentrations of the calibration standard were 0.5, 1, 5, 10, 25, 50, 75 and 100 ng.mL^{-1}. Three concentrations of QC samples representing the entire range of the standard curve were prepared at 2.00, 40.00 and 80.00 ng.mL^{-1}. Both CS samples and QC samples were maintained at −25°C until processing. All of the spiked plasma samples were then treated according to sample preparation procedure. The CS samples and the QC samples were applied in the method validation and the pharmacokinetic study.

Sample preparation

Plama samples (100 μL) in 1.5-mL test tubes were brought to room temperature before adding 10 μL internal standard. Then, these tubes were mixed on a vortexer for 60 seconds, add 1 mL tert butyl methyl ether, vortexed for 2 minutes then centrifuged for 10 minutes at 13,000 rpm, 4°C. The supernatant (approximately 600 μL was collected and dried under nitrogen gas flow at room temperature. The residues were then dissolved in 500 μL of mobile phase, vortexed for 1 minute and centrifuged at at 13,000 rpm, 4°C, then 5 μL of the supernatant was injected into the LC-MS/MS system.

Method validation

The method was validated for selectivity, linearity, accuracy, precision, recovery, stability, and matrix effect of samples according to the "Guideline on Bioanalytical Method Validation" recommended by the US Food and Drug Administration (FDA).[22]

Selectivity: The selectivity was evaluated by analyzing rat blank plasma, blank plasma spiked with phyllanthin, and a rat plasma sample after dosing. The method was established without interference from endogenous peaks existing at the peak region of phyllanthin in the blank plasma.

Linearity: Calibration curves were established by analyzing different concentrations of calibration samples on three consecutive days. The linear regressions of the peak areas ratio (phyllanthin/IS) of each concentration versus the nominal concentration (x) of phyllanthin were fitted over the range of 0.5 - 100 ng.mL^{-1}. Linearity was evaluated at 7 levels in the concentration range of 0.5-100 ng.mL^{-1}.

Precision and accuracy: Assessment of the intra-day and inter-day accuracy and the precision across the quantitation range for method standardization is essential and involves analyzing QC samples at multiple concentrations across the assay range. Method validation experiments for estimating accuracy and precision should include a minimum of three levels (2.00, 40.00, 80.00 ng/mL for phyllanthin) and six independent runs conducted on the same day and six consecutive days. Accuracy was calculated by dividing the measured mean drug concentration by the theoretical drug concentration. Precision was expressed as the coefficient of variation (CV, %), calculated as the ratio of the standard deviation to the measured mean drug concentration. Acceptable ranges of intra-day and inter-day precision and accuracy were below 15% bias or CV.[22] The sensitivity of detection was evaluated as the lower limit of quantification (LLOQ), which is the drug concentration corresponding to a peak area five times greater than the baseline noise. At the LLOQ, the precision and accuracy were required to be within 20%.[22]

Recovery and matrix effect: The recovery of phyllanthin was estimated by comparison of the peak area responses of the QC samples (n=6) that were added before extraction at 4 concentrations (0.5, 2.0, 40.0, 80.0 ng.mL^{-1}), with those obtained when the corresponding phyllanthin was added after the extraction step. Matrix effects were evaluated by utilizing the peak area ratios of the blank matrix spiked with phyllanthin to a standard solution at the same QC concentration. The matrix effect and recovery were investigated using six lots of blank matrix from individual sources at three concentration levels.

Stability: The stability of phyllanthin in rat plasma was determined by analysis of the QC solutions (2.0 and 80.0 ng/mL) exposed to different time and temperature conditions. The QC samples were prepared in sufficient volume to provide for multiple aliquots to be tested in replicate (n = 5) for each condition. The results were then compared with those of freshly prepared QC samples, and the percentage concentration deviation was calculated. The stability study included protocols for both short- and long-term stability. Short-term stability was studied under the following conditions: (a) three freeze/thaw cycles on consecutive days; (b) exposure to room temperature (bench temperature) for 6 h, 1 day, and 2 days; (c) exposure to 4°C (refrigerator) for 1 day; (d) exposure to -25°C (deep freezer) for 1 day and 3 days; (e) two- and four-fold dilution, using blank plasma. The short-term stability of a phyllanthin standard solution at room temperature for 12 h was also analyzed. Long-term stability was studied under the following conditions: (a) samples that were frozen at -25°C and thawed after 30 days; (b) phyllanthin standard solutions that were refrigerated (4°C) for 30 days.[22]

Animals

Healthy Sprague Dawley rats of either sex weighing about 220–250 gm were supplied by the Medical Laboratory Animal Unit at Vietnam Military Medical University (Hanoi, Vietnam). Rats were acclimated for at least 3 days to a room with a temperature of 25 ± 1 °C, humidity of 50–80% and 12-h light/12-h dark cycle. The animals were allowed free access to normal food and water for at least 3 days before the experiments. Pharmacokinetic studies were carried out in Preclinical Centre, Vietnam Military Medical University and were approved by institutional animal ethics committee (IRB-VN01015/2018-a18) for experimentation on animals.

Pharmacokinetic analysis

Two groups of Sprague Dawley rats were fasted for 12 h with free access to water prior to oral administration with the PAE or PAE-PC equivalent to 2 mg.kg^{-1} of phyllanthin. To determine the drug concentrations and calculate the pharmacokinetic parameters, blood samples (0.4 ml) were withdrawn from retro-orbital plexus at 0, 0.083, 0.167, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 12 h and 24 h after oral administration and were collected into centrifuge tubes containing heparin; within 30 min following blood withdrawal, the samples were centrifuged at 15,000 rpm for 15 min at 4°C. and resulting plasma was kept at –25°C before analysis. The pharmacokinetic parameters of phyllanthin in Sprague Dawley rats were calculated by Winnonlin software (Version 2.1; Pharsight Inc., CA, USA). The data are presented as mean ± SD.

Statistical analysis

Mean concentrations and pharmacokinetic parameters of phyllanthin in each formulation were analyzed using the t-test and matched-pair t-test. The standard error mean (SEM) was used to express the tendency of the data. The criterion for significant difference between parameters was a p-value less than 0.01.

RESULTS AND DISCUSSION

Optimization of UPLC/MS/MS conditions and internal standard selections

Optimal UPLC/MS/MS conditions were determined by maximizing peak resolution of the analyte versus optimal retention time. The MS/MS condition was optimized with different ESI mode, capillary voltage, cone voltage, desolvation temperature, cone gas flow and collision energy to get the maximal signal of phyllanthin. Different solvents (methanol, acetonitrile, water) and modifiers (formic acid, amoni formate, amoni acetate) at different concetrations were tried to select the mobile phase. The results showed that aqueous 10 mM amonium acetate and acetonitrile (10:90; v/v) give the optimal separation for the analysis. Different internal standards were evaluated such as meloxicam, amlodipin, roxithromycin, felodipin in which felodipin showed stable signal as well as highly symetrical peak was selected as the internal standard.

Sample preparation optimization

The sample optimization method was carried out on QC samples at two different concentrations (2.0 and 80.0 ng/mL). Different sample preparation methods were investigated including protein precipitation with perchloric acid, methanol and acetonitrile; liquid-liquid extraction with different solvents such as diethyl ether, ethyl acetate, chloroform, tert-butyl methyl ether and the mixture of them at different ratio. The results showed the best method was liquid-liquid extraction with tert-butyl methyl ether as described above.

Method validation

Selectivity

Figure 1 represents the MRM chromatograms of a blank plasma sample, zero sample, a blank plasma sample spiked with phyllanthin (50 ng.mL^{-1}), plasma sample after oral administration of PAE-PC. The peaks of phyllanthin appeared at 0.8 min. No interfering peaks were found in the MRM chromatogram of phyllanthin and IS. There was increased sensitivity and selectivity when LC-MS/MS as used for the quantitative determination of phyllanthin compared to the traditional

HPLC method. The total runtime was 1.5 min per sample. This method proved to be very efficient.

Calibration curve

A linear relationship was observed in the calibration curves over the concentration range of 0.5 - 100 ng/mL for phyllanthin in rat plasma. The regression equation is expressed as y = 0.0019 x – 0.0008; r=0.9990, where y represents the peak area ratio of phyllanthin/IS and x represents the concentration of phyllanthin in rat plasma. The LLOQ was 0.5 ng/mL concentration of phyllanthin in rat plasma, which demonstrated that the assay could detect the trace amount of phyllanthin in rat plasma. In our method, the LLOQ was only 0.5 ng/mL, compare with HPLC-FD method,[10] the LOQ was 4.88 ng/mL, our method was almost 10 times more sensitive than this HPLC-FD method. In the report of Madhukiran *et al*[11], the LOQ of phyllanthin was up to 169.99 ng/mL wih the use of HPLC-PDA, our method demonstrated up to 340 times more sensitive than HPLC-PDA method.

Accuracy and precision

As shown in Table 1, the results of intra- and inter-day precision assessed by the relative standard deviation (RSD) were no more than 7.08% and 7.55%, respectively. The accuracy was in the range of 99.01-107.90%.

Recovery and matrix effects

As can be seen from Table 1, the recovery for the method was in the range of 90.05 % - 97.84 % with matrix effect within the range of 90.02 - 97.07 %. The results indicate reasonable recoveries with a negligible matrix effect for this method.

Stability

The stability studies for phyllanthin in the rat plasma under the different storage conditions mentioned above (n=6). As can be seen from Table 2, the RSDs were ≤ 6 % in all stability tests range of phyllanthin, which indicated reliable stability behavior for phyllanthin under the different storage conditions.

Pharmacokinetics

The mean plasma concentration-time curves for phyllanthin after oral administration are shown in Figure 2. The main pharmacokinetic parameters after oral administration of PAE and PAE-PC (equivalent to 2.0 mg/kg of phyllanthin) based on non-compartment model analysis are presented in Table 3. The AUCo-t of phyllanthin of PAE compared with that of PAE-PC was increased 2.29 times. Compared with that of PAE, the Cmax of phyllanthin of PAE-PC was increased 2.75 times. All these results showed that the bioavailability of phyllanthin in rat was increased significantly after oral administration of phospholipid complex of *P.amarus* extract comparing with raw *P.amarus* extract. Thus, it would be expected that phyllanthin could be developed for oral administration in a phospholipid complex form and used for treatment in the future.

The bioavailability of lipophilic drugs orally taken as solid dosage forms is notoriously poor. Among several reasons responsible for this, a particularly widespread rationale is poor absorption due to slow and/or incomplete drug dissolution in the lumen of the gastro-intestinal tract. In this case, the innovative approach for the improved bioavailability can be carried out via delivery systems, which can magnify the rate and/or the extent of drug solubilizing into aqueous intestinal fluids. Phospholipids play a major role in drug delivery technology. Phospholipid molecules having natural amphipathic property are considerably soluble in aqueous and oily mediums, which could be a solubilizing property carrier system.[15] In addition, phospholipids are one of the major structural units of the cell membrane in mammalian animals, which facilitates them penetrate the cell membrane and enter the mammalian cell cytoplasma without distorting the cellular lipid bilayer.[16] There are several advantages of phospholipids in conjunction with solubilizing property while taking into account them for a drug carrier system. Phospholipids are amphipathic molecules having considerable
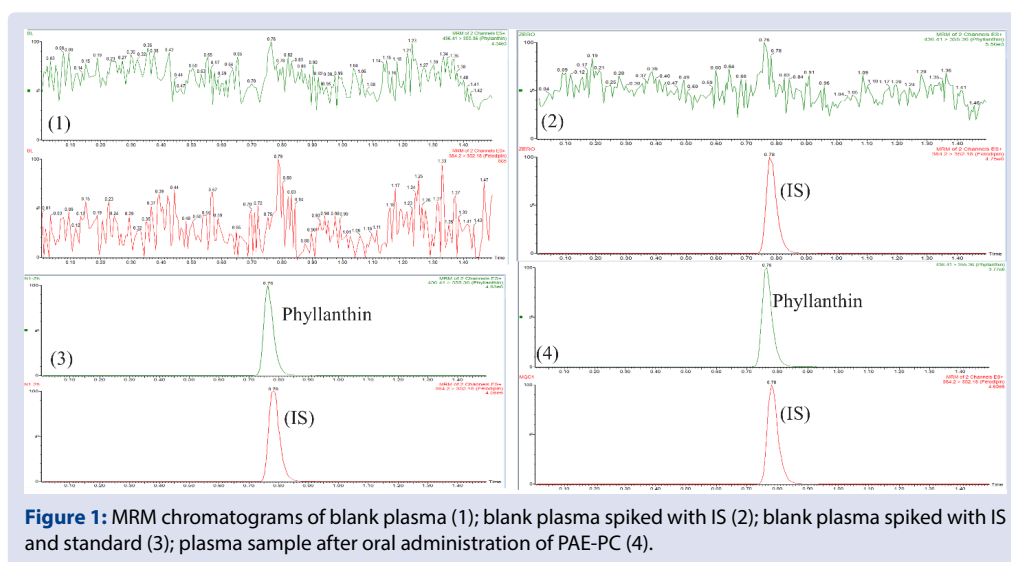
Figure 1: MRM chromatograms of blank plasma (1); blank plasma spiked with IS (2); blank plasma spiked with IS and standard (3); plasma sample after oral administration of PAE-PC (4).

Table 1: Precision, accuracy, extraction recovery and matrix effect of phyllanthin (n=6).

Spiked concentration (ng/mL)	Precision (RSD %)		Accuracy (%)		Extraction Recovery		Matrix effect	
	Intra-day	Inter-day	Intra-day	Inter-day	Mean (%)	RSD (%)	Mean (%)	RSD (%)
0.50	6.45	6.70	100.92	99.23	90.05	8.60	90.02	2.80
2.00	4.30	3.51	99.79	107.90	93.95	6.40	94.82	2.46
40.00	4.45	7.55	103.23	106.10	97.84	5.25	96.14	2.11
80.00	7.08	6.69	100.71	99.01	97.71	1.88	97.07	1.90

**Table 2: Stability of phyllanthin under various storage conditions (n=6).**

Conditions		Phyllanthin	
		(2.0 ng/mL)	(80.0 ng/mL)
Control (freshly prepared)		0 ± 4.2	0 ± 3.6
Freeze/thaw (-25 °C, 3 cycles)		-4.5 ± 1.3	3.4 ± 1.8
Bench (room temp.)	6h	3.9 ± 4.4	-3.5 ± 3.6
	1day	0.6 ± 5.2	-1.7 ± 3.9
	2days	-0.6 ± 3.9	0.6 ± 3.8
Freezer (-25 °C)	1 day	1.9 ± 2.6	2.5 ± 2.8
	2 days	2.8 ± 2.4	2.4 ± 2.6
	30 days	2.5 ± 1.3	2.2 ± 1.7
Sample dilution	2 times	-5.6 ± 3.4	-3.3 ± 3.6
	4 times	4.4 ± 4.8	4.8 ± 3.2
Standard solution	room, 12 h	3.4 ± 2.7	1.9 ± 2.7
	4 °C, 30 days	3.6 ± 4.2	3.8 ± 2.4

Data are expressed as percent deviation and SD [(analyzed - control)/control x 100 ± SD]

Table 3: Pharmacokinetic parameters after oral administration of PAE and PAE-PC dosages equivalent to 2.0 mg/kg of phyllanthin in rats (Mean ± SD, n=12).

Parameters	PAE	PAE-PC
AUC_{0-t} (h.ng.mL^{-1})	18.07 ± 1.99	41.43 ± 2.02*
$AUC_{0-\infty}$ (h.ng.mL^{-1})	22.38 ± 1.67	45.73 ± 0.90*
$t_{1/2}$ (h)	5.24 ± 0.21	8.32 ± 0.15*
$K_{elimination}$ (h^{-1})	6.95 ± 2.18	4.66 ± 0.82*
t_{max} (h)	0.25	0.25
C_{max} (ng.mL^{-1})	11.44 ± 1.15	31.44 ± 2.57*
Cl/F (L.h^{-1})	0.90 ± 0.06	0.44 ± 0.01*

*$p<0.01$ compared with phyllanthin in PEA by t-test.
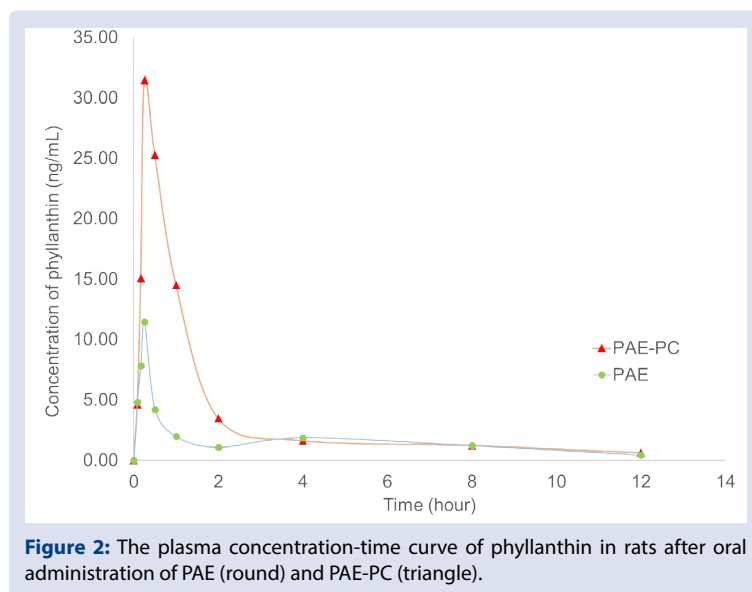
Figure 2: The plasma concentration-time curve of phyllanthin in rats after oral administration of PAE (round) and PAE-PC (triangle).

solubility in aqueous and oily mediums. The phospholipids, especially those containing phosphatidylcholine have shown to be incorporated in the cell membrane to replace cellular phospholipids and thus affect the fluidity of the membrane.[23] Internalization of phospholipid complex vesicles via anonymous mechanisms is suggested to be due to the favorable interactions of the biologically similar dietary phospholipids, with the cellular plasma membrane thus piloting the complexed drug across the membrane into the blood circulation.[24]

CONCLUSION

A simple, sensitive and robust method using UPLC-MS/MS for the quantitative analysis of phyllanthin in rats plasma was developed and fully validated. The method offers sample extraction from only 100 µL of plasma using liquid-liquid extraction technique and was successfully applied to the pharmacokinetic analysis of phyllanthin after oral administration of PAE and PAE-PC in rats. Phyllanthin

from PAE-PC showed showed significantly higher AUC and C_{max} and a longer half-life than that from PAE, indicating higher bioavailability of the phyllanthin from phospholipid complex. The phospholipid complex of *P. amarus* extract could be a novel formulation to replace the conventional formulation of medicinal plant *P. amarus*.

CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

ACKNOWLEDGMENT

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GRAPHICAL ABSTRACT
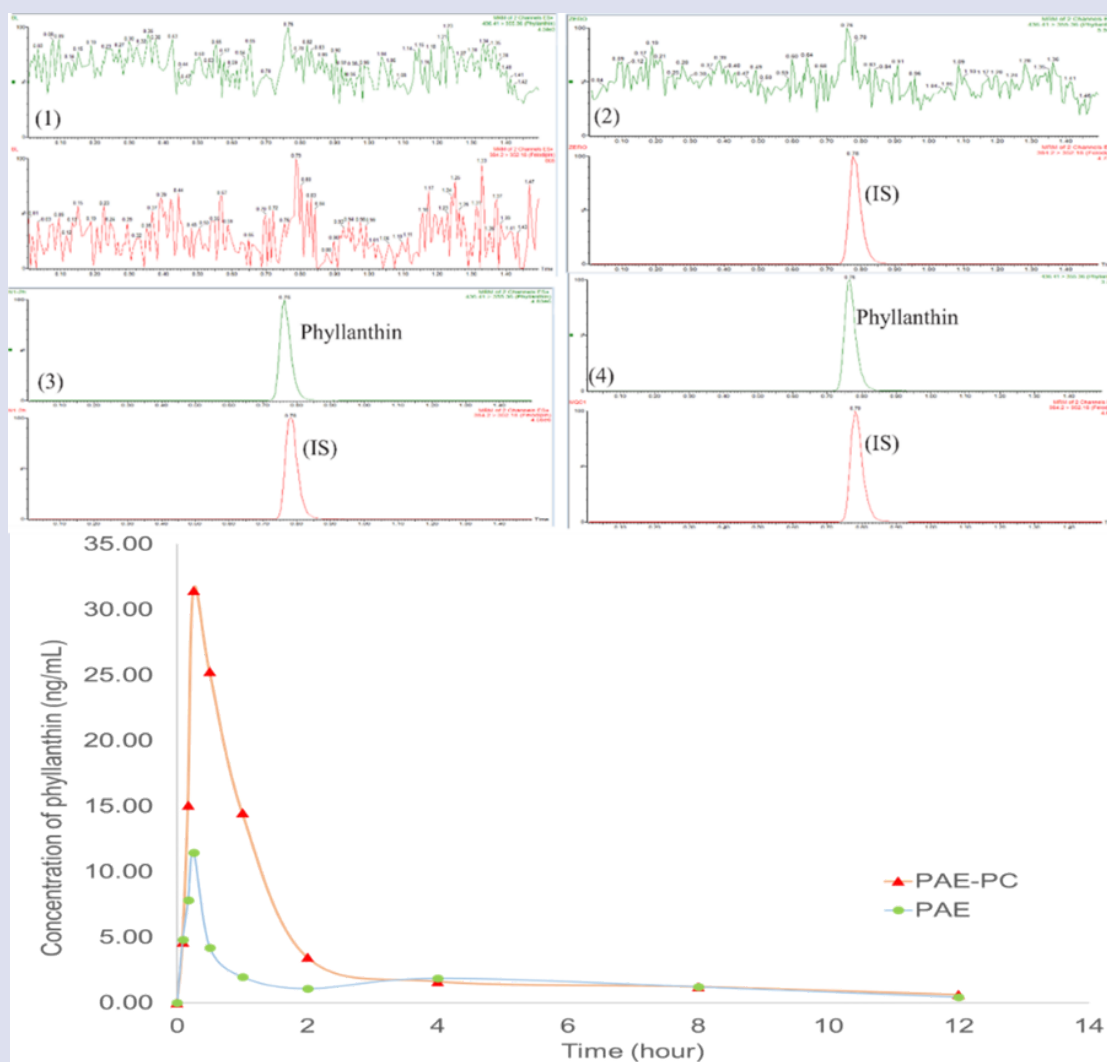
SUMMARY

A simple, sensitive and robust method using UPLC-MS/MS for the quantitative analysis of phyllanthin in rats plasma was developed and fully validated. The method offers sample extraction from only 100 µL of plasma using liquid-liquid extraction technique and was successfully applied to the pharmacokinetic analysis of phyllanthin after oral administration of *P. amarus* extract (PAE) and PAE-Phospholipid complex (PAE-PC) in rats. Phyllanthin from PAE-PC showed showed significantly higher AUC and Cmax and a longer half-life than that from PAE, indicating higher bioavailability of the phyllanthin from phospholipid complex. The phospholipid complex of *P. amarus* extract could be a novel formulation to replace the conventional formulation of medicinal plant *P. amarus*.

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