

Original Article

An alternative high performance liquid chromatographic method for determination of piperine in serum: Application to pharmacokinetic studies

Piracha Jumba-ngern*, Somboon Kiettinun*, **, Intouch Sakpakdeejaroen*, Anurak Cheomung***, Kesara Na-Bangchang***, ***

Abstract

Introduction: Piperine, a major alkaloid found in Benjakul remedy of the spice black and long peppers *Piper longum* Linn. and *Piper nigrum* Linn., is used as a traditional medicine in several tropical countries particularly Asia. The aim of the study was to establish an alternative high performance liquid chromatography (HPLC) with ultraviolet (UV) detection method, which is relatively simple, rapid, sensitive, accurate and reproducible method for determination of piperine in human serum.

Methods: Piperine and the internal standard β -17-estradiol acetate were separated from serum on a ZORBAX Eclipse XDB-C18 (4.6 x 250 mm, 5 μ m particle size) column, with the mobile phase consisting of a mixture of 25 mM KH_2PO_4 and acetonitrile at the ratio of 35:65 (v:v) running at a flow rate of 1.0 mL/min. Ultraviolet detection was at the wavelengths of 340 and 280 nm for piperine and β -17-estradiol acetate, respectively. Retention times of piperine and β -17-estradiol acetate were 4.6 and 10.0 min, respectively. Sample preparation was done by extraction of protein with the mixture of 9:1 (v:v) ethylacetate and propanol. The developed method was validated according to guidance for industry bioanalytical method validation.

Results: Good precision and accuracy were obtained for both within-day repeatability and day-to-day reproducibility. Limit of quantification (LOQ) for piperine was 25 ng/mL using 500 μ L sample. The mean recoveries for piperine were between 86.44-99.30%. The method was successfully applied for a pharmacokinetic study of piperine in healthy Thai males.

Discussion and Conclusion: An alternative analytical method for the determination of piperine in serum using HPLC with UV detection established in this study meets the criteria for application to routine clinical drug level monitoring or pharmacokinetic study. The developed method is relatively rapid, simple, and sensitive.

Key words: Piperine, HPLC-UV, Pharmacokinetics, Benjakul

Received: 30 January 2013

Accepted: 29 March 2013

* Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University

** International College of Medicine, Thammasat University

*** Thailand Center of Excellence in Drug Discovery and Development (TCEDDD), Thammasat University

Corresponding author, Kesara Na-Bangchang, Institute / Address: International College of Medicine and Thailand Center of Excellence in Drug Discovery and Development (TCEDDD), Thammasat university (Rungsit campus) 99 Moo 18 Paholyothin road, Khlong Luang, Pathum Thani, Thailand 12121, Telephone number: 0-2564-4440-79 ext. 1803, Email: kesaratmu@yahoo.com

Introduction

Piperine, a major alkaloid of the spice black and long peppers *Piper longum* Linn. and *Piper nigrum* Linn., is used as a traditional medicine in several tropical countries particularly Asia. It has been established as a bioavailability enhancer of drugs and other substances.^{1,2} In addition, piperine and the crude plant extracts have been shown to exhibit a wide range of biological and pharmacological activities including cytotoxic, antioxidant, anti-inflammatory, analgesic and anti-pyretic, central nervous system depressant, anti-platelet, anti-hypertensive, hepatoprotective, antithyroid, immuno-stimulating (promoting natural killer cell activity) activities, as well as inhibitory activity on nitric oxide production.^{3,6}

A number of analytical methods have been reported for measuring piperine and its derivatives in biological fluids (plasma, serum of rats and humans), cell cultures, as well as plant extracts or pharmaceutical formulations. These include HP-TLC,⁷⁻⁹ HPLC coupled with ultraviolet (UV) detection,^{3,10-16} GC-MS,¹⁷ LC-MS,¹⁸ LC-NMR-MS,¹⁹ LC-MS-MS,²⁰ UFLC,²¹ UPLC-qTOP-MS²² and UFLC-ESI-MS-MS.²³ Among these methods, only few methods were reported for the quantification of piperine in biological fluids.^{13, 15, 23} Although the methods involving mass spectrometry (MS) is highly sensitive (≤ 1.0 ng/mL), their application for clinical pharmacokinetic studies is limited due to their high cost and sophistication of the assay procedures.

In the present report, we describe an alternative HPLC with UV detection method, which is relatively simple, rapid, sensitive, accurate and reproducible method for determination of piperine in human serum. The method has been applied successfully for pharmacokinetic study of piperine in healthy Thai males following a single oral dose of 200 mg Benjakul formulation, an ethanolic extract of parts from five plants, i.e., *Piper chaba* Hunter.(fruit), *Piper sarmentosum* Roxb.(root), *Piper interruptum* Opiz. (stem), *Plumbago indica* Linn.(root), and *Zingiber officinale* Roscoe.(rhizome).

Materials and methods

Chemicals

Piperaquine [1-[5-[1,3-benzodioxol-5-yl]-1-oxo-2,4-pentadienyl] piperidine], (Figure 1a) and the internal standard β -17-estradiol acetate (Figure 1b) (99% pure) were purchased from Merck Co. Ltd. (Darmstadt, Germany) and Sigma-Aldrich (CA, USA). Acetonitrile, methanol, ethylacetate, and propanol were of HPLC grade, which were purchased from Labscan Co. Ltd., Bangkok, Thailand. The following chemicals and solvents were obtained in the highest purity available: phosphoric acid, and di-potassium hydrogen phosphate-3 hydrate were obtained from Analytical Sciences Co. Ltd., Bangkok, Thailand. Deionized double distilled water was used for the preparation of working nicotine standard solutions. Serum from healthy volunteers used for standard curves was provided from the blood bank of Thammasat Chalermprakiet Hospital.

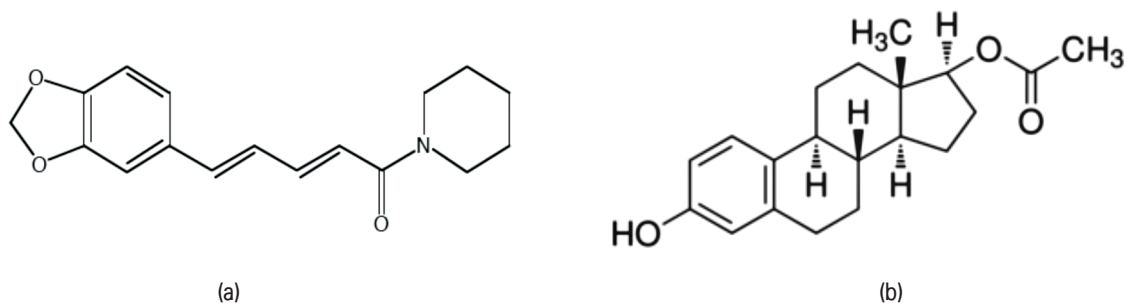


Figure 1 Chemical structures of piperine (a) and β -17-estradiol acetate (b).

Preparations of standards

Stock solutions of piperine and β -17-estradiol acetate at the concentration of 1,000 ng/ μ L by dissolving 5 mg of the compound in 5,000 μ L methanol and stored at -20° C until used. Working standard solutions were prepared by diluting the stock standard solutions with methanol. Standard solutions were stored at -20°C until analysis.

Seven aliquots of blank control serum were spiked with piperine working solution in a serial dilution to obtain the standard calibration at the concentrations of 25, 50, 100, 250, 500, and 1,000 ng/mL, with 3,000 ng/mL internal standard (3,000 ng/ μ L β -17-estradiol acetate).

Chromatography

Piperine and the internal standard were separated on a ZORBAX Eclipse XDB-C18 (4.6 x 250 mm, 5 μ m particle size) (Agilent Technologies[®], CA, USA). The HPLC system was operated under an isocratic mode at a flow-rate of 1 mL/min. The mobile phase was a mixture of 25 mM KH₂PO₄ (pH 4.5, adjusted with orthophosphoric acid) and acetonitrile at the ratio of 35:65 (v:v). The HPLC system consisted of a solvent delivery system (Spectra-System[™] P4000 Quaternary solvent delivery/controller: Thermo fisher scientific, CA, USA) equipped with solvent degasser (SpectraSystem SCM1000 solvent degasser: Thermo fisher scientific, CA, USA), and an autosampler (SpectraSystem AS3500: Thermo fisher scientific, CA, USA) and a UV detector (SpectraSystem UV/Vis 3000: Thermo fisher scientific, CA, USA). The wavelength of UV-Vis detector was operated at 340 and 280 nm for piperine and β -17-estradiol acetate, respectively.

Sample preparation

Five hundred microliters (μ L) of unknown serum samples, or quality control samples were transferred to polypropylene tubes, and 100 μ L internal standard (3,000 ng/ μ L) was added to each tube. After thoroughly mixing, distilled water (500 μ L) and 12 mM phosphate buffer pH 3.4 (100 μ L) were added. After vortex mixing, the mixture was then extracted with 6 mL of the mixture of 9:1 (v:v) ethylacetate and propanol by vortex mixing for 10 minutes. The upper organic phase was separated

through centrifugation at 2,000 x g for 5 min. Organic solvents were evaporated to dryness at 40°C in a parallel evaporator. The residue was reconstituted with 100 μ L of methanol, and 40 μ L portion was injected onto the column.

Detector linearity

Solutions of piperine in distilled water at concentrations ranging from 25 to 2,000 ng/mL were injected into the HPLC system in order to assess detector linearity. Peak heights were plotted against the quantities of all standards injected. All were found to be linear ($r^2 > 0.999$) over the concentration range observed.

Calibration curves

Calibration curves of piperine were prepared by replicate analysis of six serum samples (500 μ L each) spiked with varying concentrations of piperine (25, 50, 100, 250, 500, and 1,000 ng/mL) and a fixed concentration of the internal standard (3,000 ng/ μ L). Samples were analyzed as described above.

Data analysis

Concentrations of piperine were determined from the peak height ratios (peak heights of piperine/peak heights of internal standard), which corresponded to the known piperine concentrations in a calibration curve as described above. Peak detection, peak height integration, peak height ratio calculation were performed by the Millennium 2000 Chromatograph[™] software (Waters, Milford, MA, USA). Peak height ratios of piperine/internal standard were calculated. Concentrations of piperine in serum samples were determined by matching peak height responses against a calibration curve of response ratio (height of piperine/height of internal standard) vs concentration, obtained from standard sample injection.

Method validation

The developed method was validated according to guidance for industry bioanalytical method validation.²⁴

Precision: The precision of the method based on *within-day repeatability* was determined by replicate analysis of six sets of serum samples spiked at six different concentrations of piperine (25, 50, 100, 250, 500, and 1,000 ng/mL). The *reproducibility (day-to-day*

variation) of the method was validated using the same concentration range of serum as described above, but only a single determination of each concentration was made on six different days. Coefficient of variation (CV) were calculated from the ratios of standard deviation (SD) to the mean and expressed as percentage.

Accuracy: The accuracy of the method was determined by replicate analysis of six sets of serum samples spiked at four different levels of piperine (25, 50, 100, 250, 500, and 1,000 ng/mL) and comparing the difference between the spiked value and that actually found (theoretical value).

Recovery: The analytical recovery of sample preparation procedure for piperine was estimated by comparing the peak heights obtained from serum samples prepared as described above, with those measured with equivalent amounts of piperine in methanol. Triplicate analysis was performed at piperine and internal standard concentrations of 50, 500, and 1,000 ng/mL, respectively.

Selectivity: The selectivity of the assay was demonstrated by checking for the absence of endogenous interferences at the retention times of piperine in human blank serum obtained from six different lots, after subjecting them to sample preparation procedures.

Limit of quantification: The limit of quantification (LOQ) of the assay procedure was determined from the lowest concentration of piperine (in spiked serum sample) that produced a peak height ten times the baseline noise (absorbance unit full scale) in a 500 μ L sample.

Quality control: Quality control (QC) samples for piperine were made up in serum using a stock solution separated from that used to prepare the calibration curve, at the concentrations of with 50 (low), 500 (medium), and 1,000 ng/mL (high) piperine and 3,000 ng/mL internal standard. Samples were aliquoted into cryovials, and stored frozen at -20° C for use with each analytical run. The results of the QC samples provided the basis of accepting or rejecting the run. At least four of the six

QC samples had to be within $\pm 20\%$ of their respective nominal values. Two of the six QC samples could be outside the $\pm 20\%$ of their respective nominal value, but not at the same concentration.

Application of the method to biological samples

The method was applied to the investigation of the pharmacokinetics of piperine in serum samples in a total of 5 Thai males (aged 20-25 yr, weighing 55-74 kg) following a single dose of 200 Benjakul formulation (1 tablet, 12 mg piperine). This part of the study was approved by the Human ethics committee of faculty of medicine, Thammasat University, Thailand. Written informed consents for study participation were obtained from all patients. Venous blood samples (5 mL) were collected into plastic tubes at the following time points: 0 (before dose), and at 0.5, 1, 2, 4, 6, 9, 12, 18, 24 and 48 hr after drug administration. All samples were stored immediately after collection at -80° C until analysis.

Results

Chromatographic separation

Under the chromatographic condition described above, the chromatograms of piperine and internal standard (β -17-estradiol acetate) were free from any interference peak, with good resolution and sharp peaks. Blank serum sample showed little noise fluctuation. The retention times of piperine and β -17-estradiol acetate were 4.6 and 10.0 min, respectively (Figure 2).

Sample preparation

A number of sample preparation procedures were investigated to optimize the extraction of piperine and the internal standard β -17-estradiol acetate from serum samples. The optimal sample preparation procedure used in this study was simple involving only a single step liquid-liquid extraction by organic solvents (ethylacetate: propanol = 9:1 v:v), which resulted in clean chromatograms (Figure 2).

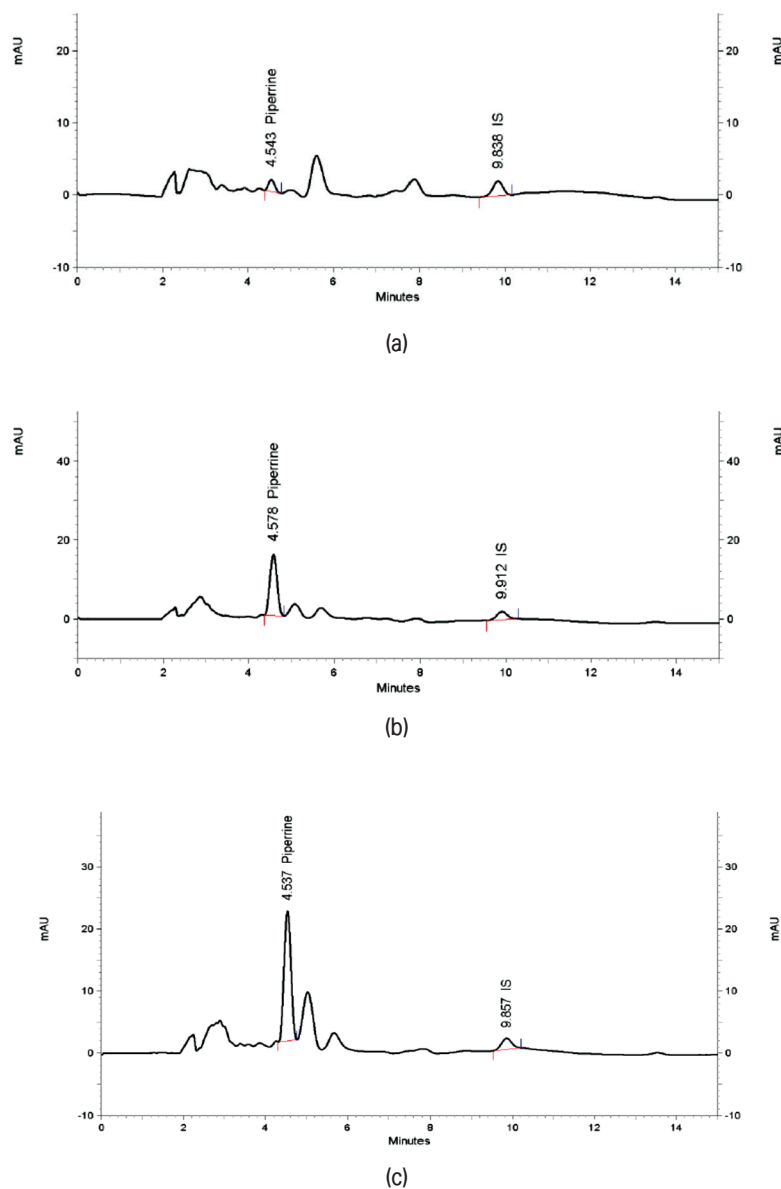


Figure 2 Chromatogram of (a) blank serum, (b) serum spiked with 500 ng/mL piperine and 3,000 ng/mL internal standard, and (c) serum sample collected from one subject after one hour of the administration of a single oral dose of 200 mg Benjakul formulation (spiked with 3,000 ng/mL internal standard).

Calibration curves

Serum analysis was calibrated using concentration range of 25-1,000 ng/mL. All calibration ranges yielded linear relationships with correlation coefficients ($r^2 > 0.999$)

or better (Figure 3). The linear regression equation obtained from the mean of the six calibration curves was $y = 0.25x + 3.201$, where y is the peak height ratio and x is the analyte concentration in ng.

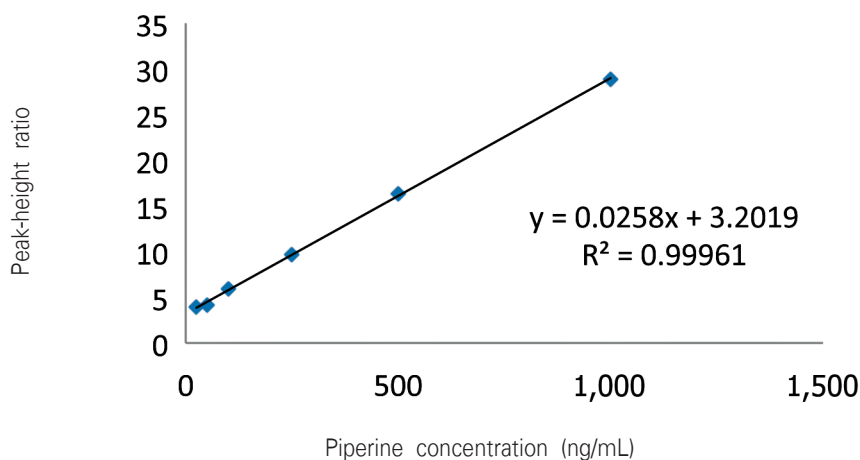


Figure 3 Calibration curve of piperine standard at concentration 25-1,000 ng/mL.

Method validation

Precision Little variation of piperine assay in serum samples was observed; coefficients of variation (CV) for six analysis at the concentration range of 25-1,000 ng/mL were all below 15%. The intra-day assay CV of piperine in serum samples varied between 3.0 and 14.07%. The inter-day assay CV varied between 1.18 and 19.11%. (Table 1).

Accuracy Good accuracy was observed from both the intra-day or inter-day assays, as indicated by the minimal deviation of mean values found with measured samples from that of the theoretical values (actual amount added). For intra-day assay validation, the mean deviation from the theoretical values (MDV) varied between -14.96 and +19.68%. The inter-day assay MDV varied between -14.22 and +11.98% (Table 1).

Recovery The mean recoveries for piperine at 50, 500 and 1,000 ng/mL were 90.13, 90.06 and 97.18%, respectively. The recovery of β -17-estradiol acetate at the concentration of 3,000 ng/mL was $99.47 \pm 0.15\%$ (Table 2). The results reflect essentially high recovery for all compounds from the spiked serum and indicate lack of interference from sample preparation procedure.

Selectivity Selectivity of the chromatographic separation was demonstrated by the absence of interferences from endogenous peaks and samples collected from one healthy male following a single oral dose of 200 mg Benjakul formulation (Figure 2).

Limit of quantification The limit of quantification (LOQ) in human serum samples for piperine was accepted as 25 ng/mL using 500 μ L blood.

Table 1 Intra-day and inter-day precision and accuracy of piperine assay

Concentration added (ng/mL)	Precision (%CV) ¹		Accuracy (%DMV) ²	
	Intra-day	Inter-day	Intra-day	Inter-day
25	3	19.11	19.68	0.48
50	2.23	1.18	-14.96	-14.22
100	14.07	3.41	3.93	4.25
250	13.59	4.79	0.74	11.98
500	9.31	2.40	1.61	6.00
1,000	10.78	1.28	-0.43	-2.18
Mean \pm SD	8.83 \pm 5.13	5.36 \pm 6.87	1.76 \pm 11.06	1.05 \pm 8.92

¹CV : coefficient of variation (%)

²DMV : deviation of mean value from theoretical value (%)

Table 2 Recovery of piperine in serum at different concentrations and internal standard (IS).

	% Recovery of piperine		% Recovery of IS	
	50 ng/mL	500 ng/mL	1,000 ng/mL	3,000 ng/mL
1	90.97	86.44	98.76	99.52
2	86.48	86.01	93.47	99.30
3	92.92	97.72	99.30	99.60
Mean \pm SD	90.13 \pm 3.30	90.06 \pm 6.64	97.18 \pm 3.22	99.47 \pm 0.15

Application of the method to biological samples

The method appears to be robust and has been applied to the investigation of the pharmacokinetics of piperine in serum samples in a total of 5 Thai males following a single dose of 200 Benjakul formulation (1 tablet, 12 mg piperine). Figure 4 showed mean and each

individual concentration-time profiles of piperine in all subjects. Median $C_{\max-1st}$ (first peak of maximum concentration) and AUC_{0-48hr} (area under the concentration time curve from 1 to 48 hr) values were 1,078 ng/mL and 10,216 ng.hr/mL, respectively.

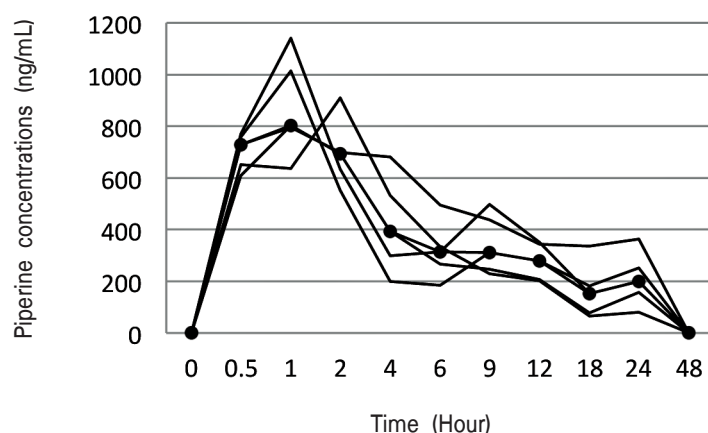


Figure 4 Serum concentration-time profiles of piperine in serum samples in a total of 5 Thai males following a single dose of 200 Benjakul formulation (1 tablet, 12 mg piperine). Data were presented as median (circle) and individual (line) values.

Conclusion and discussion

An alternative analytical method for the determination of piperine in serum using HPLC with UV detection established in this study meets the criteria for application to routine clinical drug level monitoring or pharmacokinetic study.²⁴ The developed method is relatively rapid, simple, and sensitive. It is noted however that the ubiquitousness of piperine in the human diet and the presence of lipids in the plasma of humans may interfere with piperine assay in human serum.

Acknowledgements

The study was supported by the national research council of Thailand. We would like to thank Thammasat Chalermprakit Hospital, Faculty of medicine, and Pharmacology and toxicology unit, Faculty of Allied Health Sciences, Thammasat University for supporting the facilities for sample collection and laboratory.

References

1. Bano G, Raina RK, Zutshi U, Bedi KL, Johri RK, Sharma SC. Effect of piperine on bioavailability and pharmacokinetics of propranolol and theophylline in healthy volunteers. *Eur J Clin Pharmacol* 1991;41:615-17.
2. Atal CK, Dubey RK, Singh J. Biochemical basis of enhanced drug bioavailability by piperine: Evidence that piperine is a potent inhibitor of drug metabolism. *J Pharmacol Exp Ther* 1985;232:258-62.
3. Wu S, Sun C, Pei S, Lu Y, Pan Y. Preparative isolation and purification of amides from the fruits of *Piper longum* L by upright counter-current chromatography and reversed-phase liquid chromatography. *J Chromatogr A* 2004;1040:193-204.
4. Vaghasiya Y, Nair R, Chanda S. Investigation of some *Piper* species for anti-bacterial and anti-inflammatory property. *Int J Pharmacol* 2007;3:400-5.
5. Bhitre MJ, Fulmali S, Kataria M, Anwikar S, Kadri H. Antiinflammatory activity of the fruits of *piper longum* Linn. *Asian J Chemistry* 2008;20:4357-60.
6. Veeru P, Kishor MP, Meenakshi M. Screening of medicinal plant extracts for antioxidant activity. *J Med Plant Res* 2009;3:608-12.
7. Bhat BG, Chandrasekhara N. Determination of piperine in biological tissues by thin-layer chromatography and ultraviolet absorption densitometry. *J Chromatogr* 1985;338:259-63.
8. Verma JK, Joshi AV. Rapid HPTLC method for identification and quantification of curcumin, piperine and thymol in an ayurvedic formulation. *J Planar Chromatogr* 2006;19:398-400.
9. Gopu CL, Aher S, Mehta H, Paradkar AR, Mahadik KR. Simultaneous determination of cinnamaldehyde, eugenol and piperine by HPTLC densitometric method. *Phytochem Anal* 2008;19:116-21.
10. Wood AB, Barrow ML, James DJ. Piperine determination in pepper (*Piper nigrum* L.) and its oleoresins—a reversed-phase high-performance liquid chromatographic method. *Flavour Frag J* 1988;3:55–64.
11. Verzele M, Mussche P, Qureshi SA. High performance liquid chromatographic analysis of the pungent principles of pepper and pepper extracts. *J Chromatogr* 1979;172:493-97.
12. Sunkara G, Mada SR, Vobalaboina V. Pharmacokinetics and tissue distribution of piperine in animals after i.v. bolus administration. *Pharmazie* 2001;56:640-42.
13. Bajad S, Singlab AK, Bedia KL. Liquid chromatographic method for determination of piperine in rat plasma: application to pharmacokinetics. *J Chromatogr B Analyt Technol Biomed Life Sci* 2002;776:245-9.
14. Ternes W, Krause EL. Characterization and determination of piperine and piperine isomers in eggs. *Anal Bioanal Chem* 2002;374:155-60.
15. Sethi P, Dua KV, Mohanty S, Mishra KS, Jain R, Edwards G. Development and validation of a reversed phase HPLC method for simultaneous determination of curcumin and piperine in human plasma for application in clinical pharmacological studies. *J Liq Chromatogr Rel Technol* 2009;32:2961-74.
16. Kamal YT, Musthaba SM, Singh M, Parveen R, Ahmad S, Baboota S, et al. Development and validation of HPLC method for simultaneous estimation of piperine and guggulsterones in compound Unani formulation (tablets) and ananoreservoir system. *Biomed Chromatogr* 2012;26:1183-90.
17. Marutoiu C, Gogoasa I, Oprean I, Marutoiu FO, Moise LM, Tigae C, et al. Separation and identification of piperine and chavicine in black pepper by TLC and GC-MS. *J Planar Chromatogr* 2006;19:250-52.
18. Scott IM, Puniani E, Jensen H, Livesey JF, Poveda L, Sanchez-Vindas P, et al. Analysis of piperaceae germplasm by HPLC and LCMS: a method for isolating and identifying unsaturated amides from *Piper* spp extracts. *J Agric Food Chem* 2005;53:1907-13.
19. Bajad S, Coumar M, Khajuria R, Suri OP, Bedi KL. Characterization of a new rat urinary metabolite of piperine by LC/NMR/MS studies. *Eur J Pharm Sci* 2003;19:413-21.

20. Bajad S, Khajuria R, Suri OP, Bedi KL. Characterisation of a new minor urinary metabolite of piperine, an omnipresent food component by LC-MS/MS. *J Sep Sci* 2003;26:943-46.
21. Kakarala M, Dubey SK, Tarnowski M, Cheng C, Liyanage S, Strawder T, et al. Ultra-low flow liquid chromatography assay with ultraviolet (UV) detection for piperine quantitation in human plasma. *J Agric Food Chem* 2010;58:6594-9.
22. Sachin BS, Najar IA, Sharma SC, Verma MK, Reddy MV, Anand R, et al. Simultaneous determination of etoposide and a piperine analogue (PA-1) by UPLC-qTOF-MS: evidence that PA-1 enhances the oral bioavailability of etoposide in mice. *J Chromatogr B Analyt Technol Biomed Life Sci* 2010;878:823-30.
23. Liu J, Bi Y, Luo R, Wu X. Simultaneous UFLC-ESI-MS/MS determination of piperine and piperlonguminine in rat plasma after oral administration of alkaloids from *Piper longum* L.: application to pharmacokinetic studies in rats. *J Chromatogr B Analyt Technol Biomed Life Sci* 2011;879:2885-90.
24. U.S. Department of health and human services, food and drug administration. Guidance for industry bioanalytical method validation. [Internet]. 2001 [cited 2012 January 10]. Available from: <http://www.fda.gov/downloads/Drugs/.../Guidances/ucm070107.pdf>.

บทคัดย่อ

การใช้เทคนิค HPLC ในการหาปริมาณ piperine ในซีรัม และการประยุกต์ใช้ในการศึกษาทางเภสัชจลนศาสตร์
 พิรชา จำปาเงิน*, สมบูรณ์ เกียรตินันท์***, อินทัช ศักดิ์ภักดีเจริญ*, อนุรักษ์ เชื้อมั่ง***, เกศรา ณ บางช้าง*****

* สาขาวิชาการแพทย์แผนไทยประยุกต์ คณะแพทยศาสตร์ มหาวิทยาลัยธรรมศาสตร์ (ศูนย์รังสิต)

** วิทยาลัยแพทยศาสตร์นานาชาติ มหาวิทยาลัยธรรมศาสตร์ (ศูนย์รังสิต)

*** ศูนย์วิจัย คันคว่ำ และพัฒนายา มหาวิทยาลัยธรรมศาสตร์ (ศูนย์รังสิต)

บทนำ: Piperine เป็นสารอัลคาลอยด์ที่พบมากที่สุดในตำรับยาเบญจกูล ซึ่งพบในพริกไทย ดีปลี และข้าพหลู และมีการใช้ในการแพทย์พื้นบ้านในประเทศเขตร้อน โดยเฉพาะในเอเชีย วัตถุประสงค์ของการศึกษานี้คือเพื่อพัฒนาวิธีซึ่งเป็นทางเลือกในการวิเคราะห์หาปริมาณ piperine ในซีรัมของมนุษย์ด้วยเทคนิค HPLC-UV ซึ่งเป็นวิธีที่ง่าย รวดเร็ว ถูกต้อง แม่นยำ และเชื่อถือได้

วิธีการศึกษา: แยกสาร piperine และ internal standard (β -17-estradiol acetate) ในซีรัมโดยใช้คอลัมน์ชนิด ZORBAX Eclipse XDB-C18 (4.6 x 250 mm, 5 μ m particle size) และสารละลายเคลื่อนที่ซึ่งประกอบด้วย ๒๕ มิลลิโมลาร์โพแทสเซียมไดฮัยโดรเจนฟอสเฟต และอะซิโตนไนโตร ในอัตราส่วน ๓๕:๖๕ (ปริมาตร/ปริมาตร) ด้วยอัตราเร็ว ๑ มิลลิเมตร/นาที โดยตั้งเครื่องวัด UV-VIS ที่ความยาวคลื่น ๓๔๐ นาโนเมตร สำหรับ piperine และ ๒๘๐ นาโนเมตร สำหรับ internal standard ซึ่งสามารถแยกสาร piperine และ internal standard ที่เวลา (retention time) ๔.๖ และ ๑๐.๐ นาที ตามลำดับ การเตรียมตัวอย่างซีรัมที่เก็บจากอาสาสมัครทำการตกตะกอนโปรตีนด้วยเอธิลอะซิเตทและโพรพานอลผสมกันด้วยอัตราส่วน ๔:๑ (ปริมาตร/ปริมาตร) โดยวิธีที่พัฒนาขึ้นนี้ได้ตรวจสอบความเหมาะสมของวิธีการวิเคราะห์ปริมาณ piperine ในซีรัม ตามหลักเกณฑ์ของ guidance for industry bioanalytical method validation

ผลการศึกษา: วิธีที่ใช้ในการวิเคราะห์ปริมาณ piperine นี้ มีความถูกต้อง เทียบตรง และเชื่อถือได้ ความเข้มข้นต่ำสุดที่สามารถวิเคราะห์ปริมาณ piperine ได้ คือ ๒๕ นาโนกรัม/มิลลิลิตร โดยใช้ตัวอย่างซีรัม ๕๐๐ ไมโครลิตร และมีประสิทธิภาพในการสกัดสารอยู่ในเกณฑ์ที่ยอมรับได้คือ ร้อยละ ๘๖.๔๔-๙๙.๓๐ ซึ่งวิธีนี้สามารถนำมาประยุกต์ใช้ในการศึกษาเภสัชจลนศาสตร์ของ piperine ในอาสาสมัครชายสุขภาพดีได้

วิจารณ์ และสรุปผลการศึกษา: วิธีการวิเคราะห์ที่พัฒนาขึ้นนี้ รวดเร็ว ง่าย และมีความไวในการวิเคราะห์ ซึ่งจะเป็นทางเลือกหนึ่งในการวิเคราะห์หาปริมาณ piperine ในซีรัมโดยใช้ HPLC-UV ซึ่งเป็นไปตามเกณฑ์ที่กำหนด และสามารถนำไปประยุกต์ใช้ในการหาระดับยา หรือการศึกษาทางเภสัชจลนศาสตร์ในมนุษย์ต่อไปได้

คำสำคัญ: Piperine, HPLC-UV, เภสัชจลนศาสตร์, เบญจกูล