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Marmara Pharmaceutical Journal

COUNTRY

Turkey

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Medicine
- Pharmacology (medical)

Pharmacology, Toxicology and Pharmaceutics
- Pharmacology, Toxicology and Pharmaceutics (miscellaneous)

PUBLISHER

Marmara University

Marmara University in Scimago Institutions Rankings

H-INDEX

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Journals

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SCOPE

Journal of Research in Pharmacy is the official scientific journal of Marmara University Faculty of Pharmacy. The journal is the continuation of the former "Journal of Pharmacy of University of Marmara" which was published between 1985 and 1997. Since 2010, the journal has been published online bimonthly (January-March-May-July-September-November). It is an open access, peer-reviewed journal devoted to the publication of papers in pharmacy and pharmaceutical sciences. The articles may be either in English or in Turkish. The journal aims at providing a medium for the dissemination of interdisciplinary papers of interest for many different specialists. Journal of Research in Pharmacy publishes original research papers, review articles and scientific commentaries on all aspects of pharmaceutical sciences depending on their conceptual novelty and scientific quality. The journal welcomes articles in this multidisciplinary field, with a focus on topics relevant for drug action, drug discovery and development, conventional and emerging fields related to pharmaceutical sciences. Articles, which cannot be associated with pharmaceutical issues in any way, might be returned to authors without processing. Scientific commentaries and review articles are generally evaluated by invitation or assent of the Editors. Proceedings of scientific meetings may also be published as special issues or supplements to the Journal, upon decision by the editors. Manuscripts submitted to Journal of Research in Pharmacy are only accepted on the comprehension that (I) they are subject to editorial review (at least by two independent referees); (II) they have not been, and will not be fully or partially published elsewhere; (III) the recommendations of the most recent version of the Declaration of Helsinki, for humans, and the European Community guidelines as accepted principles for the use of experimental animals have been adhered to.

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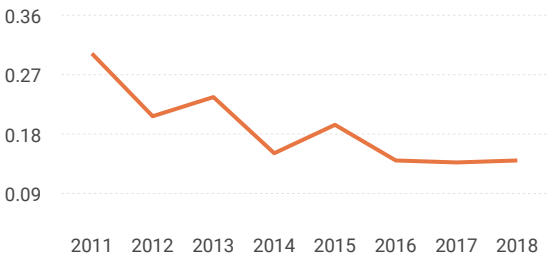

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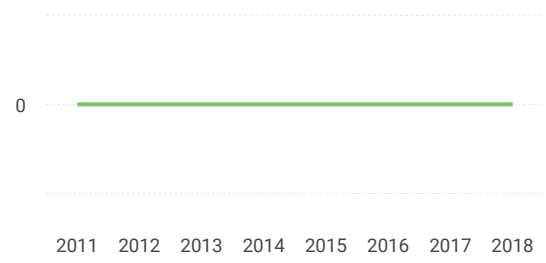

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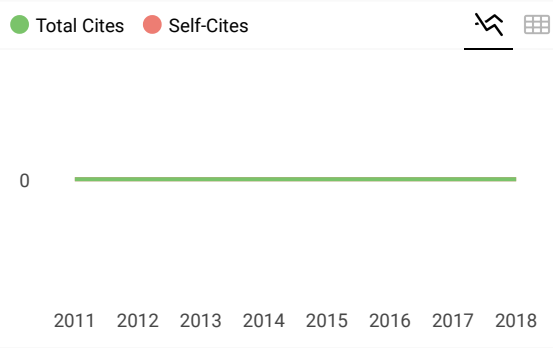

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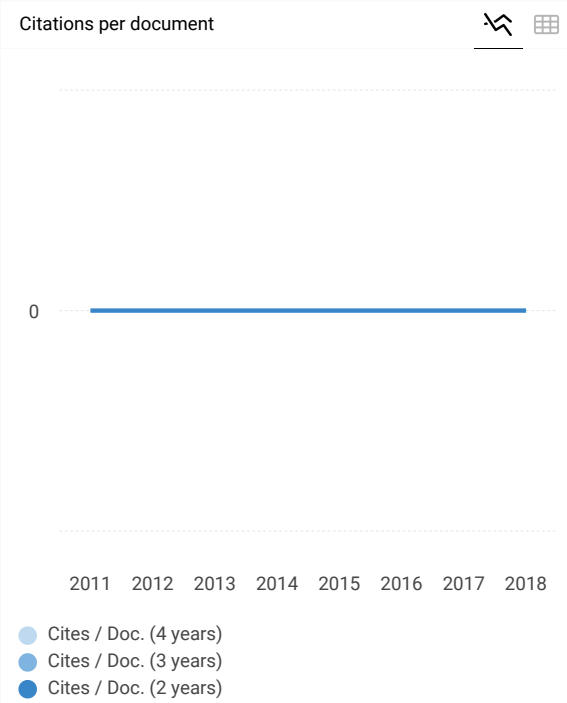

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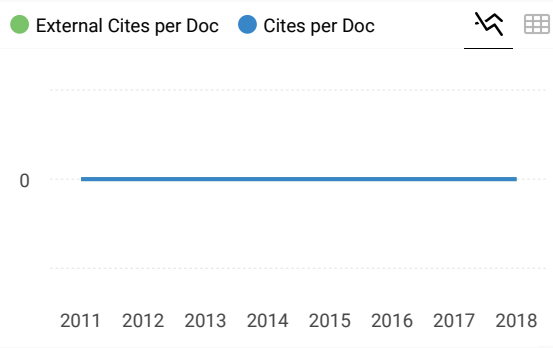

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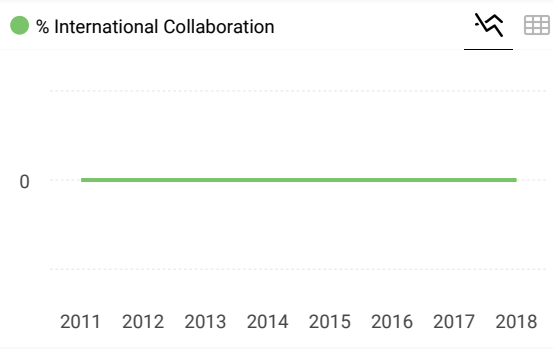

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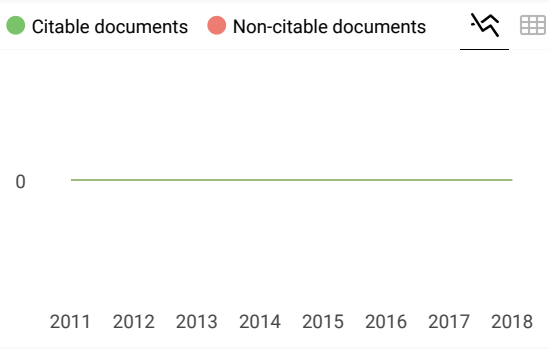

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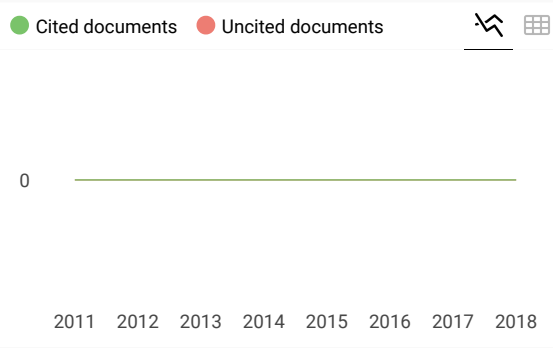

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Journal of Research in Pharmacy 2023 , Vol 27 , Issue 4

Articles (33)

Articles

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Pages 1329-1337

Siska Ayu PURNAMASARI,Dewi SETYANINGSIH

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Pages 1338-1345

Inci KAZKAYASI,Merve DENIZALTI

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Comparative Anatomical Investigations on Some Centaurea (Asteraceae) Taxa from Türkiye (abstract.php?id=1304)

Articles

Pages 1346-1355

Ebru ÖZDEMİR NATH,Şükran KÜLTÜR

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Chemometrics-assisted UV-spectroscopy for simultaneous determination of curcumin and piperine in solid dispersion-based microparticles containing Curcuma longa and Piper nigrum extracts (abstract.php?id=1305) *Articles*

Pages 1356-1365

Dewi SETYANINGSIH,Yustina Sri HARTINI,Dion NOTARIO

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Pharmacophore-based Virtual Screening: Identification of Selective Sirtuin 2 Inhibitors (abstract.php?id=1309) *Articles*

Pages 1366-1379

Berin KARAMAN MAYACK,Muhammed Moyasar ALAYOUBI

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Pages 1380-1387

Ayşegül HOŞ,Pınar Sinem OMURTAG ÖZGEN,Ayşe İNCİ,Buse AVCI,Ceyda CEYLAN

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Neuroprotective Effect of Ethanolic Extract of Pedalium murex Linn Leaf in 3-Nitropropionic acid Induced Neurodegeneration (abstract.php?id=1307) *Articles*

Pages 1388-1401

Suresh VELAYUTHAM,Arunprasanth Sundararaju,Thamotharan GOVINDHASWAMY,Muthukumaran Gurupackiyam,Anbukarasi Balakrishnan,Mohamed Yasar ABDUL,Chetan ASHOK,Srikanth JEYABALAN

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Pages 1402-1413

Shubhangi SUTAR,Snehal KORPALE,Sameer NADAF,Santosh GHULE,Sachinkumar PATIL

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Pages 1414-1420

Sapri SAPRI,Florentinus Dika Octa RISWANTO,Erna Tri WULANDARI

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Pages 1421-1430

Berin KARAMAN MAYACK,Muhammed Moyasar ALAYOUBI,Mikail Hakan GEZGINCI

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Pages 1431-1437

Oluwaseun Adenike ORUGUN,Emmanuel Maduabuchi URONNACHI,Joseph ISSAC,Zwanden Sule YAHAYA,Nnabuike Didacus NNAMANI

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Rahma NAFI'AH,Yeyet Cahyati SUMIRTAPURA,Sasanti Tarini DARIJANTO,Maria Immaculata IWO

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Pages 1451-1462

Avinash CHAUDHARY,Gaurav SHRANGARE,Sachin BHUSARI

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Pages 1463-1473

Timur Hakan BARAK,İrem DÖĞENCİ,Hilal BARDAKCI

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Pages 1474-1487

Cansu GONCUOGLU,Nesligul OZDEMIR,Emre KARA,Aygin BAYRAKTAREKINCIOGLU

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Pages 1488-1498

Olubunmi Jumoke OLAYEMI,Lucy Binda JOHN-AFRICA,Rashida ABDULLAHI,Christianah Yetunde ISIMI

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Nining NINING,Anisa AMALIA,Fatimatuz ZAHROK

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Pages 1513-1523

Cebrail AKYUZ,Emine ERDAG,Ayliz Velioglu-OGUNC,Nurettin ABACIOGLU,Ahmet Ozer SEHIRLI

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Pages 1524-1533

Fadilaturahmah FADILATURAHMAH,Resti RAHAYU,Putra SANTOSO

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Pages 1534-1546

Ilil Maidatuz ZULFA,Silfiana Nisa PERMATASARI

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Pages 1560-1566

Erkan CIVELEK,Dilek OZTURK CIVELEK

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Pages 1567- 1576

Gizem TATAR YILMAZ,Bedriye Seda KURŞUN AKTAR,Emine Elçin ORUÇ-EMRE

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Ravish PATEL,Rashesh DESAI,Amit PATEL,Shailvi SHAH,Bhupendra PRAJAPATI,Viral PATEL,Amit ALEXANDER

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Pages 1594-1604

Yener KURMAN,Görkem DÜLGER,Özge PASİN

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Pages 1605-1614

Jaikanth CHANDRASEKARAN,Pavadai PARASURAMAN,Panneerselvam THEIVENDREN,Krishnan SUNDAR,Damodar NAYAK AMMUNJE,Rex DEVASAHAYAM AROKIA BALAYA,Selvaraj KUNJIAPPAN

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Pages 1617-1625

Ayfer BECEREN,Sezgin AYDEMİR,Beste Melek ATASOY,Esin AK,Feriha ERCAN,T. Emre ŞENER,Göksel ŞENER

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Pages 1626-1637

Erhan KILICOGLU,Ayse Nur HAZAR-YAVUZ,Gul Sinemcan KABATAS,Yusufhan YAZIR,Levent KABASAKAL

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Pages 1638-1644

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Nagehan SALTAN,Yavuz Bülent KÖSE,Damla KIRCI,Fatih GÖGER,Betül DEMİRCİ

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Chemometrics-assisted UV-spectroscopy for simultaneous determination of curcumin and piperine in solid dispersion-based microparticles containing *Curcuma longa* and *Piper nigrum* extracts

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ABSTRACT: Piperine and curcumin can be combined in a mixture as piperine has been known as a bioenhancer. The piperine-curcumin combination was formulated in a solid dispersion-based microparticle containing *Piper nigrum* and *Curcuma longa* extracts. The aim of the study was to simultaneously determine piperine and curcumin concentrations in a combined dosage form of solid dispersion-based microparticles using UV-Vis spectrophotometry. A UV-Vis spectrophotometry was combined with a partial least square (PLS) approach with a central composite design (CCD) to develop a calibration series consisting of 36 standard mixtures of piperine and curcumin at concentrations ranging from 0 to 6 g/mL. The model of the calibration series was validated for determination coefficient (R^2), root means square of error prediction/cross-validation (RMSEP/RMSECV) dan predicted residual sum of square (PRESS). Accuracy and precision were determined as per ICH guidelines. The PLS model was successfully validated and applied for resolving overlaid spectra of piperine and curcumin at 206 – 408 nm. Accuracy and precision studies of prepared samples containing a mixture of piperine and curcumin at low, medium, and high concentrations conducted on different days were met with the AOAC International requirements. The limit of detection (LOD) was determined using a pseudo-variates model, and the limit was found to be 0.25 µg/mL and 0.33 µg/mL for piperine and curcumin, respectively. The proposed method is suitable for simultaneously determining piperine and curcumin that appeared in a mixture of *P. nigrum* and *C. longa* extracts in the solid dispersion-based microparticle samples.

KEYWORDS: a spectrophotometric method; curcuma; Partial Least Squares; piperine; solid dispersion

1. INTRODUCTION

Curcumin is a polyphenolic compound found in the *Curcuma longa* and *Curcuma xanthorrhiza* plants. It is a fundamental component of JAMU, an Indonesian traditional medicine believed to treat and prevent various maladies such as liver disease, digestive problems, and dysmenorrhea [1]. Curcumin/curcuminoids have demonstrated antioxidant, anti-inflammatory, and anticancer properties in preclinical and clinical research. Although curcuminoids have been shown to have a wide range of therapeutic properties with various biological targets and interactions, the clinical application of curcuminoids in formal therapy is limited by their poor bioavailability after oral administration. The bioavailability problem is caused by low water solubility, poor dissolution, absorption, and extensive metabolism once absorbed [2].

Piperine is a high lipophilic, weakly basic alkaloid component found in black pepper (*Piper nigrum*) extract that has been acknowledged as a bio-enhancer. It improves drug absorption, bioavailability, and bio-efficacy by stimulating gastrointestinal amino acid transporters and blocking drug-metabolizing enzymes [3]. Given the bioavailability-enhancing mechanism, combining piperine with curcumin in a formulation can combat curcumin's low bioavailability [4]. Because both compounds have poor water solubility, the solid dispersions approach is the preferred strategy for increasing solubility and dissolution. Solid dispersion-based microparticles containing *C. longa* and *P. nigrum* were developed in these studies, addressing product quality

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control. In the manufacturing stage, determining content in the final product is a part of quality assurance. Therefore, the amount of piperine and curcumin in the solid dispersion-based microparticle must be determined accurately. This research looked into an analytical approach that could quickly estimate piperine and curcumin concentrations in their combination in the formulation during regular laboratory analysis.

There is no official method in any pharmacopeia for simultaneously estimating piperine and curcumin present as a mixture in a dosage form [5]. Literature studies reveal that several analytical methods have been developed to simultaneously quantify piperine and curcumin in dosage forms, polyherbal formulations, and plasma samples. There is reverse-phase high-performance liquid chromatography (HPLC) equipped with a UV-Vis detector [6], Liquid Chromatography/Mass Spectroscopy (LC/MS) method for enabling simultaneous quantification of curcumin and piperine for pharmacokinetic evaluation [7,8], and high-performance thin layer chromatography (HPTLC) for simultaneous detection of piperine, curcumin and boswellic acid in a polyherbal transdermal patch [9]. While chromatographic methods have been shown to be selective for quantifying curcumin and piperine concentrations, the published methods require time-consuming sample extraction procedures and a substantial volume of organic solvents, making them less cost-effective and environmental risks due to the solvent waste.

The spectrophotometric method is one of the most preferred approaches for pharmaceutical analysis because it provides simplicity and inexpensiveness compared to other analytical methods due to natural native convenience and usefulness in most quality control studies of drugs. Spectroscopic combined with the application of Vierordt's equation was reported for determining piperine and curcumin concentrations in binary mixture samples measured at the same time analysis. The method was validated for simultaneous quantification of piperine and curcumin in dissolution and nanoparticle formulation samples following spectral measurement of the samples on the maximum wavelength of piperine and curcumin [5,10]. Despite the advantages of the spectroscopic method over the chromatographic method in terms of simplicity and the possibility of using Vierordt's equation for simultaneous measurement in multicomponent formulations, its widespread use poses challenges in the quality control phase of the manufacturing process. Recently, the application of multivariate models in a chemometric approach combined with spectrophotometry is the choice in multicomponent analysis.

Partial Least Square (PLS) is one of the multivariate models acknowledged to be used in many quantitative assays of pharmaceutical formulations among the numerous chemometric techniques applied to multicomponent analysis [11]. PLS are generally used to set up the multivariate model based on two data sets (of the same objects), the chemical values, and the spectra. PLS regression aims to establish a model that allows the analysis of an unknown sample. The PLS has been examined as a chemometric technique for resolving the resolution of overlapping spectra in multicomponent analysis resulting from UV-Vis spectrophotometry. Furthermore, when combined with chemometric data, the PLS methodology allows quantification in a multicomponent mixture with findings that are equivalent to HPLC and in agreement with HPLC results with an accuracy of 98-103 percent [12].

To the best of our knowledge, there are no publications for the simultaneous determination of piperine and curcumin in a formulation based on the UV–Vis spectroscopy and multivariate calibration methods. The work is the first study for the simultaneous analysis of piperine and curcumin in combined pharmaceuticals using chemometrics-assisted spectrophotometric methods based on multivariate calibration techniques, mainly PLS. The study's objective was to develop a UV-Vis spectroscopic method with a PLS approach for simultaneously determining piperine and curcumin concentrations in a combined dosage form of solid dispersion-based microparticles.

2. RESULTS

2.1 Wavelength scanning of piperine-curcumin

Figure 1 indicates the absorption spectra of piperine and curcumin in methanol as individual reference standard compounds of piperine (3µg/mL) or curcumin (3µg/mL), their mixture (3/3 µg/mL) in methanolic solution and the synthetical solid dispersion-based microparticle containing *P. nigrum* and *C. longa* dissolved in methanol. In the measurement range of 200-600 nm, the spectra of piperine (Figure 1 c) overlap with curcumin spectra (Figure 1 a) at 300 – 400 nm. Given that the maximum absorption spectra of piperine in this study was found at 343 nm (Figure 1c), the determination of piperine in the co-existence of curcumin (Figure 1 b, d) using the conventional spectroscopic method leads to significant analytical error. Therefore, combining spectrophotometry with chemometric techniques was necessary for such determination due to the significant interference of piperin and curcumin spectra.

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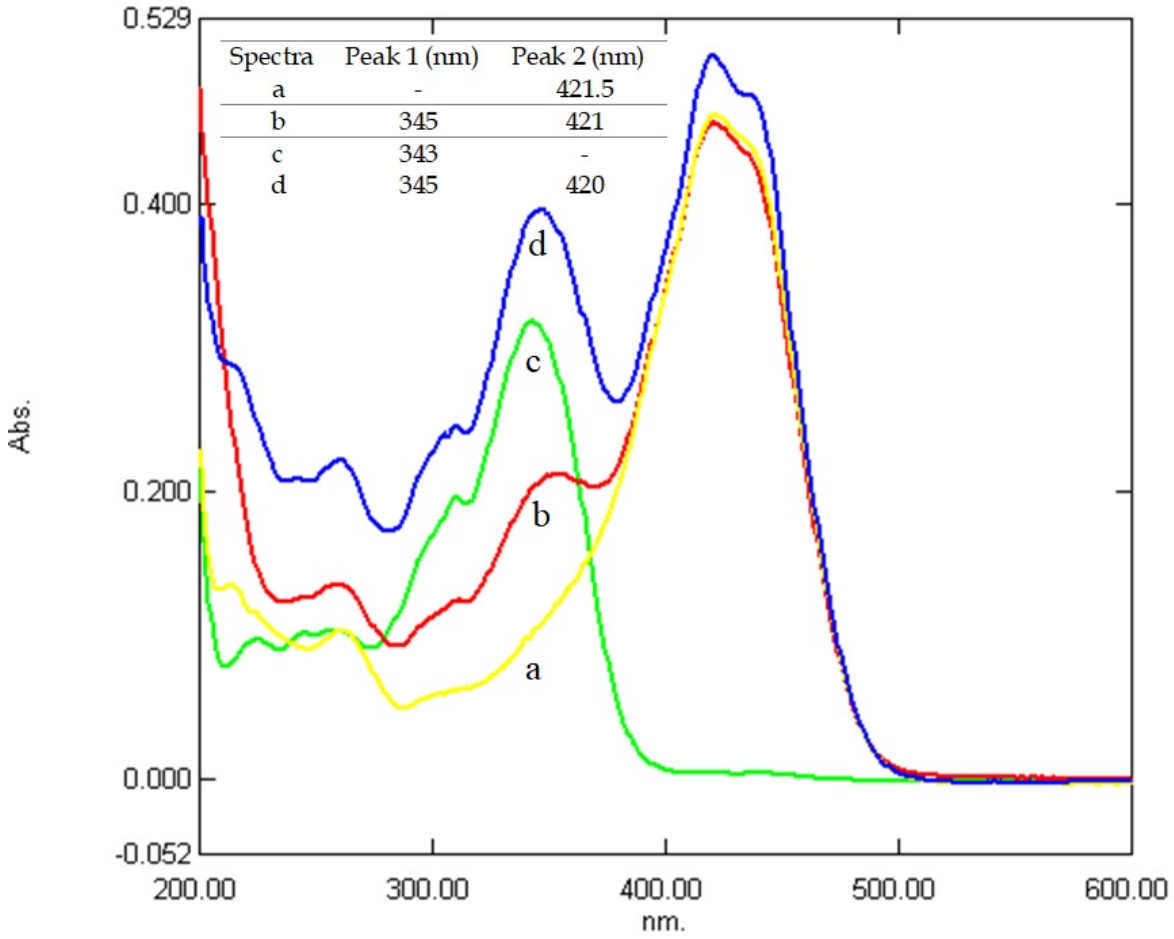

Figure 1. Representative UV-vis spectra of piperine and curcumin in pure and mixture standard solutions and synthetical sample. All samples were diluted in blank solution.
Note: a = curcumin 3 µg/ mL; b = synthetical sample of SD formulation containing *P. nigrum*/*C. longa*. c = piperine 3 µg/mL; d = mixture of piperine/curcumin standard (3/3 µg/mL)

2.2 Chemometric approach: PLS-assisted spectrophotometry

PLS were used in a chemometric-aided spectrophotometry approach to resolve strong overlapping absorption spectra of piperine and curcumin for the simultaneous identification of both chemicals in a mixed dose form. Among other chemometric models, such as Principal Component Regression (PCR) and Principal Component Analysis (PCA), PLS has been regarded as a powerful tool for resolving the interference between multiple overlapping spectra of many compounds, which necessitates the determination of multiple components as a mixture [12]. In addition, Palur, Archakam, and Koganti (2020) discovered that PLS-assisted spectrophotometry had high method selectivity for the simultaneous detection of paracetamol, diphenhydramine, caffeine, and phenylephrine concentrations in a tablet dosage form. That data was comparable to the HPLC approach [13].

To create a PLS model of the calibration, this study used 36 samples of piperine and curcumin at various concentrations, including the blank sample. The calibration samples' UV-vis spectra in the 200-600 nm range were pre-treated by deleting the less informative data, and the wavelength region offering the most usable data was chosen to develop the PLS model. The selection of a spectra region was reported to improve the prediction accuracy (Kambira et al., 2020). The spectra region to build the PLS model for piperine was the wavelengths (nm) of 206, 211, 220, 221, 223, 249, 250, 258, 283, 299, 300, 314, 316, 320, 322, 328, 332, 342, 346, 350, 355, 356, 369, 385, 396, 399, 403, 412, 422, 447. PLS of curcumin calibration was selected at the wavelength (nm) region of 209, 288, 290, 291, 292, 296, 300, 306, 314, 319, 323, 345, 346, 359, 373, 375, 379, 394, 395, 402, 405, 457, 463, 464, 482. The wavelengths below 206 nm were excluded since their contribution to the measurement

was considered minor. Furthermore, wavelengths greater than 482 nm were avoided since, while curcumin's spectra absorption is minimal, any absorbance value greater than 482 would introduce noise into the calibration, thereby increasing imprecision.

The number of principal components (NComp) is critical for PLS regression development because the number of components should account for as much of the experimental data as possible without overfitting [12]. In this study, the number of components was determined using a cross-validation method leaving out one sample at a time technique [14]. The optimum number of components found in these studies is 7 for piperine and 6 for curcumin (Table 1).

Table 1. Parameters of regression on PLS model

Parameters	Piperine	Curcumin
NComp	7	6
R^2	0.998	0.998
RMSECV (µg/mL)	0.071	0.093
RMSEP (µg/mL)	0.179	0.156
PRESS (ug/mL)	0.176	0.305
Wavelength (nm)	206, 211, 220, 221, 223, 249, 250, 258, 283, 299, 300, 314, 316, 320, 322, 328, 332, 342, 346, 350, 355, 356, 369, 385, 396, 399, 403, 412, 422, 447	209, 288, 290, 291, 292, 296, 300, 306, 314, 319, 323, 345, 346, 359, 373, 375, 379, 394, 395, 402, 405, 457, 463, 464, 482

The PLS regression model of piperine and curcumin calibrations was cross-validated using the leave-one-out technique. The actual sample concentrations (measured concentrations) were plotted against the expected concentrations of all calibration samples (Figure 2). This internal validation of the PLS model was done by determining the goodness of fit parameters for the simultaneous piperine and curcumin calculation, such as coefficient of determination (R^2), RMSECV, RMSEP, and PRESS. The RMSECV was used a diagnostic test to examine the errors in the predicted concentrations. It denotes the precision and the accuracy of predictions [12]. The number demonstrating the lowest RMSECV, RMSEP, and PRESS values was selected for building the PLS calibration model [15]

Furthermore, the PLS regression of the calibration model was considered to be good, with the best prediction if the correlation value R^2 is greater (greater than 0.91 or close to 1) [16]. Table 1 shows the final RMSECV, RMSEP, PRESS, and R^2 values. As shown in Table , the R^2 values were found to be 0.998 for piperine and curcumin. Altogether, the validation parameters determined in this study indicate that the PLS model of piperine and curcumin calibration demonstrates strong predictive capacity.

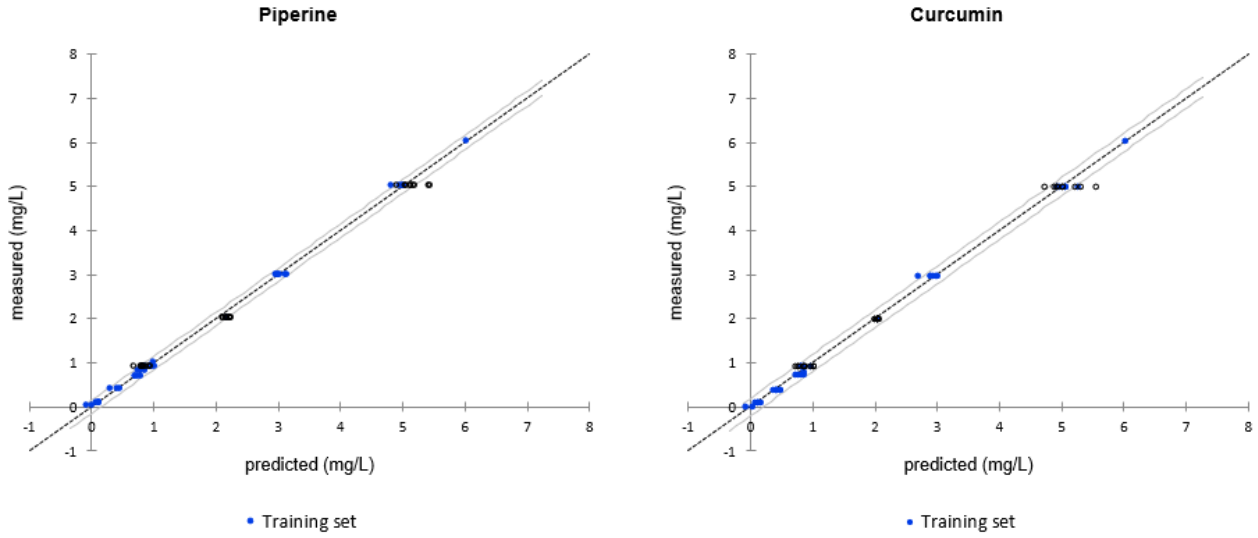

Figure 2. Validation plot of PLS regression models of piperine and curcumin

2.3 Accuracy and precession

Accuracy and precision analyses were conducted to validate the selected PLS regression model of the piperine and curcumin. The accuracy and precision studies were conducted on three concentrations of independent samples containing piperine and curcumin. Concentrations of 0.9, 2, and 5 µg/mL were prepared to represent low, middle, and high concentrations of piperine and curcumin. Table 2 summarizes the accuracy and precision parameters. The recovery/RSD values of piperine were 84.07% - 110.90%/0.56%-10.31% for intra-day assay and 92.99%-107.87%/2.65%-10.26% for inter-day assay. The intra-inter-day determination of recovery/RSD values of curcumin resulted in the value of 83.27%-110.85%/0.27%-10.31% (intraday) and 96.31%-101.20%/1.29% - 12.85% (inter-day). A higher RSD value of 12.85% was demonstrated by the sample containing curcumin at 0.9 µg/mL obtained on inter-day studies. A calculation on Predicted Relative Standard Deviation ($PRSD_R$) using Horwitz formula [17] demonstrated that the maximum RSD_R value at a concentration of 0.9 µg/mL is 16%. For the inter-day analysis, the RSD should consider the $PRSD_R$. From this number, it can be concluded that the PLS-developed method was accurate and precise and demonstrates excellent reproducibility as shown by the data of inter-day assay.

2.4 Model sensitivity

The PLS regression model sensitivity was determined based on the pseudo-univariate method [18]. From the pseudo-univariate line, the limit of detection (LOD) values was found at 0.25 µg/mL and 0.33 µg/mL for piperine and curcumin, respectively. A low LOD demonstrated from this study indicates the high sensitivity of the PLS regression method developed in this study, which allows the determination of piperine and curcumin in commercial samples primarily prepared from the extract forms.

Table 2. Accuracy and precision studies

Compound	Concentration (mg/L)	Day analysis	Intraday (n=3)		Inter-day (n=9)	
			Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Piperine	0.9	Day 1	84.07	10.31	92.99	10.26
		Day 2	96.01	4.12		
		Day 3	98.89	9.49		
	2	Day 1	110.90	0.65	107.87	2.65
		Day 2	107.92	1.64		
		Day 3	104.79	1.00		
	5	Day 1	102.65	1.82	103.58	3.30
		Day 2	102.58	0.56		
		Day 3	105.50	5.57		
Curcumin	0.9	Day 1	110.85	2.59	96.31	12.85
		Day 2	83.27	3.20		
		Day 3	94.80	4.95		
	2	Day 1	102.10	1.31	101.20	1.29
		Day 2	101.06	1.67		
		Day 3	100.44	0.27		
	5	Day 1	97.49	3.00	101.11	5.00
		Day 2	98.88	1.21		
		Day 3	106.98	3.44		

2.5 Assay on synthetical and commercial samples

The validated PLS regression model was used to assess piperine and curcumin concentrations in commercial samples and the solid dispersion-based microparticle containing *P. nigrum* and *C. longa* extracts. Table 3 displays the assay results on solid dispersion-based microparticles containing *P. nigrum* and *C. longa* extracts. The high value of recovery of 98.42 % and 103.09%, respectively, and the low value of RSD of 3.76 % and 4.26%, respectively, show that the results match the intended piperine and curcumin concentrations in the synthetical samples.

Further application of the proposed PLS regression model was conducted to measure piperine and curcumin concentrations in the commercial sample of tablet dosage containing *P. nigrum* and *C. xanthorrhizae.* The assay is presented in Table 4 and is revealed the concentration of 0.07% w/w and 0.08% w/w of piperine and curcumin in the dosage form with the RSD values of 12.13% and 9.32% for piperine and curcumin, respectively. A relatively higher RSD values of piperine and curcumin found in this sample were thought to be related to the concentrations of the low analytes at which the these monitored concentrations were around the limit of detection. Referred to the procedure of the commercial sample preparation in the method section of this manuscript, with each commercial tablet containing 20 mg of *C.xanthorrhizae* rhizome and 2.5 mg of *P.nigri* Fructus extracts, the samples subjected to analysis in this study could contain the maximum concentrations of 0.72 µg/mL and 5.74 µg/mL of *P.nigri* Fructus and *C.xanthorrhizae* rhizome extracts. Given that the extract may contain components other than piperine (*P.nigri* Fructus) and curcumin (*C.xanthorrhizae*) and the LOD values found in the proposed method, the maximum extract concentrations of the sample could have meager amounts of piperin or curcumin at which it may around the detection limits.

Table 3. Analysis of synthetical samples

Replication	Piperine (%w/w)		Curcumin (%w/w)	
	Amount (mg)	Concentration (%w/w)	Amount (mg)	Concentration (%w/w)
1	1.87	9.37	6.27	31.33
2	1.98	9.93	6.80	34.02
3	1.83	9.16	6.45	32.23
4	9.75	1.95	6.84	34.18
5	2.00	10.00	7.21	36.04
6	1.93	9.64	7.08	35.40
Mean (%w/w)	1.87	9.37	6.87	34.37
RSD (%)	3.76	3.76	4.26	4.26
Recovery (%w/w)		98.42		103.09

Table 4. Analysis of commercial samples

Replication	Piperine		Curcumin	
	Amount in tablet (mg)	Concentration (%w/w)	Amount in tablet (mg)	Concentration (%w/w)
1	0.23	0.06	0.29	0.07
2	0.29	0.07	0.36	0.09
3	0.26	0.06	0.33	0.08
4	0.29	0.07	0.35	0.08
5	0.32	0.08	0.34	0.08
6	0.25	0.06	0.28	0.07
Mean (%w/w)	0.27	0.07	0.33	0.08
RSD (%)	12.13	12.13	9.32	9.32

3. CONCLUSION

It can be concluded that PLS is a very efficient method for the simultaneous determination of substances with overlapped spectra in mixtures when the contributions of components to the composite spectra are much disparate. The proposed PLS regression model is suitable for the simultaneous determination of piperine and curcumin in a synthetical solid dispersion-based microparticle containing *P. nigrum* and *C. longa* using the simple spectrophotometric method without any separation works in the sample preparation. The method is considered a selective, sensitive, rapid, and accurate analytical method employing UV-Vis spectroscopy combined with the chemometric approach and is applicable for routine analyses.

4. MATERIALS AND METHODS

4.1 Chemicals

USP-grade reference standard compounds of curcumin and piperine were purchased from Sigma-Aldrich (St. Louis, USA). *C. longa* extract was obtained as a gift from PT Phytochemindo Reksa, Bogor, Indonesia (purity of 97.56% w/w curcumin analyzed by spectrophotometry). *P. nigrum* extract (purity of 98.97% w/w of piperine as determined by HPLC) was isolated using the reported method [19]. PVP K30 was a gift from PT Konimex, Solo, Indonesia. Pro-analytical grades of methanol and ethanol were obtained from Merck (Darmstadt, Germany). De-ionized water was prepared using a Milli-Q IQ water purification system. Synthetical samples of solid dispersion-based microparticles containing *C. longa* rhizome and *P. nigrum* fructus extracts were prepared using a solid dispersion technique, a solvent evaporation method, in a Büchi mini-spray-dryer (Büchi, Flawil, Switzerland) as described previously [6]. Commercial tablets containing curcumin and piperine of *C. xanthorrhiza* dan *P. nigrum* extracts were obtained through the local pharmacy in Yogyakarta, Indonesia (batch number of 20H0152, Expiry date of 12-2022). Each tablet contains 20 mg of *C. xanthorrhizae* rhizome and 2.5 mg of *Piperis nigri* Fructus extracts. The weighted average of 20 tablets was determined in our laboratory and was found to be 417.75 ± 6.41 mg.

4.2 Preparation of stocks solutions dan calibration graph

The individual stock of reference standard solutions of curcumin and piperine were prepared in methanol at 1 mg/mL concentrations under sonication for 15 minutes in an Ultrasonic Bath (Fisher FS140H). The stock solution was stored under -20°C for a maximum of 3 weeks.

4.3 Preparation of calibration series

In order to obtain a suitable calibration set, a systematic experimental design was used. For designing the multilevel concentrations in calibration series, this study employed a Central Composite Design (CCD) combined with some replication samples at specific concentrations, as presented in the run of Table 1. R-studio software of 3.5.1 version was used to generate the CCD in the runs of the calibration series. The calibration samples were prepared by spiking the blank with the stock solutions of curcumin and piperine to achieve compositions of curcumin and piperine in mixture solutions, as described in Table 1. The solid dispersion carrier's methanolic solution, a PVP K30 (70% w/w) solution, was used as the blank sample. All calibration samples which were filtered through a 0.45 µm filter size, were scanned in the wavelength range of 200-600 nm with a reading interval of 1 nm (UV-VIS 1800 Shimadzu, Japan).

4.4 Generating Partial Least Square (PLS) model on calibration data

A calibration model based on PLS model was developed for the simultaneous quantification of curcumin and piperine in solid dispersion-based microparticles and commercial samples. Calibration series, including blanks samples, were used to construct PLS model using an XL-STAT add in Excel software of 2020.4.1.1027 version. A step of backward elimination was conducted to choose the latent variable of the wavelength in the range of 200-600 nm. Replicated samples at certain concentrations shown in were used as internal validation set for the PLS-generated model. Leave-One-Out-Cross-Validation (LOOCV) based on Jack-Knife method was conducted to select the suitable PLS model. R^2, Root Mean Square Error of Cross-Validation (RMSECV), and Root Mean Square Error of Cross-Validation Prediction (RMSEP) are the parameter to assess the calibration and were calculated on the XL-STAT. The lowest number of the components and wavelength resulting coefficient of determination (R^2) above 0.91, smaller RMSECV/RMSEP, and lowest predicted residual sum of square (PRESS) were selected [16].

The selected PLS model obtained by the internal validation method was subjected to an external validation step using an accuracy and precision test. The samples were prepared independently from the calibration series to conduct the accuracy and precision tests. The samples were prepared as a mixture of piperine and curcumin at three concentration levels by spiking the blank sample to result in the concentrations of piperine and curcumin of 0.9-0.9, 2-2, and 5-5 µg/mL. These three levels represented low, middle, and high sample concentrations. The accuracy and precision studies were conducted on three consecutive days. Recovery and Relative Standard Deviation (RSD) values obtained from the three replications in every concentration level were judged according to the Guidelines for Standard Method Performance Requirements in the Association of Official Agricultural Chemists (AOAC)[17].

Table 5. Calibration series of curcumin and piperine mixture

Run	CUR (µg/mL)	PIP (µg/mL)	Run	CUR (µg/mL)	PIP (µg/mL)	Run	CUR (µg/mL)	PIP (µg/mL)
1	0.9	0.9	**13**	5	0.9	**25**	0.4	0
2	0.9	0.9	**14**	3	6	**26**	0.1	0.1
3	0.9	0.9	**15**	6	3	**27**	0.1	0.1
4	3	3	**16**	3	3	**28**	0.1	0.1
5	5	5	**17**	3	3	**29**	0.7	0.7
6	5	5	**18**	3	3	**30**	0.7	0.7
7	5	5	**19**	0.8	0.4	**31**	0.7	0.7
8	0	3	**20**	0.4	0.8	**32**	0	0.7
9	0.9	5	**21**	0.4	0.8	**33**	0.1	0.4
10	3	0	**22**	0.4	0.8	**34**	0.4	2
11	5	0.9	**23**	0.7	0.1	**35**	2	1
12	5	0.9	**24**	0.4	0.4	**36**	0	0

4.5 Pharmaceutical sample preparation

4.5.1. Synthetical SD samples

A 20.0 mg of synthetical samples of solid dispersion-based microparticle formulation containing *C. longa* and *P. Nigrum* extracts in PVP K30 matrix was accurately weighed and dissolved in 25.0 mL methanol in a volumetric flask. The samples were 100 times diluted with methanol and filtered through 0.45 µm size, and analyzed for spectrophotometry on 200-600 nm at 1 nm reading interval.

4.5.2 Commercial sample

Twenty commercial tablets were weighed individually and ground into finely powdered. A 100.0 mg powder was accurately weight and dissolved in 100 mL of methanol. A volume of 0.6 mL was transferred into a 5 mL volumetric flask and diluted with methanol. The samples were filtered through a 0.45 µm filter before the spectral measurement on 200-600 nm using a spectrophotometer at a 1 nm reading interval.

4.5.3 Concentrations determination

The sample spectrum data were analyzed on the validated PLS calibration model to predict then unknown curcumin and piperine concentrations in synthetical and commercial SD samples.

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Conflict of interest statement: The authors declared no conflict of interest in the manuscript.

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