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Research Article

CHEMOMETRICS-ASSISTED UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF LOPINAVIR AND RITONAVIR IN SYRUP

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ABSTRACT

Lopinavir and ritonavir are effective HIV-1 protease inhibitors. Chemometric-assisted spectrophotometric method was proposed for the determination of these two drugs in syrup. A set of calibration mixtures (calibartion set) was design by the Central Composite Design (CCD). The UV spectra results obtained from the calibration set were subjected to principle component regression (PCR) and partial least squares regression (PLS-1) to construct the prediction models for lopinavir and ritonavir in unknown samples. A set of synthetic mixtures was randomly selected and used as samples to validate the models. The prediction models were used to analyze two drugs in syrup and compared with an HPLC method. The results of PCR and PLS-1 methods were agreed with the results obtained from HPLC method.

Keywords: Chemometrics, PCR, PLS-1, Lopinavir, Ritonavir

INTRODUCTION

Two protease inhibitors, lopinavir and ritonavir, are important drugs in highly active anti-retroviral therapy (HAART). Protease inhibitor can prevent viral replication by inhibiting the activity of HIV-1 protease enzyme which is necessary to cleave nascent proteins for final assembly of new virons. Lopinavir and ritonavir are manufactured as a fixed combination by Abbott Laboratories under the trade name Kaletra®. Lopinavir is approximately ten times more potent than ritonavir against wild-type HIV. In order to prohibit the metabolism of CYP3A4, small amount of ritonavir is used as pharmacokinetics booster in Kaletra® [1].

For analysis of lopinavir and ritonavir, high-performance liquid chromatography (HPLC)[2-4], high-performance liquid chromatography coupled to tandem mass spectrometry (LC/MS)[5-9], LC/MS/MS[10], matrix-assisted laser desorption/ ionization tandem time-of-flight (MALDI-TOF/TOF)[11], HPTLC[12] and immunoassay[13] methods have been reported for quantitative determination of lopinavir and ritonavir. All of these, chromatographic method, high-performance liquid chromatography (HPLC) in conjunction with UV detection was the most widely used. Analysis method utilizing spectrophotometric measurement without prior chromatographic separation is interesting but overlapping of absorbance spectra is a problem. However, chemometrics is a key and may solve this limitation. Chemometrics was introduced in 1972 by Svan Wold[14]. It is a chemical discipline which uses mathematical and statistical methods to design or select optimal measurement procedures and experiments and provide maximum chemical information by analyzing chemical data[15]. In brief, chemometrics concerns with chemical data and extraction of relevant information from it by using suitable statistical and mathematical methods. Multivariate calibration method (e.g., multiple linear regression (MLR), principle component regression (PCR) and partial least squares (PLS)) utilizing spectrophotometrics data are the important chemometrics approach for determination of mixtures including drugs combination[16].

In this work, two chemometric-assisted spectrophotometric methods based on the application of principle component regression (PCR) and partial least squares (PLS-1) were proposed for analysis of lopinavir and ritonavir in synthetic mixtures (test set) and true sample (syrup). Especially, this was the first study for determination of lopinavir and ritonavir by PCR and PLS-1 approaches**.** An HPLC method was employed to determine the same syrup for comparison.

MATERIALS AND METHODS

Apparatus and software

The absorbance spectra were recorded by a Shimadzu (UV-160A) UV-Vis spectrophotometer combined with a 1 cm quartz cell. Chromatography was performed on a high-performance liquid chromatography system (Shimadzu corporation, Kyoto, Japan) consisting degasser DGU-12A, liquid chromatograph LC-10 AD, communications bus module CBM-10A, UV-Visible detector SPD-10A and data processing (class LC-10). The analytical column was a BDS Hypersil C18, 250 x 4.6 mm i.d., 5µm (Thermo scientific, USA). Manual injection was made by using a Rheodyne model 7725 injector with a 20-µl loop. Unscrambler® program was purchased from Charpa Techcenter Co., Ltd. (Bangkok, Thailand). Data analysis, PCR and PLS-1 modeling were performed by Unscrambler® program.

Reagents

Standard lopinavir and ritonavir, syrup containing 80 mg/mL of lopinavir and 20 mg/ml of ritonavir and syrup blank were kindly supported from the Government Pharmaceutical Organization (GPO) of Thailand. Acetonitrile and methanol (HPLC grade) were purchased from Lab-Scan, Thailand. Ammonium acetate (Analytical grade) was obtained from Ranken, India. Acetic acid and ammonium hydroxide (Analytical grade) were obtained from Lab-Scan, Thailand. Stock solutions of drugs (1 mg/ml) were prepared by dissolving appropriate amount of solutes in methanol. Working solutions of lower concentrations were prepared by proper dilution from the stock standard solutions and diluted to volume with 50% methanol. Sample preparation was prepared by dilution of syrup with methanol until the final concentration (8 µg/ml of lopinavir and 2 µg/ml of ritonavir) was obtained. Syrup blank working solution was prepared by 100 times dilution of syrup blank with methanol.

One component calibration

To find the linear dynamic concentration range of each drug, one component calibration was performed. Linear dynamic ranges were studied in the concentration range of 4-24 µg/ml for lopinavir and 1-6 µg/ml for ritonavir. Absorbance values were recorded at λ_{max} of each drug (259 nm for lopinavir and 239 nm for ritonavir) in 1-cm quartz cell and used 50% methanol as blank. Linear dynamic range for each compound was determined by least-square linear regression of concentration and the corresponding absorbance.

Binary standards solutions

Two sets of standard solutions, calibration set and test set were prepared. According to Table 1 and 2, 20 mixtures solutions and 15 mixtures solutions were used in calibration set and test set, respectively. The concentrations of calibration set were selected by mean of central composite design (CCD) and those of test set were randomly selected. A 100 µl of syrup blank working solution was added to all solution in calibration and test sets.

HPLC method for analysis of lopinavir and ritonavir

An HPLC method was previously developed for simultaneous determination of lopinavir and ritonavir[17]. The optimum chromatographic condition used a BDS Hypersil C18, 250 x 4.6 mm i.d., 5µm as stationary phase. A mixture of 5mM ammonium acetate buffer, pH 7 and acetonitrile (50:50, v/v) was used as mobile phase and the flow rate was 1 ml/min. The presences of lopinavir and ritonavir were detected by UV detector at 245 nm. This HPLC method was used to determine lopinair and ritonavir in test set and syrup samples for comparison with PCR and PLS-1 models.

RESULTS AND DISCUSSION

Quantitative determination of compounds in sample is usually performed by HPLC[18-21]. Spectrophotometric method is more simple but sometime has limitation for using in the mixture. Lopinavir and ritonavir absorb UV in the same region (Fig. 1), this was not allowed for simultaneous determination of these compounds by conventional univariate calibration methods. Therefore, multivariate calibration methods such as PCR and PLS-1 were employed for simultaneous analysis of them.

The standard solutions used in the multivariate calibration methods are mixtures of analytes. Some important parameters should be considered in preparing of these standard solutions[22] .The first one is that the concentration of each analyte must be in its linear dynamic range. The concentration of the analytes in the calibration samples (Table 1) must be orthogonal. The absorbance of calibration samples should not exceed the maximum absorbance reading of the instrument, and the concentration of the prediction mixtures (test set) should be the same range as that of the calibration mixtures.

Table 1: Compositions of CCD design for construction the determination models of lopinavir and ritonavir (calibration set)

Sample	Concentrations (µg/ml)		Volume of placebo stock solution (µl)
	Lopinavir	Ritonavir	
1	0.00	2.00	100
2	16.00	2.00	100
3	8.00	0.00	100
4	8.00	4.00	100
5	2.30	0.60	100
6	13.60	0.60	100
7	13.60	3.40	100
8	2.30	3.40	100
9	8.00	2.00	100
10	8.00	2.00	100
11	8.00	2.00	100
12	8.00	2.00	100
13	8.00	2.00	100
14	8.00	2.00	100
15	8.00	2.00	100
16	8.00	2.00	100
17	0.00	0.00	100
18	0.00	0.00	100
19	0.00	0.00	100
20	0.00	0.00	100

Table 2: Compositions of samples for test set

Sample	Concentrations (µg/ml)		Volume of placebo stock solution (µl)
	Lopinavir	Ritonavir	
1	4.00	1.00	100
2	7.00	1.75	100
3	8.00	2.00	100
4	9.00	2.50	100
5	12.00	3.00	100
6	10.00	0.60	100
7	12.00	1.00	100
8	4.00	3.00	100
9	2.40	2.00	100
10	15.00	1.50	100
11	7.00	3.75	100
12	6.00	2.50	100
13	8.00	1.75	100
14	13.00	0.75	100
15	3.00	3.15	100

The resulted univariate calibration equations for the analytes at λ_{max} (259 and 239 nm for lopinavir and ritonavir, respectively) were linear in the ranges of 4-24 µg/ml for lopinavir and 1-6 µg/ml for ritonavir (Fig. 2). To prevent obtained solutions with overload absorbencies, the concentrations of lopinavir and ritonavir in the mixtures were taken in the ranges of 0-16 and 0-4 µg/ml, respectively. The composition of the test samples (Table 2) was selected randomly according to the linear dynamic ranges.

Results of PCR and PLS-1 analysis

The PCR and PLS-1 models were developed in Unscrambler® program. Model development was performed by using calibration

standards. Leave-one-out cross-validation (LOO-CV) was used to validate PCR and PLS-1 models in model development and obtaining optimum latent variables (number of factors) of model. The resulted models were also validated to predict the concentration of analytes in a separate test set that did not contribute to the model development steps. The results of prediction and the percentage of recoveries are represented in Table 3. As observed, there is a very good agreement between the predicted (calculated) and actual concentrations of drugs. The mean recoveries for lopinavir and ritonavir are 105.5% and 92.5% for PCR models and 105.3% and 94.8% for PLS-1 models, confirming the high prediction power of the resulted models. Then the suitable PCR and PLS-1 models were employed to analyze both drugs in syrup and three determinations were performed. The determination results are presented in Table 4. As seen from this Table 4, the data indicate excellent reproducibility of the prediction by the proposed models with the standard deviation (SD) less than 0.33 for all cases.

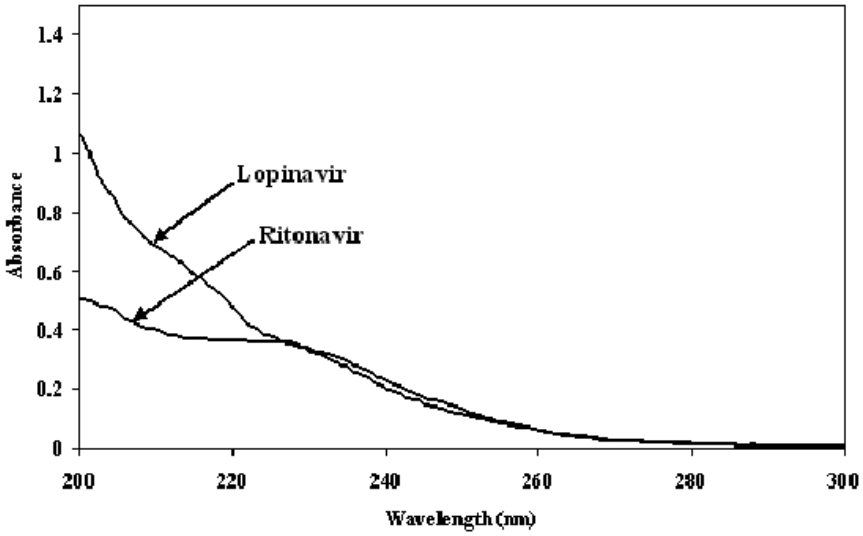

Fig. 1: UV spectra of lopinavir and ritonavir

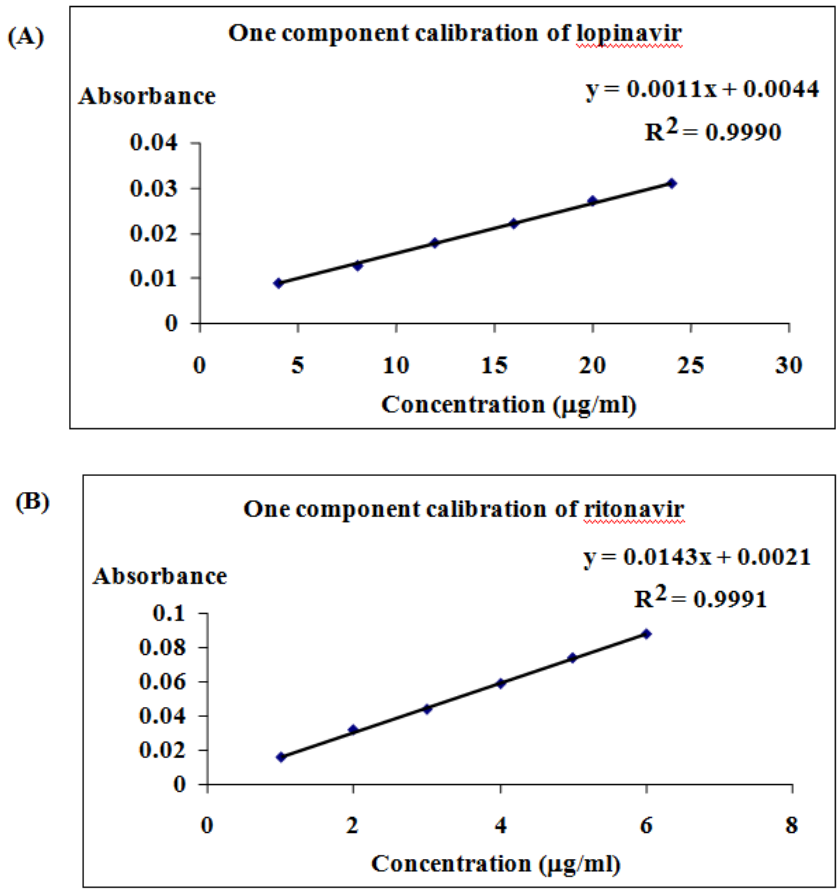

Fig. 2: (A) Absorbance values at 259 nm versus concentrations (µg/ml) of standard lopinavir and (B) Absorbance values at 239 nm versus concentrations (µg/ml) of standard ritonavir

Table 3: Comparison of PCR and PLS-1 models for determination of lopinavir and ritonavir in test set

Test set number	Lopinavir (% recovery)		Ritonavir (% recovery)	
	PLS-1	PCR	PLS-1	PCR
1	102.7	104.2	92.0	71.0
2	104.1	104.3	91.4	87.4
3	106.7	107.1	88.0	99.0
4	105.9	106.0	101.8	95.6
5	104.9	104.9	95.7	99.0
6	102.1	102.4	86.7	121.7
7	102.5	102.5	89.0	104.0
8	111.5	111.7	83.3	93.0
9	106.7	107.5	91.0	87.0
10	102.0	100.9	112.7	83.3
11	108.0	106.7	85.9	76.5
12	102.7	103.2	97.6	93.6
13	104.9	105.0	97.7	96.0
14	99.0	98.7	121.3	90.7
15	115.3	117.0	88.0	89.5
Average	105.3	105.5	94.8	92.5
SD	4.1	4.4	10.4	11.8

Comparison of the PCR and PLS-1 models with HPLC

In order to compare the results of the proposed PCR and PLS-1 models for determination of lopinavir and ritonavir in syrup, an HPLC method[17] was also employed. The same sample solutions used for PCR and PLS-1 models were applied by HPLC method. The determination results of PCR, PLS-1 and HPLC methods are presented in Table 4. The determination data were expressed in term of µg/ml. As shown in this Table, amount of drugs analyzed by HPLC method were closed to those obtained by PCR and PLS-1 models, especially for lopinavir. In case of ritonavir, the results obtained from PLS-1 and PCR models were significantly less than that of HPLC method. This may be due to the fact that the UV spectrum of ritonavir is absolutely covered with the spectrum of lopinavir (Fig. 1) and is not allow for application of multivariate calibration model. In the other word, multivariate calibration model may suitable for the mixtures which their UV spectra are partially overlapping.

Table 4: Syrup determination results of PLS-1, PCR and HPLC methods

Sample	Lopinavir (µg/ml)			Ritonavir (µg/ml)		
	PLS-1	PCR	HPLC	PLS-1	PCR	HPLC
1	8.06	8.02	8.01	1.68	1.62	2.00
2	8.00	7.98	7.46	1.63	1.64	1.86
3	7.93	7.89	8.06	1.58	1.47	2.01
Average	8.00	7.96	7.85	1.63	1.58	1.96
SD	0.07	0.07	0.33	0.05	0.09	0.08

CONCULSION

In summary, principle component regression (PCR) and partial least-square regression (PLS-1) models were developed for determination of lopinavir and ritonavir in a standard mixture set (test set) which was not contribute in the calibration step. Similar accuracy was obtained from two multivariate calibration methods. The same results were also performed when multivariate calibration models were applied to determine drugs in syrup. To evaluate the results obtained by multivariate calibration methods, an HPLC procedure was also used. The results obtained from PLS-1 and PCR models closed to those obtained from HPLC method. This implies that the proposed PCR and PLS-1 models are simple, accurate and precise procedures.

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REFERENCES

1. Dickinson L, Robinson L, Tjia J, Khoo S, Back D. Simultaneous determination of HIV protease inhibitors amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir and saquinavir in human plasma by high-performance liquid chromatography-tandem mass spectrometry. J Chromatogr B 2005; 829: 82-90.
2. Albert V, Modamino P, Lastra CF, Mariño EL. Determination of saquinavir and ritonavir in human plasma by reversed-phase high-performance liquid chromatography and the analytical error function. J Pharm Biomed Anal 2004; 36: 835-840.
3. Notari S, Bocedi A, Ippilito G, Narciso P, Pucillo LP, Tossini G, Donnorso RP, Gasparrini F, Ascenzi P. Simultaneous determination of 16 anti-HIV drugs in human plasma by high-performance liquid chromatography. J Chromatogr B 2006a; 831: 258-266.
4. Rebiere H, Mazel B, Civade C, Bonnet PA. Determination of 19 antiretroviral agents in pharmaceuticals or suspected products with two methods using high-performance liquid chromatography. J Chromatogr B 2007; 850: 376-383.
5. Colombo S, Beguin A, Telenti A, Biollaz J, Buclin T, Rochat B, Decosterd LA. Intracellular measurements of anti-HIV drugs indinavir, amprenavir, saquinavir, ritonavir, nelfinavir, lopinavir, atazanavir, efavirenz and nevirapine in peripheral blood mononuclear cells by liquid chromatography coupled to tandem mass spectrometry. J Chromatogr B 2005; 819: 256-276.
6. D´Avolio A, Siccardi M, Sciandra M, Lorena B, Bonora S, Trentini L, Perri GD. HPLC-MS method for the simultaneous quantification of the new HIV protease inhibitor darunavir, and 11 other antiretroviral agents in plasma of HIV-infected patients. J Chromatogr B 2007; 859: 234-240.

7. DiFrancesco R, DiCenzo R, Vicente G, Donnelly J, Martin TM, Colon LA, Schifito G, Morse GD. Determination of lopinavir cerebral spinal fluid and plasma ultrafiltrate concentrations by liquid chromatography coupled to tandem mass spectrometry. J Pharm Biomed Anal 2007; 44: 1139-1146.
8. Heine R, Rosing H, van Gorp ECM, Mulder JW, van der Steeg WA, Beijnen JH, Huitema ADR. Quantification of protease inhibitors and non-nucleoside reverse transcriptase inhibitors in dried blood spots by liquid chromatography-triple quadrupole mass spectrometry. J Chromatogr B 2008; 867: 205-212.
9. Temghare GA, Shetye SS, Joshi SS. Rapid and sensitive method for quantitative determination of lopinavir and ritonavir in human plasma by liquid chromatography-tandem mass spectrometry. E-J Chem 2009; 6(1): 223-230.
10. Ehrhartd M, Möck M, Haefeli .E, Mikus G, Burhenne J. Monitoring of lopinavir and ritonavir in peripheral blood mononuclear cells, plasma, and ultrafiltrate using a selective and highly sensitive LC/MS/MS assay. J Chromatogr B 2007; 850: 249-258.
11. Notari S, Mancone C, Tripodi M, Narciso P, Fasano M, Ascenzi P. Determination of anti-HIV drug concentration in human plasma by MALDI-TOF/TOF. J Chromatogr B 2006b; 833: 109-116.
12. Sulebhavikar AV, Pawar UD, Mangoankar KV, Prabhu-Navelkar ND. HPTLC methods for simultaneous determination of lopinavir and ritonavir in capsule dosage form. E-J Chem 2008; 5(4): 706-712.
13. Azoulay S, Nevers MC, Créminon C, Heripret L, Garraffo R, Durant J, Dellamonica P, Grassi J, Guedj R, Duval D. An enzyme immunoassay for the quantification of plasma and intracellular lopinavir in HIV-infected patients. J Immunol Methods 2004; 295: 37-48.
14. Lavine B, Workman J. Chemometrics. Anal Chem 2008; 80: 4519-4531.
15. Einax JW. Chemometrics in analytical chemistry. Anal Bioanl Chem 2008; 390: 1225-1226.
16. Dinte E, Tomuta I, Mut EM, Iovanov RI, Leucuta SE. Chemometric methods for the simultaneous assay of chloramphenicol, chlorhexidine and metronidazole during *in vitro* dissolution of drugs from mucoadhesive buccal gels. FARMACIA 2010; 58(5): 572-582.
17. Phechkrajang CM, Thin EE, Sratthaphut L, Nacapricha D, Wilairat P. Quantitative determination of lopinavir and ritonavir in syrup preparation by liquid chromatography. Mahidol University Journal of Pharmaceutical Sciences 2009; 36(1-4): 1-12.
18. Singh B, Patel DK, Ghosh SK. A reversed-phase high performance liquid chromatographic method for determination of chlorthalidone in pharmaceutical formulation. Int J Pharm Pharm Sci 2009; 1(2): 24-29.
19. Lakshmi KS, Rajesh T, Sharma S. Simultaneous determination of metformin and pioglitazone by reversed phase HPLC in pharmaceutical dosage forms. Int J Pharm Pharm Sci 2009; 1(2): 162-166.
20. Sreekanth N, Baburao CH, Mukkanti K. RP-HPLC method development and validation of ropinirole hydrochloride in bulk and pharmaceutical dosage forms. Int J Pharm Pharm Sci 2009; 1(1): 186-192.
21. Sreekanth N, Baburao CH, Mukkanti K. RP-HPLC method development and validation of rimonabant hydrochloride in bulk and pharmaceutical dosage forms. Int J Pharm Pharm Sci 2009; 1(1): 199-205.
22. Hemmateenejad B, Rezaei Z, Khabnadideh S, Saffari M. A comparative study between PCR and PLS in simultaneous determination of diphenyl amine, aniline and phenol:Effect of wavelength selection. Spectrochimica Acta Part A 2007; 68: 718-724.