

RESEARCH

Open Access



# Chemometric method development for the determination of naringin and verapamil

Sadhana Sharma<sup>1</sup>, Bhupendra Shrestha<sup>1</sup>, Nihar Ranjan Bhuyan<sup>1</sup>, Subhabrota Majumdar<sup>2</sup>,  
Sailee Chowdhury<sup>3</sup> and Rana Mazumder<sup>4\*</sup>

## Abstract

**Background** By resolving complicated spectra from drug combinations, chemometric techniques are valuable for multi-component investigation. The capacity to properly estimate combinations of components without separating drugs from their mixture is one of the benefits of chemometric analysis approaches over traditional analytical methods. These approaches are easy to use and sensitive even to the lowest concentrations. They are also practical, affordable, and cost-effective. In the current study, the chemometric aided spectrophotometric approach was used to evaluate the two drugs naringin and verapamil. The approach is multidimensional and based on chemometrics, which includes an orthogonal partial least square method that is a new refinement of the partial least squares regression analysis method. With this technique, no conversions are made to the spectrum that overlaps the two drugs. The tools UV-PROBE, SIMCA version 17, and excel were used to process the chemometric data.

**Results** According to results from an orthogonal partial least square model, the mean percent recovery and relative standard deviation for the combination of verapamil with naringin were 100.80/1.19 and 100.836/1.35, respectively. The calibration model was used to predict known synthetic mixtures. This method shows good consistency in recovery ranging between 98.92 and 103.59% for VER and from 96.21 to 101.84% NAR. As saying the synthetic mixture revealed that it had a high percentage of purity.

**Conclusions** The proposed chemometric method can estimate the quantitative amount of pharmaceuticals based on their dosage forms. This approach meets the requirements for the international conference on harmonization's (ICH) analytical criteria, such as precision and accuracy.

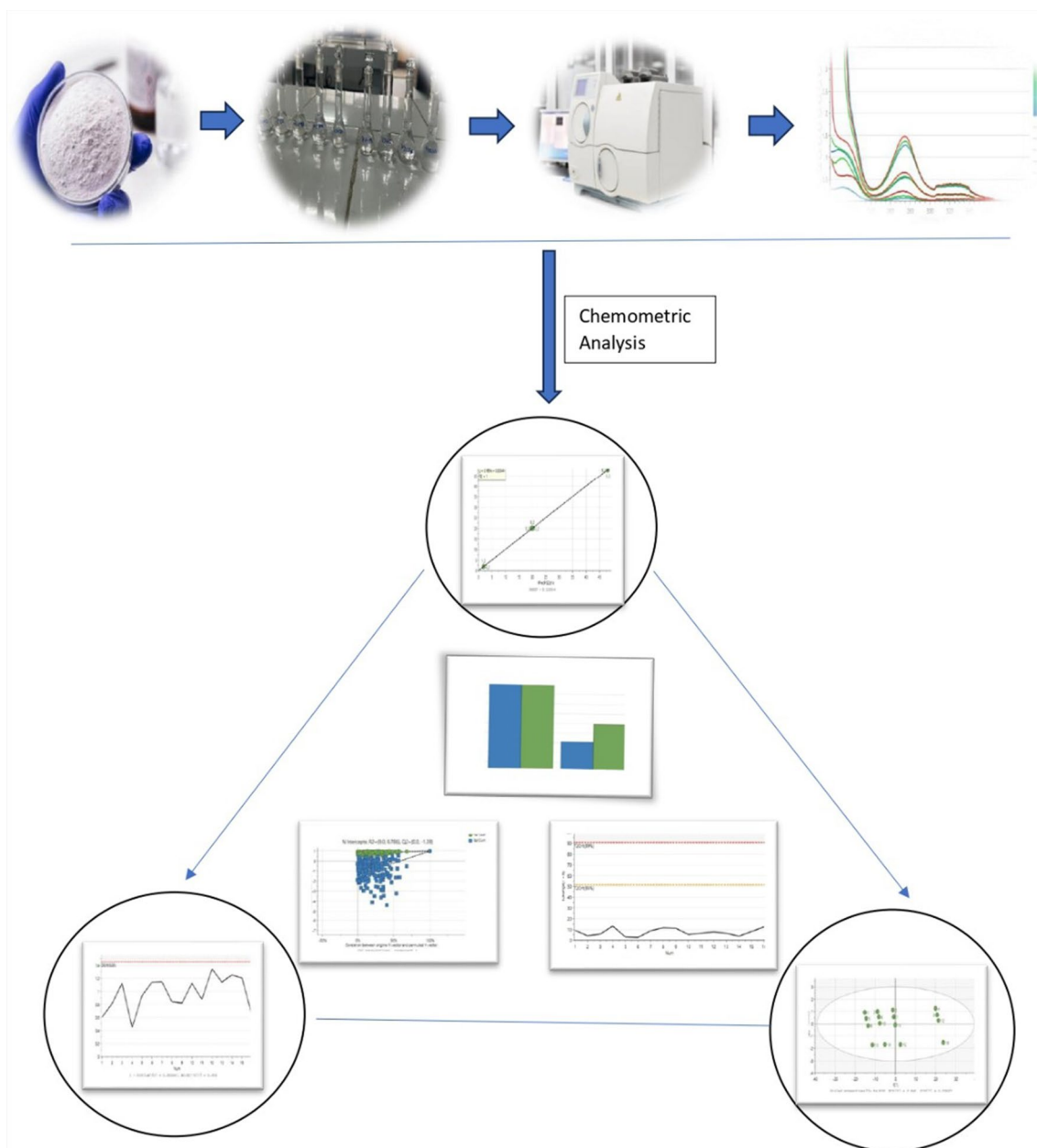
**Keywords** Spectrophotometry, Chemometrics, Verapamil, Naringin, Orthogonal partial least square (OPLS), Root mean square for cross-validation, Root mean square error of prediction

\*Correspondence:

Rana Mazumder  
ranapharma.mazumder@gmail.com

Full list of author information is available at the end of the article

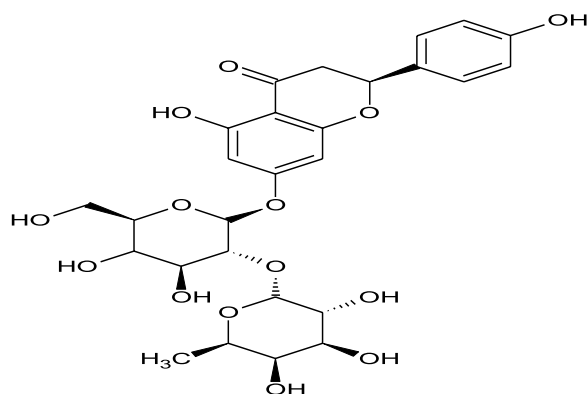
## Graphical Abstract



## Background

Chemometric methods are useful for analyzing multi-components by solving their complex spectrums from mixtures (Dinc et al. 2002). Compared to conventional analysis methods, chemometrics can accurately determine the combination of medications without isolating them from the mixture. These techniques have many other advantages, including being easy to use, sensitive

even to the lowest concentrations and practical. They are also cost-effective compared with the alternative techniques used for simultaneous identification. These procedures do not affect the analysis or calibration of pharmaceuticals due to the concentration of the other components. It is also faster to identify each component in the combination (Prachi et al. 2013). Chemometric methods for measuring naringin and



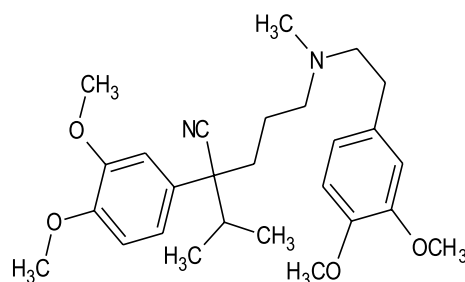
**Fig. 1** Chemical structure of naringin

verapamilin synthetic combinations were refined and verified. These methods are easy to use, precise, accurate, and economical.

Citrus flavonoid naringin (NAR), one of the most promising substances in the human diet, is readily available. Citrus fruits contain the abundant flavone glycoside naringin (4,5,7-trihydroxyflavanone-7-rhamnoglucoside). Chemical structure of naringin is shown in (Fig. 1), which has intriguing biological and pharmacological effects (Opinion et al. 2011). The bitterness of naringin is due to its glucose moiety. Its contents are naringenin (an aglycone) and neohesperidose, linked with the C-7 hydroxyl. Naringin can now be added in foods without restriction because it has been classified as a flavoring compound (Sharma et al. 2011).

Naringin contain a flavanone-7-o-glycoside which has a number of pharmacological actions, including antioxidant, anti-carcinogenic, and anti-diabetic properties. Additionally, it blocks some cytochrome P450 enzymes, such as CYP3A4 and CYP1A2, which can lead to a variety of medication interactions in-vitro (Tulasi et al. 2019, Kim et al. 2023).

Verapamil is chemically known as 2-(3,4-dimethoxyphenyl)-5-[2-(3,4-dimethoxyphenyl)ethyl-methylamino]-2-propan-2-ylpentanenitrile. Verapamil's chemical structure is illustrated in Fig. 2. Verapamil is a P-glycoprotein Inhibitor, cytochrome P450 3A4 and cytochrome P4503A inhibitor. Verapamil increases the size of the coronary arteries and reduces the contractility of the myocardium by blocking transmembrane calcium ion input into smooth muscle and myocardial cells. This substance also blocks drug efflux pumps P-glycoprotein, which are overexpressed by some tumors resistant to many drugs and can enhance the effect of certain antineoplastic agents (National Center for Biotechnology Information 2023).



**Fig. 2** Chemical structure of verapamil

Verapamil has been analyzed by various methods like UV (Trivedi et al. 2017), High-performance liquid chromatography (HPLC) (Auti et al. 2019), Ultra performance liquid chromatography (UPLC) (Vijayabaskar et al. 2017), thin layer chromatography (Bhushan et al. 2005), LC-MS/MS (Ravi et al. 2019), chiral capillary electrophoresis (Cârcu-Dobrin et al. 2021). Similarly, Naringin also has been estimated by various methods like Chromatography-Tandem Mass Spectrometry (Xiong et al. 2014), Capillary electrophoresis (Akiyama et al. 2000), HPLC (Ishii et al. 1997), HPTLC (Alam et al. 2014), UPLC-MS/MS (Wen et al. 2012), thin layer chromatography and fourier transform infrared spectroscopy (FTIR) (Hakim et al. 2019). Each method has yet to be developed to estimate naringin together with verapamil. In the present work, chemometric techniques have been effectively used to estimate verapamil and naringin simultaneously by OPLS.

## Methods

### Materials

Verapamil and naringin ( $C_{27}H_{38}N_2O_4$  and  $C_{27}H_{32}O_{14}$ ) with purity levels of  $\geq 99\%$  were purchased from Yarrow Chem products, Mumbai. Methanol (AR Grade) was purchased from S.D fine Chemicals Ltd, Mumbai, water (Milli Q. All other reagents and materials were of AR grade and commercially available). Verapamil and Naringin concentrations were determined using a UV-visible spectrophotometer (Shimadzu-Kyoto, Japan Model 1800 double beam with matching 1 cm path-length quartz cells) using methanol as the solvent at wavelengths between 200 and 400 nm. The drug's solubility, melting point, and molar absorptivity were its distinguishing qualities. The drug was analyzed using the UV-visible technique using software UV Probe and SIMCA (Sartorius, version 17) for handling the orthogonal partial least square (OPLS) model, which satisfied the requirements set out by the International Conference on harmonization (ICH) for analytical criteria such as linearity, precision, and accuracy.

### Preparation of standard solution

Weight precisely 10.0 mg each of NAR and VER. The stock solution was prepared by dissolving the VER in 10.0 mL methanol and then adding the required volume using the same solvent. VER or NAR stock solutions were prepared using concentrations ranging between 0.5–12 and 2–48 µg/mL. The zero-order spectra were recorded at wavelengths between 200 and 400 nm compared to a blank solvent (Mazumder et al. 2020).

### One component calibration

The linear dynamic range of each drug was determined by filling a 10.0 mL volumetric container with different amounts of the stock solution. This was diluted in methanol at the correct concentration. The electronic absorption spectrum was recorded in the 200–400 nm wavelength range. Absorption values at 200 nm wavelengths and 400 nm were measured for various concentrations (Prasad et al. 2017). The light dependent resistor (LDR) was calculated by plotting the absorbance against concentration. The LDR for the two drugs was achieved between 0.5 and 50 µg/mL.

### Standard calibration and prediction samples set

The multilevel, multi-factor architecture was used to develop the calibration and validation sets. A calibration set with four component mixtures has been designed to improve the prediction of OPLS. To prepare the calibration set, sixteen samples were used, as shown in Table 1, and nine samples in Table 2, and then the volume of the methanol was adjusted to match the NAR or VER

**Table 1** Concentration of different mixtures of VER and NAR used as calibration set

Sl. No	VER (µg/ml)	NAR (µg/ml)
1	0.5	2
2	0.5	10
3	0.5	20
4	0.5	48
5	2.5	2
6	2.5	10
7	2.5	20
8	2.5	48
9	5	2
10	5	10
11	5	20
12	5	48
13	12	2
14	12	10
15	12	20
16	12	48

**Table 2** Concentration of different mixtures of VER and NAR used as prediction set

Sl. No	VER (µg/ml)	NAR (µg/ml)
1	0.5	2
2	0.5	20
3	0.5	48
4	5	2
5	5	20
6	5	48
7	12	2
8	12	20
9	12	48

concentrations. These concentrations ranged from 2–48 to 0.5–12 mg/mL, respectively, against blank methanol in a range between 200 and 400 nm at intervals of one nm. The recorded spectrum from UV Probe was transferred to SIMCA 17, using OPLS Toolbox, for data analysis. A calibration model was created (Zou et al. 2022).

### Building an OPLS model

A multivariate calibration method like OPLS could be applied with a filter derivative to address the overlap between the medicines studied. As a part of the pre-processing, data from calibration samples was automatically scaled to be used in OPLS. The cross-validation method using one sample was left out and then followed. The ideal number of components were important for the OPLS method. If the components were too many, noise was added, but if they were too few, noise was reduced. The established models of the OPLS method could accurately characterize two latent variables (Abdallah et al. 2019). A calibration curve is created by comparing projected concentrations with actual concentrations.

### Construction of calibration and prediction

To prepare the calibration and validation mixes, we combined working standard solutions from NAR and VER at different ratios based on their linearity concentration ranges. For the calibration set, the concentration was calculated using a general factorial system (2 factors with 4 levels each) and for the predictions set (2 factors with 3 levels each) (Sonawane et al. 2019). The calibration and validation mixtures totaled 16 in number and were

**Table 3** Statistical parameters for OPLS method

Drugs	R <sup>2</sup>	RMSECV	RMSEP
Verapamil	0.9978	1.22652	0.2102
Naringin	1	0.66506	0.1280

**Table 4** Composition of prediction set and their predicted results obtained in synthetic mixtures by OPLS method

Actual concentration		Predicted concentration		% Recovery	
VER (µg/mL)	NAR (µg/mL)	VER (µg/mL)	NAR (µg/mL)	VER (µg/mL)	NAR (µg/mL)
0.5	2	0.5075	1.93443	101.5	96.72
0.5	20	0.49719	20.2088	99.4	101.044
0.5	48	0.4944	47.9761	98.88	99.95
5	2	5.17955	1.97389	103.591	98.69
5	20	5.14411	19.7169	102.8	98.58
5	48	4.9674	48.1218	99.34	100.25
12	2	11.904	2.02438	99.20	101.219
12	20	12.0277	19.9711	100.23	99.85
12	48	12.187	47.9556	101.558	99.89
MEAN				100.7221	99.577
S.D				1.7125	1.3944
%RSD				1.7	1.4003

prepared independently. All mixtures were recorded in the absorption spectra range of 200–400 nm, with 1 nm increments. Calculations were made for regression equations and statistical data related to the thesis model. The calculations were also done for recoveries and mean recoveries.

**Table 5** Statistical parameters for simultaneous determination of VER and NAR by OPLS

Parameters	VER	NAR
Linearity		
Conc. range (µg/ml)	0.5–50 µg/mL	0.5–50 µg/mL
Slope	0.024	0.008
R <sup>2</sup>	0.997	1
No. of components	2.0	2.0
RMSEE	0.310189	0.175224
RMSECV	1.22652	0.66506
RMSEP	0.210237	0.128044
Q <sup>2</sup>	0.995	0.995
Accuracy		
Mean	99.6796	99.531
S.D	1.317	0.6106
Intra-day precision		
Mean	100.27	100.226
SD	1.025	1.126
%RSD	1.022	1.11
Inter-day precision		
Mean	100.438	100.505
S.D	0.998	1.367
% RSD	0.9	1.36

### Preparation of sample solution

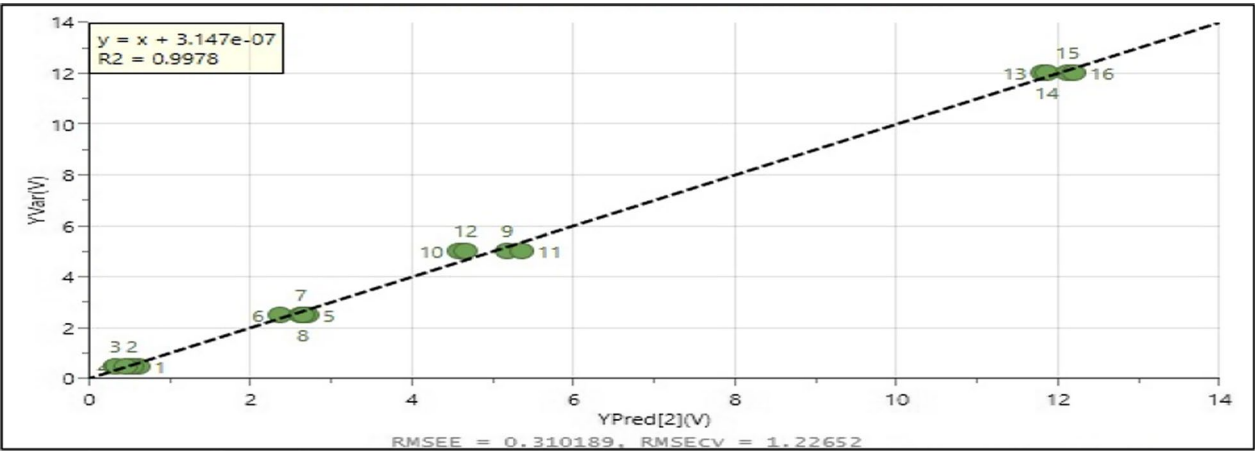
To perform the assay, a quantity of powder equal to 20.0 mg VER is weighed and placed in a calibrated 100.0 mL volumetric flask (Sonawane et al. 2019). Then it's dissolved in methanol. In a 25.0 mL volumetric flask calibrated to 0.5 mg/mL, a solution of 0.5 mg/mL was pipetted and then dissolved in the methanol. This solution was then diluted further with methanol to the desired concentration within the calibration range. The proposed chemometric method (OPLS), which was scanned in a spectrophotometer to determine sample solutions, was then used.

### Assay of synthetic mixture

To perform the assay, a quantity of powder equal to 20.0 mg VER was accurately weighed and placed in a calibrated 100.0 ml volumetric flask. Then it was dissolved in Methanol. In a 25.0 ml volumetric flask calibrated to 0.5 mg/ml, a solution of 0.5 mg/ml was pipetted and then dissolved in the Methanol. The resulting solution is further diluted using Methanol at a concentration within the calibration range. After

**Table 6** Assay data of synthetic mixture by OPLS method

Assay	VER	NAR
3.2	102.156	102.15
6	100.41	101.0
7.2	102.0	102.1
8	99.55	99.03
12	99.9	99.9
MEAN	100.802	100.836
S.D	1.202818	1.368295
%RSD	1.1932	1.35



**Fig. 3** Calibration curve of VER obtained from calibration set data by OPLS method

scanning with a spectrophotometer, the proposed chemometric method (opls) was used to determine sample solutions (Ríos-Reina et al. 2023). Table 6 shows the results of an assay.

### Results

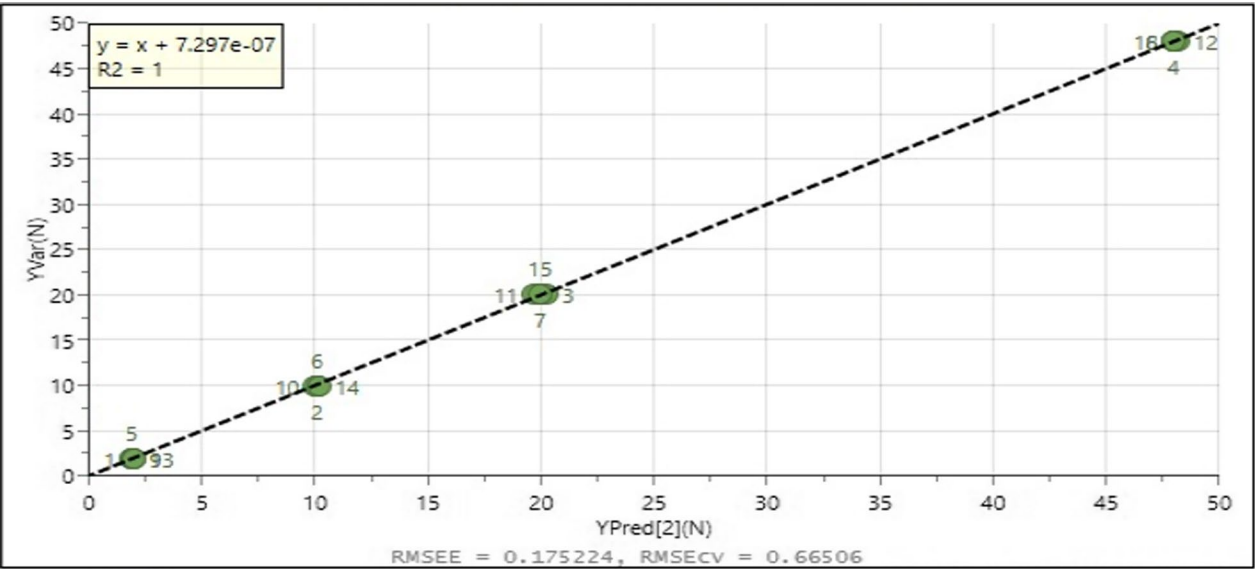
In methanol, the VER and NAR spectra overlapped with the binary combination of NAR and zero-order superimposed spectra. To overcome this problem, it is possible to use a chemometric technique to separate these two drugs from the mixture. Under conditions where the spectra overlap, chemometric techniques can be employed to estimate drugs (Biancolillo et al. 2018). Multivariate chemometrics was developed to offer a simple, reliable,

selective and valid method of determining the drugs without interference.

### Statistical parameters

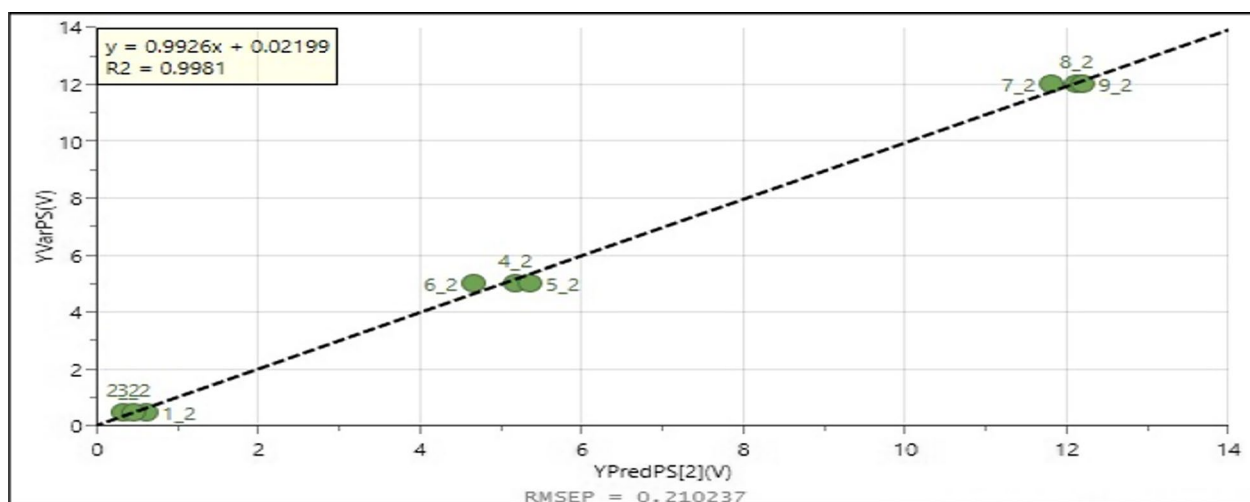
The regression model can be defined in several ways. When describing chemometric methods, RMSEP is the root mean square error of prediction, and RMSECV is the root mean square for cross-validation.

By applying the OPLS approach to the validation set of the two drug mixture, the RMSECV, RMSEP and  $R^2$  were determined. The results are shown in Table 3. Statistical parameters for simultaneous determination of VER and NAR by OPLS are shown in Tables 4 and 5.



**Fig. 4** Calibration curve of NAR obtained from calibration set data by OPLS method





**Fig. 5** Prediction curve of VER obtained from prediction set data by OPLS method

The validity of calibration procedures can also be assessed by simultaneously estimating two drugs in the set. The mean percent recovery for two drug solutions combined using the OPLS method was found within acceptable ranges (Indrayanto et al. 2018). Recovery studies were conducted using standardized drugs added in certain amounts to a synthesized mixture of a specific concentration. After defining the sample volume or concentration in which it was to be added, known amounts of standard working solutions were added. The volume of the flask was then filled with methanol. Chemometric techniques were used to evaluate the recovery of the two drugs in the mixtures (Table 6).

In Table 4, mean % and root mean square deviation (RSD) values are shown. Recovery study results showed that the procedures had successfully been proved since the % root mean square deviation (RSD) was less than 2 and the recovery study was closer to 100%. Additionally, the results shows that the suggested techniques are correct and that excipients do not affect the assessment of two drugs. By looking at various drug concentrations, the linearity for VER and NAR was evaluated. The root mean square error of cross validation (RMSECV) was calculated which can be used to determine the error in the predicted concentrations (Van Wyngaard et al. 2021). Drug concentrations in various sets of mixtures used for method development were analyzed and predicted to validate the approaches. By comparing the predicted concentrations on the graph, the method's ability for prediction was determined. Figures 3 and 4 explains variation of model that shows the peaks to be linear for both the drugs therefore the optimization of calibration set shows a good  $R^2$  value (0.997 & 1) and good prediction power  $Q^2$  value (0.210 & 0.128), minimum RMSEE (0.310

& 0.175), and RMSECV (1.226 & 0.665) for each drug. Figures 5 and 6 explains the predictive ability from which we can record the RMSEP value. It indicates OPLS- optimized technique, is good for both the drugs in the prediction set, indicating the method to be effective and has good predictive ability.

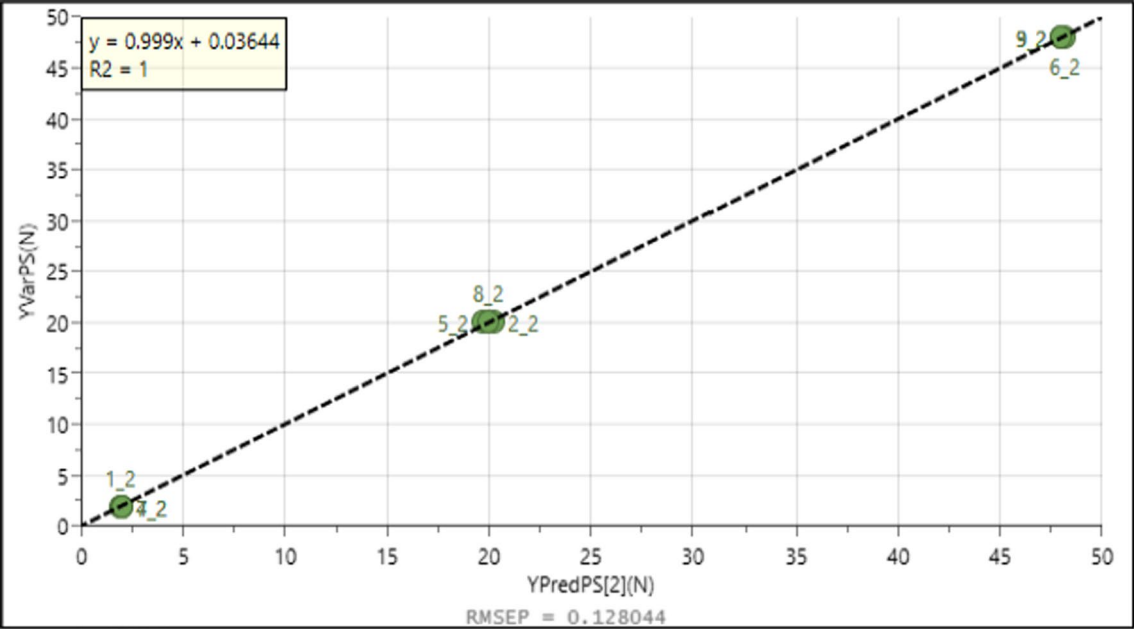
#### Model validation

The Hotelling  $T^2$  test and D.ModX were used to find weak and moderate outliers, and they encountered no deviation, indicating that no outliers were found (Shabbak et al. 2012). According to permutation analysis, all of the permuted  $Q^2$  were below 0, and  $R^2$  was below or about 1. Since  $Q^2$  and  $R^2$  were lower than the original values, it is likely that the model fitting was accurate and unlikely to have developed randomly. We can see all the samples that fall inside the Hotelling range by looking at the score plot. We get the conclusion that the model is effective since every optimisation strategy satisfy the requirements. The summary fit, score representation, Hotelling's range, D.ModX, and permutation plot are displayed in Figs. 7, 8, 9, 10, 11.

#### Discussion

It was found that the lambda max value for both the drugs i.e. naringin and verapamil was very near to each other. Since, iso-absorptive point was very near for the drugs, therefore, the Q-ratio method development was not processed.

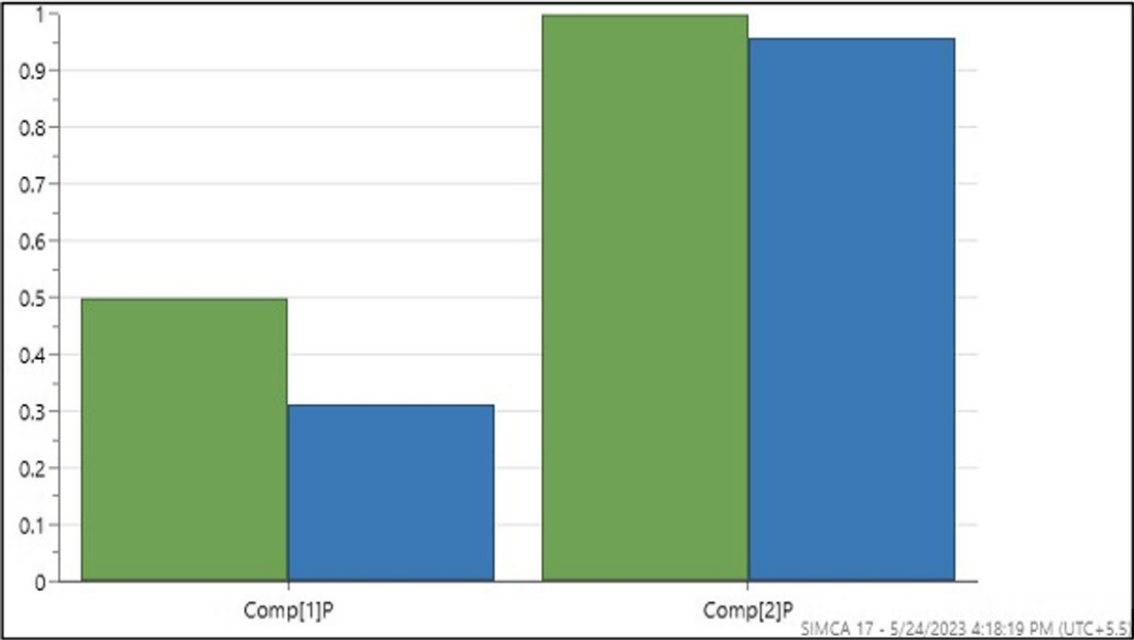
Without a successful Q-ratio method, an innovative UV spectrophotometric technique based on chemometrics was developed (Darbandi et al. 2020). The chemometrics technique can solve many spectrum



**Fig. 6** Prediction curve of NAR obtained from prediction set data by OPLS method

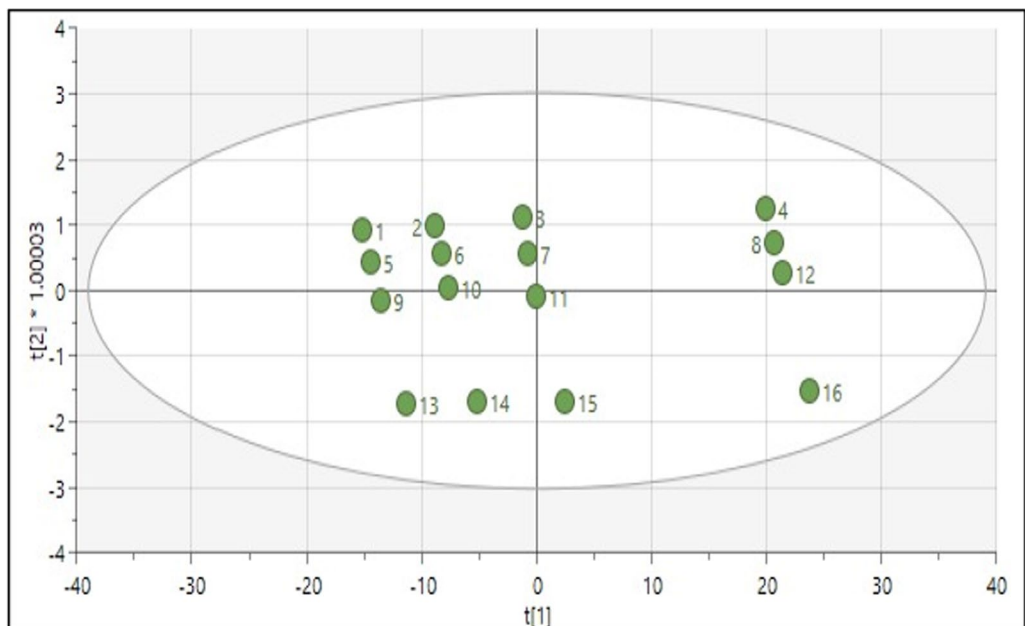
resolution problems. This technique can improve the signal-to-noise ratio, increase selectivity in determination, optimize experimental conditions, boost analytical efficiency, and give much information. Hence, it is used for the simultaneous determination of multi-component mixtures. Absorptivity and other parameters

were checked for naringin and verapamil, and methanol was selected as a solvent for quantifying the drugs in the method. This method was linear over a range of naringin 0.05–50 ug/mL and verapamil 0.5–50 ug/mL with a correlation coefficient of 0.9978 and 0.9998, respectively.



**Fig. 7** Summary of fit of the calibration set by the OPLS method





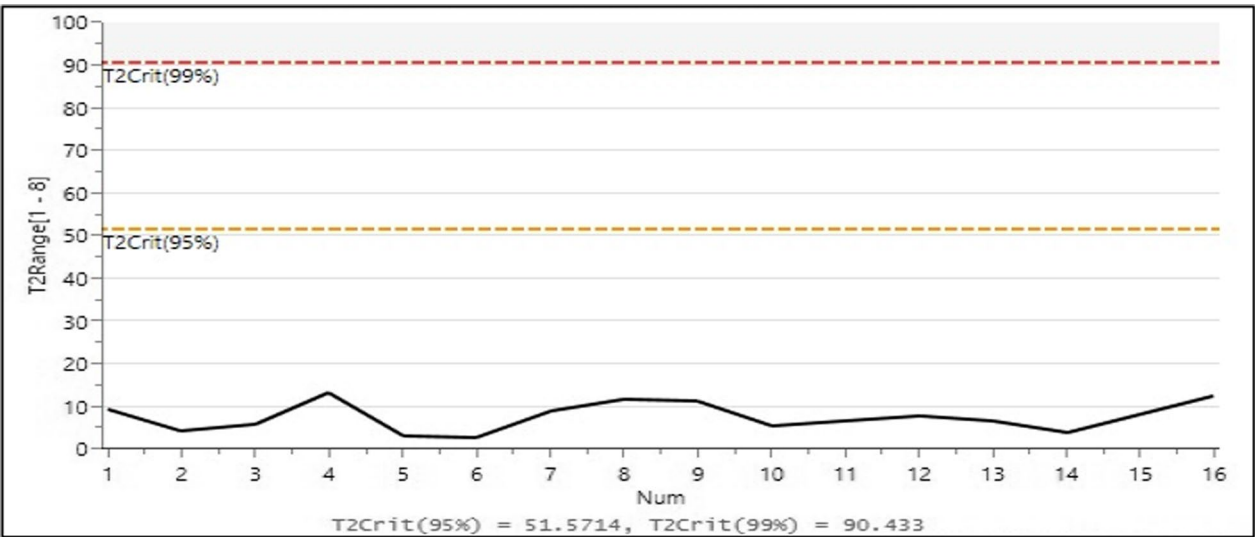
**Fig. 8** Score representation of 16 calibrations set

The calibration model prepared for OPLS is optimal because it has a good correlation coefficient and low RMSEP and RMSECV for naringin. Naringin has good linearity in the range of concentrations of 0.5–50 mg/mL, with a value of 0.9987, and verapamil has a value of 1.

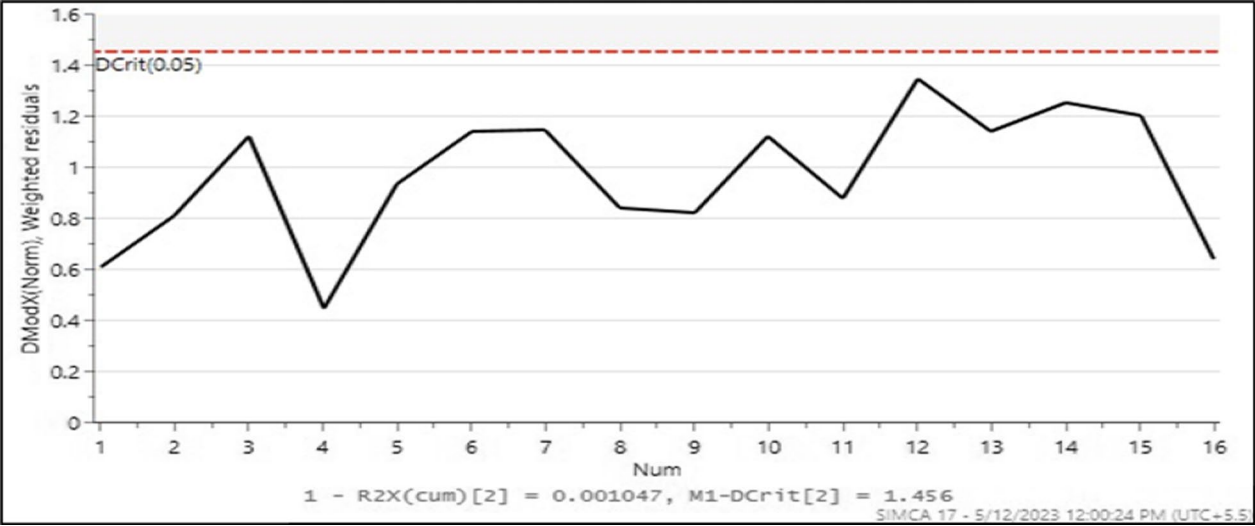
The calibration model was used to predict known synthetic mixtures where RMSEP 2. By identifying the minimum error, further assays, accuracy and precision tests were conducted. The RSD percentage for the intra-day and Inter-day precision was less than 2%, which indicates that this method is reproducible and

repeatable (Kamal et al. 2015). This method shows good consistency in recovery ranging between 98.92 and 103.59% for VER and from 96.21 to 101.84% NAR. As saying the synthetic mixture revealed that it had a high percentage of purity. The mean assay for VER and NAR using the OPLS technique was 100.80%, respectively.

The chemometric studies mainly implicated for experimental design and optimization, data treatments, sample classification, calibration for determination of concentrations and model identification. Finally Based on the results of different assessments, we can formulate some hypotheses about the reproducibility of the



**Fig. 9** Hotelling's range peak of the calibration set by the OPLS method



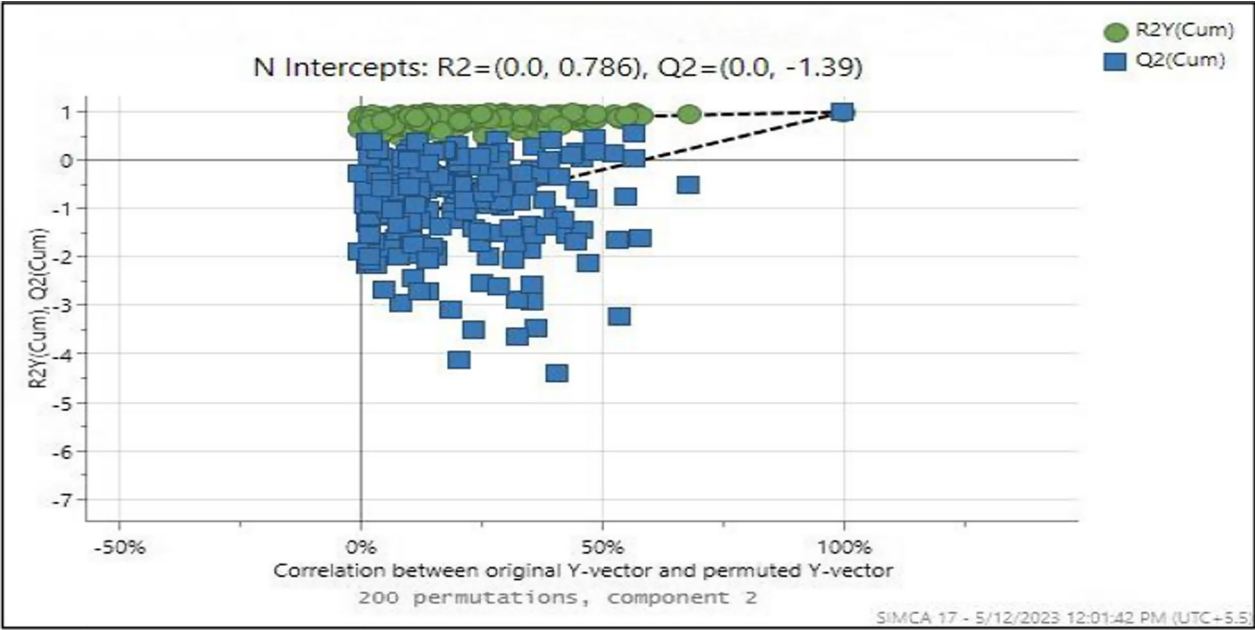
**Fig. 10** D-ModX of the calibration set by the OPLS method

methods. The suggested procedures were verified in compliance with ICH guidelines (I. H. T. Guideline 1994).

**Conclusions**

A simple, reproducible, and accurate chemometric technique was developed for the simultaneous estimation of NAR and VER. The technique is a great way to analyze commercial pharmaceuticals without interference or the need to physically separate them. The method solved the

issue of interference and merging of mixture peaks. The results of this method were validated according to ICH Guidelines and met the criteria. This method is valid and may be used to perform routine quality controls on VERs and NARs.



**Fig. 11** Permutation data of the calibration set by the OPLS method

## Abbreviations

OPLS	Orthogonal partial least square
VER	Verapamil
NAR	Naringin
OPLS	Orthogonal partial least square
UV	Ultraviolet–visible spectroscopy
HPLC	High-performance liquid chromatography
UPLC	Ultra performance liquid chromatography
LC–MS/MS	Liquid chromatography with tandem mass spectrometry
FTIR	Fourier transform infrared spectroscopy
LDR	Light dependent resistors
RMSEP	Root mean square error of prediction
RMSECV	Root mean square for cross-validation
RSD	Relative standard deviation

## Acknowledgements

The authors acknowledge Himalayan Pharmacy Institute, Majhitar, East Sikkim, for providing adequate facilities to perform the experiments and also thanks to Gitanjali College of Pharmacy, Lohapur-731237, Birbhum, West Bengal, India, Calcutta Institute of pharmaceutical Technology & A.H.S, Uluberia, Howrah and BCDA College of Pharmacy & Technology, Barasat, Kolkata for providing all necessary support.

## Author contributions

SS and BS contributed to the technique, experimental work, original draft writing, and data analysis. SM and SC contributed to the conceptualization, instructions, reviewing and formal analysis of the study. RM drafted and edited the manuscript. BS supervised the work and NRB conceived and designed of the study. All authors have read and approved the final manuscript.

## Funding

The authors have no funding to report.

## Availability of data and materials

All necessary data generated or analyzed during this study are included in this manuscript. Any additional data could be available from the corresponding author upon request.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

## Author details

<sup>1</sup>Himalayan Pharmacy Institute, Majhitar, East Sikkim, India. <sup>2</sup>Calcutta Institute of Pharmaceutical Technology & Allied Health Sciences, Banitabla, Uluberia, Howrah, West Bengal, India. <sup>3</sup>BCDA College of Pharmacy and Technology, 78/1 Jessore Road (S), Hridaypur, Barasat, Kolkata, West Bengal, India. <sup>4</sup>Gitanjali College of Pharmacy, Birbhum, West Bengal, India.

Received: 3 November 2023 Accepted: 6 January 2024

Published online: 15 January 2024

## References

- Abdallah FF, Darwish HW, Darwish IA, Naguib IA (2019) Orthogonal projection to latent structures and first derivative for manipulation of PLSR and SVR chemometric models' prediction: a case study. *PLoS ONE* 14(9):e0222197
- Akiyama T, Yamada T, Maitani T (2000) Analysis of enzymatically glucosylated flavonoids by capillary electrophoresis. *J Chromatogr A* 895:279–283
- Alam P, Siddiqui N, Al-Rehaily A, Alajmi M, Basudan O, Khan T (2014) Stability-indicating densitometric high-performance thin-layer chromatographic method for the quantitative analysis of biomarker naringin in the leaves and stems of *Rumex vesicarius* L. *J Planar Chromatogr Mod TLC* 27:204–209
- Auti P, Gabhe S, Mahadik K (2019) Bioanalytical method development and its application to pharmacokinetics studies on Simvastatin in the presence of piperine and two of its synthetic derivatives. *Drug Dev Ind Pharm* 45:664–668
- Bhushan R, Gupta D (2005) Thin-layer chromatography separation of enantiomers of verapamil using macrocyclic antibiotic as a chiral selector. *Biomed Chromatogr* 19:474–478
- Biancolillo A, Marini F (2018) Chemometric methods for spectroscopy-based pharmaceutical analysis. *Front Chem* 6:576
- Cărcu-Dobrin M, Hancu G, Papp LA, Fülöp I, Kelemen H (2021) Development of a chiral capillary electrophoresis method for the enantioseparation of verapamil using cyclodextrins as chiral selectors and experimental design optimization. *Symmetry* 13(11):2186
- Darbandi A, Sohrabi MR, Bahmaei M (2020) Development of a chemometric-assisted spectrophotometric method for quantitative simultaneous determination of Amlodipine and Valsartan in commercial tablet. *Optik* 218:165110
- Dinc E, Baleanu D (2002) Spectrophotometric quantitative determination of cilazapril and hydrochlorothiazide in tablets by chemometric methods. *J Pharm Biomed Anal* 30:715–723
- Hakim A, Loka IN, Prastiwi NW (2019) New method for isolation of Naringin compound from citrus maxima. *Nat Resour* 10:299
- I. H. T. Guideline, Note for Guidance on Toxicokinetics (1994) The Assessment of Systemic Exposure in Toxicity Studies S3A, ICH harmonization for Better Health, Geneva, Switzerland
- Indrayanto G (2018) Chapter five: validation of chromatographic methods of analysis: application for drugs that derived from herbs. In: Brittain HG (ed) Profiles of drug substances excipients and related methodology. Academic Press, pp 359–392
- Ishii K, Furuta T, Kasuya Y (1997) Determination of naringin and naringenin in human urine by high-performance liquid chromatography utilized solid-phase extraction. *J Chromatogr B Biomed Appl* 704:299–305
- Kamal A, Khan W, Ahmad S, Ahmad FJ, Saleem K (2015) Development and validation of high-performance liquid chromatography and high-performance thin-layer chromatography methods for the quantification of khellin in Ammi visnaga seed. *J Pharm Bioallied Sci* 7(4):308–313
- Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA, Thiessen PA, Yu B, Zaslavsky L, Zhang J, Bolton EE (2023) PubChem 2023 update. *Nucleic Acids Res* 51(D1):D1373–D1380
- Mazumder R, Mahanti B, Mazumder S, Pal R, Chowdhury AK (2020) Improved comprehensive analytical method for assessment of satranidazole in drug and product. *Fut J Pharmaceut Sci* 6:202054
- National Center for Biotechnology Information (2023). PubChem compound summary for CID 2520, Verapamil. Accessed 15 Jul 2023
- Opinion S (2011) Scientific Opinion on the safety and efficacy of naringin when used as a sensory additive for all animal species. *EFSA J* 9:1–12
- Prachi B, Asutosh Phatak SR (2013) Simultaneous determination of doxylamine succinate, pyridoxine hydrochloride and folic acid by chemometric spectrophotometry. *Int J Pharma Bio Sci* 4:738–749
- Prasad S, Mandal I, Singh S, Paul A, Mandal B, Venkatramani R, Swaminathan R (2017) Near UV-visible electronic absorption originating from charged amino acids in a monomeric protein. *Chem Sci* 8(8):5416–5433
- Ravi Y, Rajkamal B (2019) An improved LC-MS/MS method development and validation for the determination of trandolapril and verapamil in human plasma. *Int J Pharm Pharm Sci* 11:91–95
- Rios-Reina R, Azcarate SM (2023) How chemometrics revives the UV-Vis spectroscopy applications as an analytical sensor for spectralprint (nontargeted) analysis. *Chemosensors* 11(1):8
- Shabbak A, Habshah Midi H (2012) An improvement of the hotelling statistic in monitoring multivariate quality characteristics. *Math Probl Eng* 2012:15
- Sharma AK, Bharti S, Ojha S, Bhatia J, Kumar N, Ray R, Kumari S, Arya DS (2011) Up-regulation of PPAR $\gamma$ , heat shock protein-27 and -72 by naringin attenuates insulin resistance,  $\beta$ -cell dysfunction, hepatic steatosis and kidney damage in a rat model of type 2 diabetes. *Br J Nutr* 106:1713–1723
- Sonawane SS, Chhajed SS, Attar SS et al (2019) An approach to select linear regression model in bioanalytical method validation. *J Anal Sci Technol* 10:1

- Trivedi L, Telrandhe R, Dhabarde D (2017) Differential spectrophotometric method for estimation and validation of Verapamil in Tablet dosage form. *Int J Pharm Drug Anal* 5:419–422
- Tulasi I, Shivani G (2019) Bioavailability enhancers: an overview. *Int J Adv Res* 5:825–829
- Van Wyngaard E, Blancquaert E, Nieuwoudt H, Aleixandre-Tudo JL (2021) Infra-red spectroscopy and chemometric applications for the qualitative and quantitative investigation of grapevine organs. *Front Plant Sci* 12:723247
- Vijayabaskar S, Mahalingam V (2017) Analytical method development and validation for the analysis of verapamil hydrochloride and its related substances by using ultra performance liquid chromatography. *J Pharm Biomed Anal* 137:189–195
- Wen J, Qiao Y, Yang J, Liu X, Song Y, Liu Z, Li F (2012) UPLC-MS/MS determination of paeoniflorin, naringin, naringenin and glycyrrhetic acid in rat plasma and its application to a pharmacokinetic study after oral administration of SiNiSan decoction. *J Pharm Biomed Anal* 66:271–277
- Xiong X, Jiang J, Duan J, Xie Y, Wang J, Zhai S (2014) Development and validation of a sensitive liquid chromatography-tandem mass spectrometry method for the determination of naringin and its metabolite, naringenin, in Human Plasma. *J Chromatogr Sci* 52:654–660
- Zou L, Li H, Ding X, Liu Z, He D, Kowah JAH, Wang L, Yuan M, Liu XA (2022) Review of The application of spectroscopy to flavonoids from medicine and food homology materials. *Molecules* 27:7766

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.