DRUG FORMULATIONS AND CLINICAL METHODS

Linear Support Vector Regression and Partial Least-Squares for Determination of Dapoxetine Hydrochloride and Tadalafil in Binary Pharmaceutical Mixtures

BASMA H. ANWAR, NESSREEN S. ABDELHAMID, and MAIMANA A. MAGDY
Beni-Suef University, Faculty of Pharmacy, Pharmaceutical Analytical Chemistry Department, Alshaheed Shehata Ahmad Hegazy St, 62514, Beni-Suef, Egypt
IBRAHIM A. NAGUIB
Beni-Suef University, Faculty of Pharmacy, Pharmaceutical Analytical Chemistry Department, Alshaheed Shehata Ahmad Hegazy St, 62514, Beni-Suef, Egypt; Taif University, College of Pharmacy, Department of Pharmaceutical Chemistry, Al-Hawiah 21974, Taif, Saudi Arabia

Background: **Dapoxetine (DAP) is a serotonin-norepinephrine reuptake inhibitor, and Tadalafil (TAD) is a phosphodiesterase type-5 inhibitor. Both are coformulated as tablets called Erectafil® for treatment of erectile ejaculation.** ***Objective:*** **DAP and TAD were analyzed in their binary mixtures and pharmaceutical formulations using two multivariate calibration chemometric models.** ***Methods:*** **Partial least-squares (PLS) and linear support vector regression (SVR) models were applied using two factor-four level experimental design and UV-spectrophotometric data. They were compared to each other, and their advantages and disadvantages were discussed.** ***Results:*** **The developed methods succeeded to determine DAP and TAD in different ratios with good results regarding International Conference on Harmonization guidelines. Linearity ranges were 2–15 µg/mL and 3–30 µg/mL for DAP and TAD, respectively, with good accuracy of 100 ± 0.37 for DAP and 100 ± 0.8 for TAD regarding PLS model and 100.04 ± 0.32 for DAP and 99.89 ± 0.77 for TAD regarding SVR model. Good precision values of 0.787 for DAP and 0.793 for TAD regarding PLS model and 1.105 for DAP and 0.930 for TAD regarding SVR model were obtained. The two models were applied on the dosage forms and statistically compared with the published HPLC method with no significant difference regarding accuracy and precision.** ***Conclusions:*** **The two models can be utilized for routine analysis and QC of DAP and TAD in their bulk and pharmaceutical formulations. The SVR model gives better results and generalization ability than those of the PLS model regarding accuracy and prediction error, while the latter is better for being simpler and faster.**

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Corresponding author's e-mail: bassma.hossam@hotmail.com
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Dapoxetine hydrochloride (DAP), shown in Figure 1, is chemically identified as (S)-N,N-Dimethyl-3-(naphthalen-1-yloxy)-1-phenylpropan-1-amine (1). It is a member of the selective serotonin inhibitor family; hence, it helps to cure depression (2, 3) and treat men suffering from premature ejaculation (4, 5). Tadalafil (TAD), shown in Figure 1, is chemically identified as (6R,12aR)-6-(1,3-benzodioxol-5-yl)-2-methyl-2,3,6,7,12,12a hexahydropyrazino [1',2':1,6] pyrido [3,4-b] indole-1,4-dione (1). It is a phosphodiesterase type-5 inhibitor, which is also used to treat men suffering from erectile dysfunction and pulmonary arterial hypertension (3). Both drugs are mixed and formulated as long-last tablets called Erectafil®, which is used for treatment of erectile ejaculation problems. Different methods were reported for the estimation of DAP either as a single compound or in the presence of other compounds using different techniques such as UV-spectrophotometry (6), HPLC (7, 8), and HPTLC (9, 10), while TAD was detected by various reported methods such as UV-spectrophotometry (11, 12), HPLC (13–15), TLC (13, 16), and capillary electrophoresis (17). Only one spectrophotometric method using dual wavelength (18) and two HPLC chromatographic separations were published for estimation of DAP and TAD in their pharmaceutical dosage forms and human plasma (19, 20). This study aimed to develop and validate accurate, sensitive, and selective chemometric methods using UV spectral data for solving the spectral interference problems of DAP and TAD in their binary mixtures and pharmaceutical formulation without any need of prior separation. The aim of the multivariate calibration methods is to detect a relationship between the UV spectral information and the concentrations of the proposed drugs. Two models were used and discussed in a lot of previous literature, including partial least-squares (PLS) and linear support vector regression (SVR; 21, 22).

In PLS and SVR, two data matrices, X and Y, are related to each other by a linear multivariate model. PLS and SVR are able to analyze data and resolve many, noisy, collinear, and even incomplete variables in both X and Y. Also, in these models, the increasing number of relevant variables and observations improves the precision of the parameters. Nowadays, SVR and PLS are commonly used because of their dependence on desktop computer; hence, optimizing or changing parameters can be performed quickly and easily instead of spending days or weeks. SVR is more preferable than PLS because it is more flexible and gives the minimum errors in data prediction (23, 24).

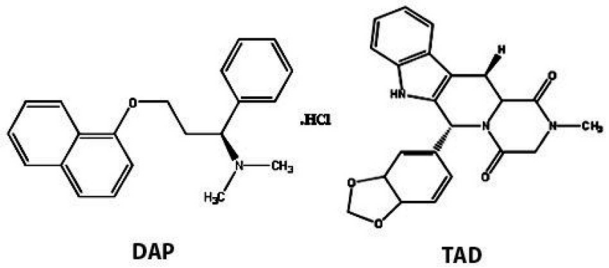

Figure 1. Chemical structures of DAP and TAD.

Experimental and Procedures

Instruments

A double beam UV-visible spectrophotometer (SHIMADZU, Kyoto, Japan) model UV 1601 PC with quartz cell of 1 cm pathlength was connected to an IBM compatible computer. The software was UVPC personal spectroscopy software version 3.7.

Material and Reagents

(**a**) *Pure samples*.—DAP was thankfully afforded by Al-Andalous Medical Company (Cairo, Egypt), with purity labeled to be 99.91%. TAD was thankfully afforded by Eva Pharma Company (Cairo, Egypt), with purity labeled to be 99.91%.

(**b**) *Pharmaceutical dosage form*.—Erectafil Long Last tablets batch No. 325-OSP are manufactured by Combitic Global Caplet (New Delhi, India). Each tablet is labeled to contain 60 mg DAP and 20 mg TAD.

(**c**) *Chemicals and reagents*.—Methanol HPLC grade was AQ 1 acquired from Fischer, United Kingdom.

Standard Solutions (Stock and Working)

(**a**) *Stock standard solutions (1 mg/mL)*.—An amount equal to 0.1 g of each drug was exactly weighed and transferred into two different 100 mL volumetric flasks; 50 mL methanol was added to each flask and shaken to dissolve, and then the volume was completed to the mark with methanol.

(**b**) *Working standard solutions (100 μg/mL)*.—Ten mL each of DAP and TAD stock standard solutions was precisely transferred into two distinct 100 mL volumetric flasks, and then the volume was completed to the mark with methanol.

Linearity

UV spectral data for different aliquots of each of DAP and TAD ranging between 2 and 15 μg/mL, and 3 and 30 μg/mL, respectively, were recorded from 221 to 325 nm, where DAP and TAD exhibited good linearity. The overlapped spectra of 10 μg/mL DAP and 30 μg/mL TAD are shown in Figure 2.

Experimental Design

(**a**) *Calibration set*.—A multifactor multilevel calibration design consisting of four levels and two factors was developed using four concentration levels that are given specific codes as −2, −1, +1, +2, and −1, which is the central value for each of the proposed drugs. The design purpose is to distribute the mixture space equally, which means that there are four mixtures for each compound at each concentration level, resulting in 16 mixtures for the training set (25). The central value was 10.5 and 4 μg/mL for DAP and TAD, respectively. Selection of the concentration for the levels of DAP and TAD was based on their calibration ranges and on their ratio in their pharmaceutical dosage form of Erectafil long-last tablets. Both drugs were involved in levels from 25 to 100% of each other based on a molar basis to cover maximum possibilities. Table 1 represents the training set concentrations.

(**b**) *Test set*.—The validity and the sensitivity of the methods AQ 2 were checked by preparing nine independent mixtures, including four mixtures repeated from the training set (4, 7, 12, and 14) and another five mixtures other than the training set mixtures, which still included in the space of the design concentration, as shown in Table 2. The two-dimensional score plot for the first two principal components of the concentration matrix is shown in Figure 3, which indicates that the training set design (presented as circles) is orthogonal, symmetric, and rotatable, while the test set mixtures (presented as stars) are included within the space of the design concentration of the training set.

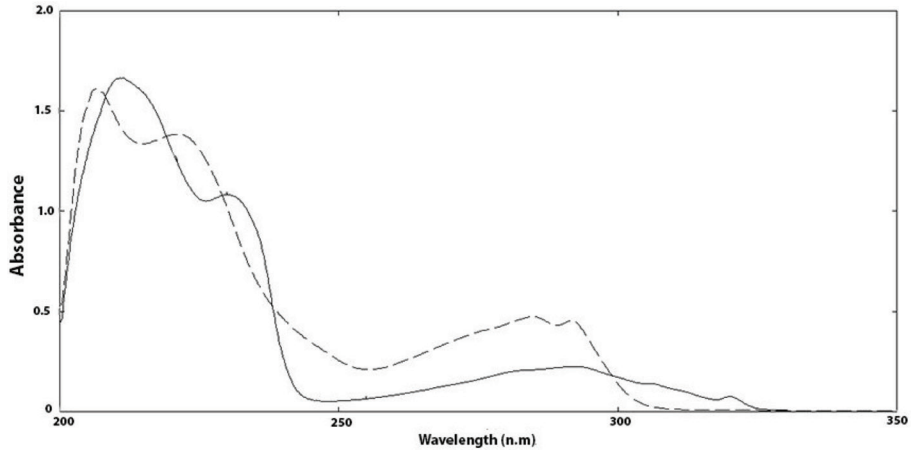

Figure 2. Zero order absorption spectra of 10 μg/mL DAP (solid line) and 30 μg/mL TAD (dashed line) using methanol as a solvent. AQ 3

Table 1. The three-level two-factor experimental design using 16 training set mixtures with 9 test set mixtures shown as concentrations of the mixture components in micrograms per milliliter

	Training set		Test set	
No.	DAP	TAD	DAP	TAD
1	9.5	4	10.5	5
2	9.5	3	9	6
3	9	3	10.5	7
4	9	6	9.5	7
5	10.5	3	9.5	6
6	9	4	9	5
7	9.5	7	10.5	5
8	11	7	10	4
9	11	3	10	5
10	9	7	—[a]	—
11	11	4	—	—
12	9.5	6	—	—
13	10.5	6	—	—
14	10.5	7	—	—
15	11	6	—	—
16	10.5	4	—	—

AQ 4 [a] — = AUTHOR PLEASE DEFINE.

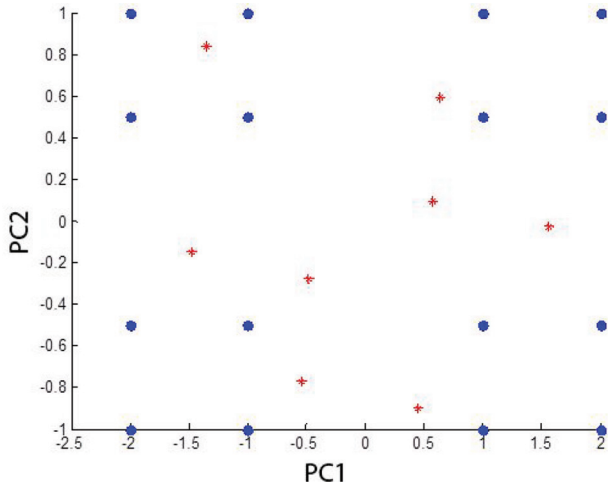

Figure 3. Two-dimensional scores plot for the mean centered 16 training set samples (circles) and 8 test set samples (stars) of concentration matrices of the three-level two-factor experimental design.

(**c**) *Analysis of Erectafil long-last tablets*.—Ten Erectafil tablets were weighed, finely grinded, and blended well. A volume equal to 60 and 20 mg of DAP and TAD, respectively, was exactly weighed and transferred into a 100 mL volumetric flask, and 75 mL methanol was added. The resulted solution was sonicated for 30 min and cooled, and the volume was

Table 2. Analysis results for the prediction of training set (auto-prediction) of Dapoxetine and Tadalafil by PLS and linear SVR chemometric methods

		Training set							
Taken, µg/mL		PLS				Linear SVR			
		DAP		TAD		DAP		TAD	
DAP	TAD	Found, µg/mL	% *R*	Found, µg/mL	% *R*	Found, µg/mL	% *R*	Found, µg/mL	% *R*
9.5	4	9.51	100.07	4.02	100.50	9.51	100.11	4.03	100.75
9.5	3	9.46	99.60	2.99	99.56	9.47	99.71	2.98	99.45
9	3	9.07	100.76	3.01	100.22	9.08	100.84	3.00	100.12
9	6	9.00	99.95	5.98	99.70	9.01	100.11	5.97	99.50
10.5	3	10.49	99.92	2.95	98.46	10.49	99.90	2.94	97.94
9	4	8.94	99.35	4.00	100.10	8.99	99.88	3.99	99.77
9.5	7	9.47	99.71	7.00	100.05	9.49	99.89	6.98	99.73
11	7	11.00	100.02	7.04	100.52	11.01	100.09	7.03	100.43
11	3	10.99	99.90	3.04	101.25	11.01	100.09	3.03	101.00
9	7	9.04	100.49	7.00	99.98	9.03	100.35	7.00	100.02
11	4	11.03	100.27	3.97	99.19	11.01	100.09	3.97	99.25
9.5	6	9.53	100.30	6.00	100.05	9.50	100.04	6.03	100.50
10.5	6	10.44	99.45	5.96	99.27	10.43	99.32	5.97	99.50
10.5	7	10.49	99.88	7.02	100.28	10.49	99.90	7.03	100.38
11	6	11.03	100.26	5.96	99.29	11.03	100.29	5.95	99.22
10.5	4	10.51	100.11	4.07	101.71	10.50	99.97	4.03	100.75
Mean, %			100.00		100.01		100.04		99.89
SD			0.37		0.80		0.32		0.77
RMSEC			0.0413		0.0397		0.03		0.0302

completed to the mark with methanol to prepare 1 mg/mL stock solution. Then, the stock solution was filtered and diluted with ethanol to obtain 100 µg/mL working solution. Finally, 0.1 and 0.35 mL each DAP and TAD working solutions, respectively, were diluted to 10 mL with methanol. The average of three resulting spectra was obtained. These procedures were repeated three times, and the resulted spectra were resolved using the presented multivariate chemometric models.

(**d**) *Software*.—Commands for PLS (PLS1 algorithm; 21), bootstrap, and grid search were written using MATLAB 7.5.0 (R2007b). The SVR algorithm commands were found on http://onlinesvr.altervista.org/. All calculations were done using an Intel Core 2 Duo CPU with 2.20 GHz and 3.00 GB RAM under Microsoft Windows 7.

(**e**) *Chemometric methods*.—(*1*) *PLS regression*.—In this model, a given number of PLS components (latent variables) is used to decompose the predictor matrix (X) and the response vector (c) according to some equations, which were described in detail in many previous works (26–28).

(2) *Optimization of the number of PLS components*.—Leave one out cross validation (LOO-CV) was used to select the optimum number of PLS components that gave the lowest AQ 5 value of RMSECV. The basis of the CV method was discovered in detail by Haaland and Thomas (27), and it was used in previous works (28).

(*3*) *SVR*.—Assume vector (c) is an output of a data matrix X ($I \times J$). Relying on X, finding a multivariate regression function $f(x)$ is the aim to predict a desired output property (e.g., the concentration of a chemical compound) from a sample (e.g., a spectrum). A full description of SVR models was reported in many previous works (29, 30).

(*4*) *Optimization of the number of linear SVR model parameters*.—ε-insensitive loss function was performed in this study to optimize the SVR model. The basics of this function was explained in detail by Gunn and Parrella (31, 32). The value primary range was 0.01–1 for ε and 30–1000 for regularization constant C.

Results and Discussion

Optimization Results of Parameters

For the PLS model, the LOO-CV method was used to detect the best PLS components number. The results were four for DAP and five for TAD, as shown in Figures 4 and 5. For SVR model, the results of the grid search that gave the minimum RMSECV value were ε = 0.01 and C = 420 for DAP and AQ 6 ε = 0.03 and C = 990 for TAD.

Data Analysis Results

The presented work aimed to determine DAP and TAD quantitatively using two common chemometric methods, including PLS and linear SVR. The two multivariate models were able to use the UV spectral data and resolve the overlapping spectra of the components shown in Figure 2. This work shows a comparative study of the two presented chemometric models through the analysis of the two drugs.

PLS and linear SVR models were successfully able to detect the concentrations of both drugs together in each mixture of the training set and the test set, provided by the high recovery percentage with low SD results as shown in Tables 2 and 3. The root mean square error of prediction (RMSEP) is an important way to measure the predictive abilities of the proposed models (Tables 2 and 3). A comparative plot of RMSEP of each of PLS and linear SVR models for the prediction of test set mixtures is shown in Figure 6. The comparison indicates the success of the presented chemometric models in the prediction of both drugs together with good accuracy, satisfied precision, and low prediction error, especially for linear SVR.

Various comparison parameters were included in this work. The first was the RMSEP, which provides the auto-predictive error value. It was noticed that linear SVR shows lower RMSEC than that of PLS as shown in Table 2, which indicates the higher accuracy of SVR than of PLS.

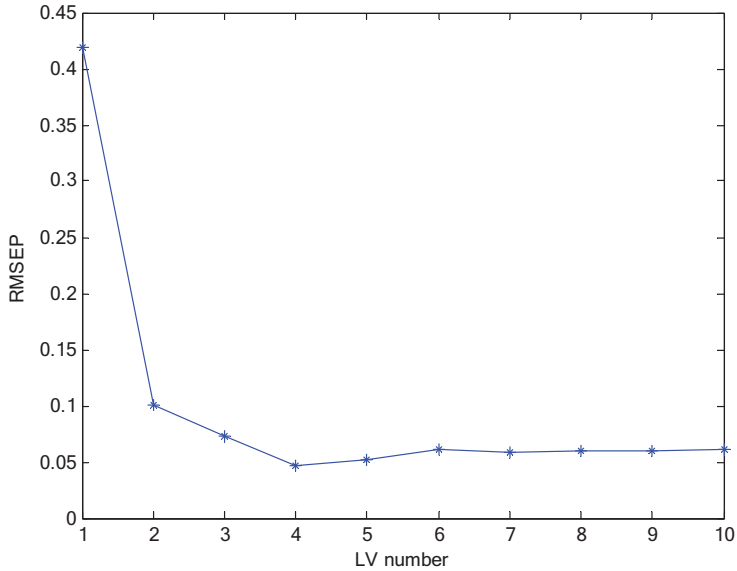

Figure 4. Selection of the optimum number of PLS components for Dapoxetine [latent variables (LV)] via plotting the number of PLS components versus the corresponding root mean square error of prediction (RMSEP) by using bootstrap technique.

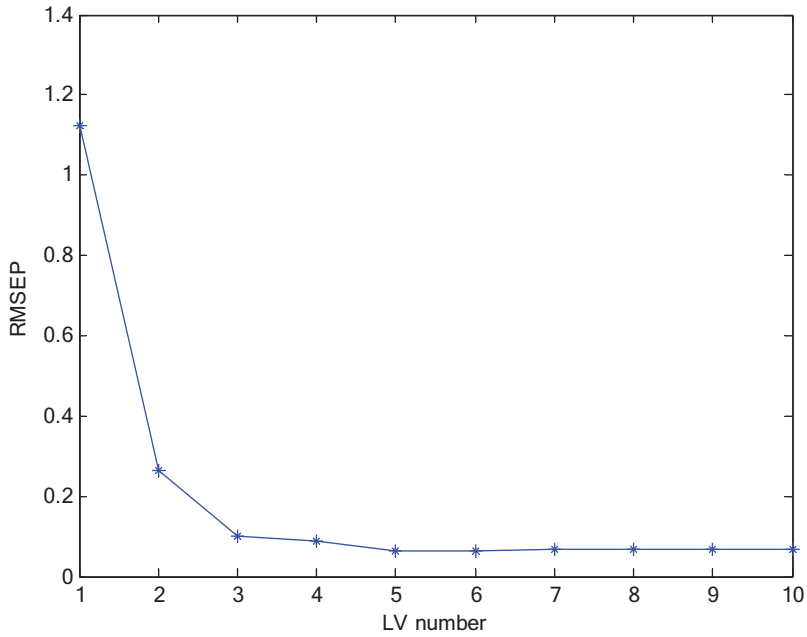

Figure 5. Selection of the optimum number of PLS components for Tadalafil [latent variables (LVs)] via plotting the number of PLS components versus the corresponding root mean square error of prediction (RMSEP) by using bootstrap technique.

Table 3. Analysis results for the prediction of eight independent test set of Dapoxetine and Tadalafil by PLS and linear SVR chemometric methods

Taken, µg/mL		Test set							
		PLS				Linear SVR			
		DAP		TAD		DAP		TAD	
DAP	TAD	Found, µg/mL	% R	Found, µg/mL	% R	Found, µg/mL	% R	Found, µg/mL	% R
10.5	5	10.54	100.42	5.07	101.34	10.54	100.43	5.06	101.28
9	6	9.05	100.58	5.97	99.48	9.05	100.57	5.98	99.62
10.5	7	10.43	99.36	7.03	100.38	10.39	98.96	7.08	101.08
9.5	7	9.54	100.46	6.98	99.66	9.52	100.20	7.00	100.06
9.5	6	9.52	100.24	5.98	99.67	9.51	100.10	6.01	100.10
9	5	9.00	100.01	4.98	99.58	8.98	99.76	5.00	99.94
10.5	5	10.41	99.12	4.93	98.64	10.36	98.67	5.00	99.94
10	4	10.04	100.42	3.93	98.23	9.99	99.94	3.98	99.42
10	5	9.89	98.90	4.93	98.60	9.85	98.51	4.98	99.51
Mean, %			99.95		99.51		99.68		100.11
SD			0.64		0.96		0.77		0.66
RMSEP			0.0613		0.0496		0.0812		0.0358

Moreover, the corresponding SD of the linear SVR model is smaller than that of PLS, indicating the higher precision of SVR than of PLS for the determination of DAP but a higher precision of PLS than of SVR for TAD, as shown in Table 2. In Figure 6, the comparative bar plot shows that the linear SVR gives lower RMSEP than PLS for the determination of DAP, while the linear PLS gives lower RMSEP than SVR for TAD. Concerning the calculations and computational procedures, PLS is simpler than SVR, as the latter requires more steps in processing and is more time consuming for optimization. However, when a comparative *t* and *F* statistical analysis of test set mixtures for both PLS and linear SVR was done, no significant difference was indicated, as shown in Table 4, which indicates the two methods are efficient for analysis of linear data sets.

From the previous discussion, the two models provide a better way to save time and cost and use simpler equipment when compared with the reported HPLC method (20). They also give good results and prediction capability.

Furthermore, the proposed chemometric methods show better sensitivity than the reported dual wavelength

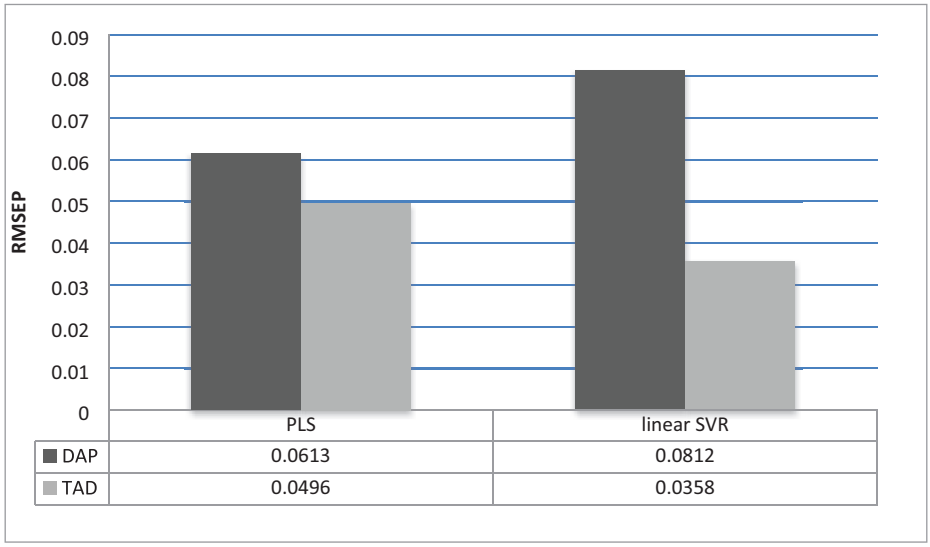

Figure 6. RMSEP plots for the prediction of the independent test set samples for Dapoxetine and Tadalafil using PLS and linear SVR models.

Table 4. Statistical analysis of the three proposed PLS and linear SVR chemometric methods and the reported HPLC method for determination of Dapoxetine and Tadalafil in pharmaceutical formulation

Parameters		PLS	Linear SVR	Reported HPLC method (20)
Mean, μg	DAP	91.94	91.51	91.51
	TAD	104.41	104.44	105.03
SD	DAP	0.37	0.74	0.63
	TAD	0.69	0.73	1.25
N	DAP	6	6	6
	TAD	6	6	6
Student's *t*-test (2.228)[a]	DAP	1.448	0.012	—[b]
	TAD	1.074	1.005	—
F-test (5.050)[a]	DAP	2.824	1.395	—
	TAD	3.259	2.950	—

[a]

AQ 7 [b] — = AUTHOR PLEASE DEFINE.

spectrophotometric method (18), as shown in Table 5. A brief comparison between the proposed chemometric methods and the reported spectrophotometric and HPLC methods is given in Table 5.

Application of the Proposed Methods to the Pharmaceutical Formulation

The developed chemometric methods were utilized for analysis of DAP and TAD in Erectafil long last tablets, and satisfied results were found with acceptable recoveries. These results were compared statistically to those of the published HPLC method (20) by applying *t*- and *F*-tests. The resulted values were lower than the theoretical ones, indicating the lack of any significant difference between the developed methods and the reported HPLC method (20) regarding precision and accuracy (Table 4).

Method Validation

Validation of the methods was carried out according to ICH guidelines (33).

Selectivity.—Using different laboratory prepared mixtures containing different ratios of DAP and TAD within their linearity ranges (training and test set), selectivity of the proposed chemometric models was assessed. Good results are shown in Table 2.

Linearity and range.—The calibration range for DAP and TAD was determined according to Beer-Lambert's law to give accurate, precise, and linear results. Linearity ranges of DAP and TAD are shown in Table 6.

Accuracy.—Using blind pure samples of the studied drugs, accuracy of the proposed methods was calculated as the percentage recoveries. The concentrations were found from the corresponding regression equations, and the results are shown in Table 6.

Precision.—(*1*) *Repeatability*.—Three concentrations of DAP (3, 5, 14 μg/mL) and TAD (10, 16, 20 μg/mL) were analyzed three times intra-daily using the proposed chemometric models. Good results were obtained, as shown in Table 6.

(*2*) *Intermediate precision*.—The previous procedures were repeated inter-daily on three different days for the analysis of the chosen concentrations. Good results were obtained, as shown in Table 6.

LOD and LOQ.—High sensitivity of the proposed chemometric models for DAP and TAD was found because of low LOD and LOQ values, as shown in Table 6.

Conclusions

The presented study was proposed to analyze DAP and TAD quantitatively in binary mixtures and pharmaceutical dosage form using different chemometric models. Moreover, it aimed to show a comparative study among PLS and linear SVR models, finding the advantages and limitations of each model. The results obtained are an encouragement for performing clever chemometric models for the quantitative determination of

Table 5. A comparison between the proposed chemometric models (PLS and linear SVR) and the reported methods for determination of Dapoxetine and Tadalafil

	Proposed PLS and linear SVR models		Reported dual wavelength method (18)		Reported HPLC method (20)	
Parameters	DAP	TAD	DAP	TAD	DAP	TAD
Linearity range, µg/mL	2–15	3–30	10–60	4–24	0.75–12	0.25–4
Used instrument	A double beam UV/Vis spectrophotometer; UVPC personal spectroscopy software version 3.7		A double beam UV/Vis spectrophotometer; UV-Probe system software		HPLC system; UV/Vis detector; Thermo Hypersil BDS–C18 column	
Analyzed samples	Bulk material and pharmaceutical formulations					

Table 6. Results of assay validation parameters of the proposed models (PLS and linear SVR) for determination of Dapoxetine and Tadalafil

	PLS		Linear SVR	
Parameters	DAP	TAD	DAP	TAD
Calibration range, µg/mL	2–15	3–30	2–15	3–30
Slope	0.1473	0.0516	0.1473	0.0516
Intercept	0.0616	0.144	0.0616	0.144
Correlation coefficient	0.9999	0.9999	0.9999	0.9999
Accuracy, mean ± SD	99.84 ± 1.13	99.85 ± 1.07	99.84 ± 1.13	99.85 ± 1.07
Precision				
Repeatability (RSD %)[a]	0.358	0.350	0.343	0.216
Intermediate precision (RSD %)[a]	0.787	0.793	1.105	0.930
LOD	0.597	0.949	0.597	0.949
LOQ	1.808	2.877	1.808	2.877

AQ 8 [a] AUTHOR PLEASE DEFINE.

various drugs with low cost and simple equipment and devices AQ 9 using the UV lamps in the spectrophotometric techniques. However, PLS is much simpler and faster and would be more suitable for routine analysis of such simple mixtures. The two models have better sensitivity than the reported dual wavelength spectrophotometric method and can save time and cost using simple equipment rather than the reported HPLC method with satisfied results and detection ability.

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Author Query Sheet

Query No.	Queries	Response
AQ 1	For “Fischer, UK,” is Thermo Fisher Scientific meant? Please revise accordingly and provide a city.	
AQ 2	Please confirm if the changes to the sentence beginning “The validity and the sensitivity of the methods were checked…” retain your intended meaning, or revise as necessary. Please also clarify “…which still included in the space…” Is “which were still included in the space…” meant instead?	
AQ 3	We edited your caption for Figure 2 to cited “solid line” and “dashed line”. Please confirm if these edits are acceptable.	
AQ 4	Please provide an explanation for the em dash used in Table 1. For example, does it mean not applicable?	
AQ 5	Root mean square error of prediction has been used as the definition for both RMSEP and RMSEC. Please confirm if RMSEC should be changed to RMSEP for consistency, or provide a different definition for RMSEC. Please also note that RMSECV is used and has not been defined. Please provide a definition for this at first use in the text.	
AQ 6	As there were no numbered equations included in the text, mention of “Eq.3” has been removed. Please confirm this change is appropriate, or revise as necessary.	
AQ 7	Please provide a footnote for the “a” label in Table 4 or remove the letter from the table. Also, please provide an explanation for the em dash used. For example, does it mean not applicable?	
AQ 8	Please provide a footnote for the “a” label in Table 6, which was originally an asterisk. If not, please confirm if this footnote symbol may be removed.	
AQ 9	Please confirm the change from lambs to lamps is correct in the sentence beginning “The results obtained are an encouragement for…” or revise as necessary.	
AQ 10	Please provide more information for reference 32, such as the University name and location, if possible.	