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Simultaneous Spectrophotometric Determination of Sacubitril and Valsartan in their Recently Approved Pharmaceutical Preparation

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ABSTRACT

Objectives: Simultaneous estimation of sacubitril and valsartan in their new pharmaceutical tablets by bivariate method and two multivariate methods namely; classical least square and principal component regression. **Methods: (A)** Bivariate method, where absorbance values were measured at two optimum wavelengths (220 nm and 250 nm). **(B)** Multivariate methods, where partial factorial design was constructed within the wavelength range from 210-290nm at 1 nm interval. **Results:** The bivariate method is based on simple mathematic algorithm using two optimum wavelengths for the analysis of the mixture. On the other hand, two multivariate methods considering all the variables at the same time have been used to improve the quality of spectral analysis of the mixture. **Conclusion:** The proposed methods have been successfully applied for the determination of the two drugs in Entresto® tablets. Statistical comparisons between the obtained results and those obtained by the reported method have been performed showing no significant differences by applying t-test and F-test.

Keywords: *Bivariate; Multivariate; Sacubitril; Valsartan*

INTRODUCTION

Entresto® (sacubitril/valsartan) is a new FDA approved mixture for the treatment of heart failure. It contains sacubitril(SAC) **Figure 1**, a prodrug that results in neprilysin inhibition and valsartan (VAL) which is angiotensin II Type-1 receptor blocker, **Figure 2**[1,2].

The resolution of multi-component preparations is often the main goal of analytical chemistry. The spectrophotometric techniques have advantages of simplicity, solvent and time saving. The only drawback is the presence of severely overlapped spectra in multi-component preparations [3,4]. Hence, several spectrophotometric methods have been developed for simultaneous estimation of these components[5-11].

Up to date, only three chromatographic methods have been reported for simultaneous determination of SAC and VAL mixture[12-14]. Hence, the aim of this work is to develop three spectrophotometric methods for simultaneous determination of SAC and VAL in their pharmaceutical tablets. The developed methods are bivariate method (BM)[15-19], classical least square (CLS) and principal component regression (PCR)[20-23].

MATERIALS AND METHODS

Experimental

Apparatus and software

Shimadzu UV-Visible 1800 Spectrophotometer, (Tokyo, Japan), equipped with 10 mm matched quartz cells. UV-Probe personal spectroscopy software version 2.43. (SHIMADZU) is used. All chemometric methods were implemented in MATLAB 8.2.0.701 (R2013b) using PLS toolbox version 2.1. The t-test and F-test were performed using Microsoft Excel (2010).

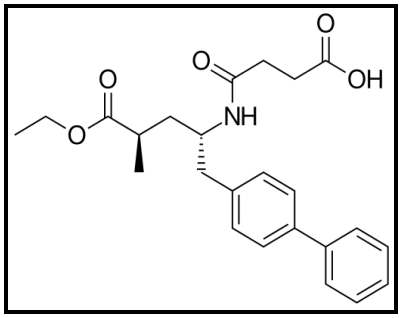

Figure 1. Structural formula of SAC.

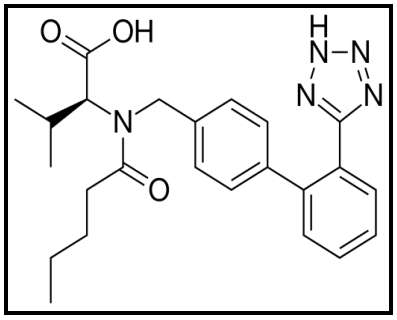

Figure 2.Structural formula of VAL.

Materials and solvents

Pure standard of SAC (99.5%) and VAL (99.4%) were kindly supplied by National Organization for Drug
Control and Research, Giza, Egypt. Entresto® tablets 97/103 (manufactured by Novartis stein AG, Switzerland), labeled to contain 97 mg of SAC and 103 mg of VAL, were kindly supplied by National Organization for Drug Control and Research, Giza, Egypt. Methanol, HPLC grade (Sigma-Aldrich, Germany).

Standard solutions

Standard stock solutions of SAC and VAL (1000 µg/mL) were prepared separately by dissolving 100 mg of each drug powder in methanol. Working solutions (100 µg/mL) were prepared before use by dilution from the stock solutions with methanol.

Procedure

For BM

Different aliquots equivalent to 10–150 µg of SAC and VAL were accurately transferred from their working standard solutions (100 µg/mL) into two separate series of 10-mL volumetric flasks and completed to volume with methanol. The absorption spectra (from 200 to 400 nm) of these solutions were recorded using methanol as a blank. The absorbance was measured at 220 and 250 nm from which the calibration graphs were constructed and then the corresponding regression equations were computed at the selected wavelengths. The obtained slope and intercept values were used for construction of two mathematical equations used for calculating the concentrations of both drugs in their binary mixture.

Assay of laboratory prepared mixtures

Laboratory prepared mixtures containing SAC and VAL in different ratios was prepared by transferring the aliquots of two drugs into 10-mL volumetric flasks and complete to volume with methanol. The absorbance values of the mixtures were recorded at 220 and 250nm and substituted in the following two equations to obtain the concentrations of both drugs:

$$C_{SAC} = (A_{M1} - e_{SAC+VAL1} - m_{VAL1}C_{VAL}) / m_{SAC1}$$

$$C_{VAL} = [m_{SAC2}(A_{M1} - e_{SAC+VAL1}) + m_{SAC1}(e_{SAC+VAL2} - A_{M2})] / m_{SAC2}m_{VAL1} - m_{SAC1}m_{VAL2}$$

Where:

- C_{SAC}, C_{VAL} are the concentration of component SAC, component VAL.
- m_{SAC1}, m_{SAC2} are the slope values of component SAC at λ_1, λ_2.
- m_{VAL1}, m_{VAL2} are the slope values of component VAL at λ_1, λ_2.
- A_{M1}, A_{M2} are the absorbance values of the binary mixture at λ_1, λ_2.
- $e_{SAC+VAL1}$, $e_{SAC+VAL2}$ are the sum of the intercepts of components SAC, VAL at λ_1, λ_2.

For CLS and PCR

Partial factorial design based on five levels and two factors was constructed [24]. The design results in 25 mixtures of different ratios of SAC and VAL, **Table 1**. Thirteen samples were used as a training set and twelve were used as a validation set. The chosen concentrations for each compound are based on its linearity. The absorption spectra of the samples were scanned from 200 - 400 nm at 1 nm interval against methanol as a blank. The noisy region from 200-210 nm and the zero absorbance after 290 nm were rejected. CLS and PCR models were constructed by transferring the spectral data to MATLAB for subsequent calculations. The 2D plot of the experimental space showing the positioning of the training set and the validation set samples, **Figure 3**.

Application to pharmaceutical preparation

Ten Entresto® tablets (each tablet labeled to contain 97 mg SAC and 103 mg VAL) were weighed and finely powdered. A portion of powder equivalent to one tablet was weighed, transferred into conical flask and dissolved in 75 mL of methanol. The solution was

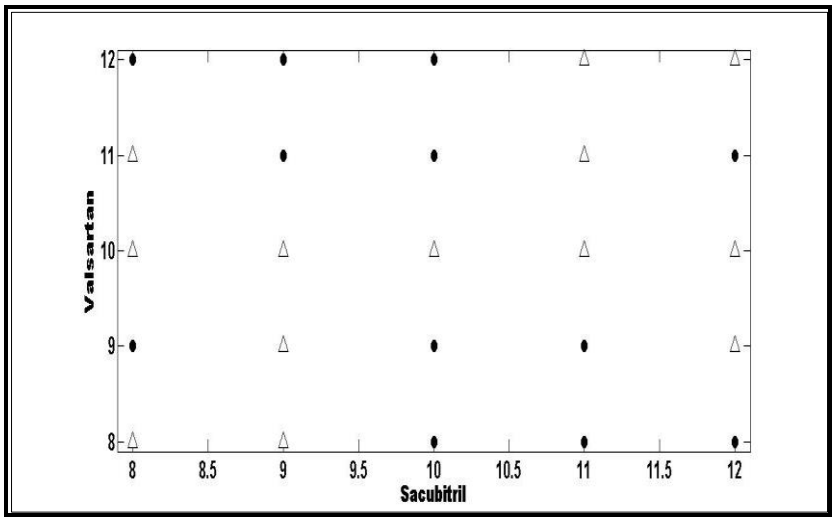

Figure 3. The 2D plot of the experimental space showing the positioning of training set (Δ) and the validation set (•) samples for SAC and VAL.

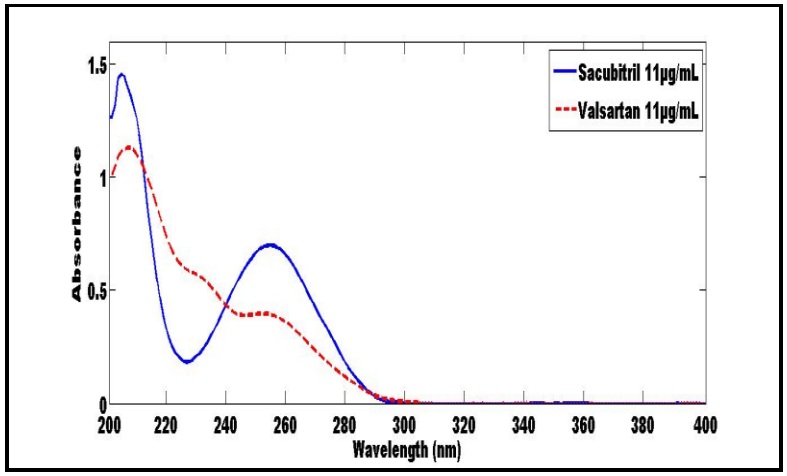

Figure 4. Absorption spectra of SAC and VAL.

shaken vigorously for 15 min then sonicated for about 30 min and filtered into 100-mL volumetric flask. The volume was completed to 100-mL with methanol to get a stock solution containing 970 µg/mL of SAC and 1030 µg/mL of VAL. The solution was suitably diluted with methanol to obtain sample solutions containing SAC and VAL in the concentrations ratio of 1:1.06 µg/mL, respectively, as in the tablet formulation. Then the procedure was completed as described under the procedure of each method.

RESULTS AND DISCUSSION

The zero order absorption spectra of SAC and VAL show sever overlap which doesn't permit direct determination of both drugs in their mixture, **Figure 4**. In order to resolve these overlap, bivariate and multivariate methods have been developed.

Bivariate method (BM)

Kaiser method [19]has been used for the selection of two optimum wavelengths for the analysis of two drugs. The absorbance of the two components individually at six different selected wavelengths was recorded in the region of overlapping; 220, 230, 240, 250, 260 and 270 nm. The slope values of the linear regression equations were estimated for both SAC and VAL at the selected wavelengths. A series of sensitivity matrices (K) was created for each binary mixture and for every pair of the preselected wavelengths:

Table 1. Experimental design of concentrations of SAC and VAL mixtures used in chemometric models

No. of Mix	SAC (µg/mL)	VAL (µg/mL)
1	10	10
2	10	8
3	8	8
4	8	12
5	12	9
6	9	12
7	12	10
8	10	9
9	9	9
10	9	11
11	11	12
12	12	11
13	11	10
14	10	12
15	12	12
16	12	8
17	8	11
18	11	8
19	8	10
20	10	11
21	11	11
22	11	9
23	9	8
24	8	9
25	9	10

The shaded rows represent the calibration set.

$$K = \begin{vmatrix} m_{SAC1} & m_{VAL1} \\ m_{SAC2} & m_{VAL2} \end{vmatrix}$$

Where, $m_{SAC1,2}$ and $m_{VAL1,2}$ are the slopes of the components SAC and VAL at the two selected wavelengths [16-18]. The determinants of these matrices were calculated and the wavelength set selected for which the highest matrix determinant value was obtained, **Table 2**. It was found that; the slopes at 220 and 250 nm gave the maximum value of K and thus chosen for the analysis.

Method validation

The proposed method was validated according to ICH recommendations [25].

- ***Linearity***

The linearity of the developed method was evaluated by analyzing different concentrations of SAC and VAL in triplicates. Beer-Lambert concentration ranges were found to be 1-15 µg/mL for both drugs. The values of coefficient of determination were close to unity indicating good linearity. The regression parameters were summarized in **Table 3**.

- ***Limit of detection (LOD) and limit of quantitation (LOQ)***

LOD and LOQ were calculated from the following equations:

$$LOD = 3.3\ Sa / slope$$
$$LOQ = 10\ Sa / slope$$

Where Sa is the residual standard deviation of a regression line. Low values of LOD and LOQ values of SAC and VAL indicated the sensitivity of the proposed methods**,** **Table 3**.

- ***Accuracy***

The accuracy of the results was checked by applying the proposed method for triplicate determination of three concentration levels covering the specified range for each drug (5, 9, 13 µg/mL). The concentrations were obtained from the constructed mathematical equations. The mean percent recovery of the concentrations was calculated. Good recovery percent of the studied concentration with acceptable value was obtained, **Table 3**. Moreover, accuracy of the method was further assessed by applying the standard addition technique to prove that the proposed methods can selectively analyze each drug without any interference from the other drug or the excipients, **Table 4**.

- ***Precision***

Three concentrations of (5, 9, 13 µg/mL) for either SAC or VAL were analyzed three times intraday for repeatability and on three successive days for intermediate precision using the proposed method. The small calculated relative standard deviations indicated the high precision of the proposed methods**,** **Table 3**.

- ***Specificity***

The specificity of the method was confirmed by the analysis of different laboratory prepared mixtures of SAC and VAL within the linearity range. Perfect determination of each drug in the presence of the other indicated by acceptable calculated recovery percent and confirmed the high specificity of the proposed methods**,** **Table 5.**

- ***Stability of solutions***

Working standard solutions of SAC and Val showed no spectrophotometric changes up to 7 days when stored at 4 °C.

Multivariate methods

Multivariate models are a useful tool for data analyses. CLS involved the application of multiple linear regressions to the classical expression of the Beer-Lambert law of spectroscopy:

$$\boldsymbol{A = KC + E}$$

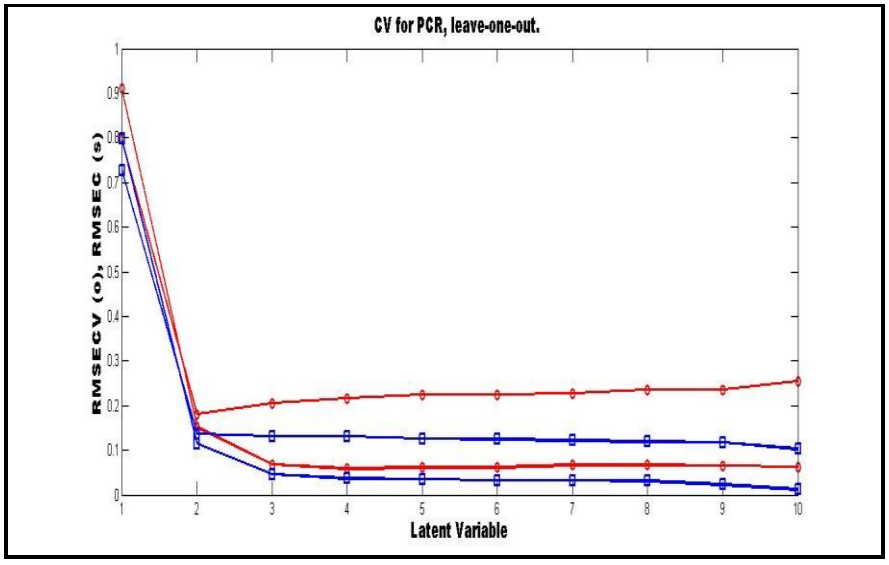

Figure 5. RMSECV plot of the cross validation results of the calibration set as a function of the number of latent variables (LVs) used to construct the PCR model.

Table 2. Values of the sensitivity matrix determinates calculated according to Kaiser's method (k x 10^6) for the mixture of SAC and VAL by the BM

λ/λ	220	230	240	**250**	260	270
220	0	140	-1562	**-2848**	-2768	-1711
230		0	-1408	-2489	-2412	-1492
240			0	-872	-924	-563
250				0	-138	-71
260					0	14
270						0

Where A is absorbance matrix, absorptivity coefficient and path length are combined in single constant K matrix, C is constituent concentrations matrix and E is matrix of absorbance error (the residual errors between the least squares fit line and the actual absorbance).

The calibration absorbance matrix (13×81) and their corresponding concentration matrix (13×2) were used to find the (k) matrix which was further used for predicting the concentrations of SAC and VAL in the validation set and in pharmaceutical preparation.

However, CLS method is the most simplest of the multivariate methods, but it is a rigid model that require the whole information of all the components in the mixture and their concentrations Unlike CLS, PCR method is more flexible and can be applied even in the presence of interfering substances. PCR is a factors analysis method which involves decomposition of spectral matrix into a set of eigenvectors and scores followed by regression of the scores against the constituent concentrations.

A cross validation method leaving out one sample at a time was used to select the optimum number of latent variables (factors) in PCR[26]. If the number of factors was more than required, more noise would be added to data and if the number of factors was too small, meaningful data that could be necessary for the calibration might be discarded[11]. The method developed by Haaland and Thomas was used for selecting the optimum number of factors[27]. It was found that two latent variables are sufficient for PCR method, **Figure 5**.

Table 6 shows percent recoveries, mean, standard deviation (SD) and root mean square error of calibration (RMSEC) of the calibration set in both models. While, **Table 7** shows percent recoveries, mean, standard deviation (SD) and root mean square error of prediction (RMSEP) of the validation set in

Table 3. Regression and validation data for determination of SAC and VAL by the BM

Parameter		BM	
		SAC	VAL
Accuracy (mean %R) *		99.60	100.06
Repeatability (%RSD) *		0.828	1.333
Intermediate precision (%RSD) *		1.109	1.585
LOD (µg/mL)	220 nm	0.115	0.276
	250 nm	0.131	0.294
LOQ (µg/mL)	220 nm	0.348	0.836
	250 nm	0.398	0.891
Range (µg/mL)		1-15	1-15
Slope ± SE	220 nm	0.0260± 0.0001	0.0606± 0.0005
	250 nm	0.0615± 0.0002	0.0338± 0.0003
Intercept ± SE	220 nm	0.0004± 0.0007	0.0252± 0.0047
	250 nm	0.0008± 0.0019	0.0167± 0.0027
Coefficient of determination (r^2)	220 nm	0.9999	0.9996
	250 nm	0.9999	0.9996

**Average of three determinations for three concentrations repeated three times.*

Table 4. Determination of SAC and VAL in Entresto® tablets by BM and application of standard addition technique

Entresto® tablets		Standard addition technique							
% Recovery* ± %RSD		Pharmaceutical(µg/mL)		Pure added (µg/mL)		Pure found (µg/mL)		% Recovery**	
SAC	VAL	SAC	VAL	SAC	VAL	SAC	VAL	SAC	VAL
100.12 ± 1.002	99.66 ± 1.078	5	5	4	4	.402	.407	100.53	101.77
				5	5	5.07	5.03	101.47	100.69
				6	6	6.11	6.05	101.85	100.80
Mean ± %RSD								101.29 ± 0.670	101.09 ± 0.586

**Average of five determinations.*
***Average of three determinations.*

Table 5. Determination of SAC and VAL in synthetic laboratory mixtures by BM

SAC (µg/mL)	VAL (µg/mL)	SAC found (µg/mL)	VAL found (µg/mL)	%Recovery of SAC	%Recovery of VAL
3	3	2.99	2.98	99.56	99.42
5	5	4.87	4.91	97.36	98.29
7	7.4	7.02	7.47	100.34	100.97
9	9.5	9.02	9.44	100.18	99.36
10	10.6	10.08	10.78	100.84	101.69
Mean				99.65	99.95
% RSD				1.366	1.365

Table 6. Percent recoveries, mean, SD and RMSEC for SAC and VAL in the calibration set by CLS and PCR models

Calibration mixture	CLS		PCR	
	SAC	VAL	SAC	VAL
1	98.59	101.27	98.59	101.28
2	102.24	99.28	102.20	99.30
3	99.69	99.23	99.47	99.44
4	102.43	101.34	102.23	101.53
5	100.87	99.17	100.89	99.14
6	99.22	100.27	99.31	100.21
7	98.07	101.29	97.98	101.07
8	99.86	98.19	99.87	98.16
9	102.09	99.15	102.26	98.92
10	98.88	101.29	99.11	101.15
11	100.16	99.17	100.18	99.14
12	99.16	99.31	99.04	99.41
13	99.44	101.25	99.53	101.91
Mean ± SD	100.18 ±1.428	99.91 ± 1.115	100.17 ± 1.419	99.89 ± 1.100
RMSEC	0.136	0.112	0.134	0.111

Table 7. Percent recoveries, mean, SD and RMSEP for SAC and VAL in the validation set by CLS and PCR models

Validation mixture	CLS		PCR	
	SAC	VAL	SAC	VAL
1	102.20	98.22	101.96	98.44
2	99.30	102.10	99.78	101.86
3	100.15	101.42	100.45	101.25
4	101.30	99.18	101.20	99.24
5	100.10	98.36	100.33	98.20
6	99.42	97.82	99.35	97.86
7	100.91	99.08	101.12	98.93
8	97.37	99.34	97.06	99.69
9	97.85	99.32	97.59	99.58
10	100.84	99.17	100.95	99.07
11	97.83	99.27	97.69	99.38
12	102.68	99.24	102.80	99.14
Mean ± SD	99.99 ± 1.715	99.38 ± 1.228	100.02 ± 1.805	99.39 ± 1.157
RMSEP	0.170	0.142	0.181	0.137

Table 8. Statistical comparison of the results obtained by applying the proposed methods and the reported method[14]

Parameters	BM		CLS		PCR		Reported method	
	SAC	VAL	SAC	VAL	SAC	VAL	SAC	VAL
N**	5	5	5	5	5	5	5	5
$\overline{X}$***	100.12	99.66	100.29	99.39	100.34	99.34	100.15	99.75
SD	1.004	1.074	1.055	0.922	1.067	0.941	1.213	1.367
% RSD	1.002	1.078	1.052	0.928	1.063	0.947	1.212	1.371
t-test (2.306)****	0.042	0.248	0.190	0.646	0.268	0.735		
F-test (6.388)****	1.462	1.620	1.322	2.200	1.294	2.113		

**HPLC determination on C18 column using mobile phase consists of acetonitrile: methanol: water (pH 3.0 adjusted with Ortho-phosphoric acid) (30: 50: 20, by volume).*
*** Number of experiments.*
**** The mean of percent recovery of pharmaceutical preparation.*
***** The values in parenthesis are tabulated values of "t "and "F" at (P = 0.05).*

Table 9. One way ANOVA testing for the different proposed methods and the reported methods used for the determination of SAC and VAL

Source of variation	Sum of Squares		Degree of Freedom		Mean of squares		F value	
	SAC	VAL	SAC	VAL	SAC	VAL	SAC	VAL
Between groups	0.166	0.618	3	3	0.055	0.206	0.05 (3.24)	0.17 (3.24)
Within groups	18.925	19.033	16	16	1.183	1.190		

both models. While, **Table 7** shows percent recoveries, mean, standard deviation (SD) and root mean square error of prediction (RMSEP) of the validation set in both models.

Statistical analysis

The developed methods have been applied for determination of SAC and VAL in Entresto® tablets and the results obtained were acceptable with small %RSD values. Results obtained by the proposed methods were statistically compared to those obtained by the reported method [14] and no significant difference was observed, **Table 8.** Moreover, a one-way analysis of variance (ANOVA) test was carried out to compare the proposed methods with each other, and no significant difference was found, **Table 9**.

CONCLUSION

In this work, bivariate and multivariate spectrophotometric methods have been successfully applied for simultaneous determination of a recently FDA approved binary mixture of sacubitril and valsartan. The developed methods have advantages over chromatographic methods that required sophisticated procedures and time consuming.

Conflict of Interest

The authors declare that they don't have any conflict of interest.

REFERENCES

1. US Food and Drug Administration. FDA approves new drug to treat heart failure.www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm453845.htm. Press release. July 7, 2015.
2. Entresto (sacubitril and valsartan) tablets [prescribing information]. East Hanover, NJ: Novartis; July 2015.
3. Badyal, P. N.; Sharma, C.; Kaur, N.; Shankar, R.; Pandey, A.; Rawal, R.K. Analytical techniques in simultaneous estimation: An Overview, *Austin J. Anal. Pharm. Chem.* **2015**, *2* (2), 1037.
4. Siddiqui, M. R.; Alothman, Z. A.; Rahman, N. Analytical techniques in pharmaceutical analysis: A review, *Arabian J. Chem.* **2017**, *10*, s1409-1421.
5. Attia, K. A. M.; El-Abasawi, N.M.; El-Olemy, A.; Abdelazim, A. H. Application of different spectrophotometric methods for simultaneous determination of elbasvir and grazoprevir in pharmaceutical preparation, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2018**, *189*, 154-160.

6. Lotfy, H. M.; Hegazy, M. A. Simultaneous determination of some cholesterol-lowering drugs in their binary mixture by novel spectrophotometric methods, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2013**, *113*, 107-114.
7. Attia, K. A. M.; El-Abasawi, N. M.; El-Olemy, A.; Abdelazim, A. H. Comparative study of different spectrophotometric methods for determination of phenazopyridine hydrochloride in the presence of its oxidative degradation product. *Anal. Chem. Letters.* **2016**, *6* (6), 863-873.
8. Hegazy, M. A.; Yehia, A. M.; Moustafa, A. A. Bivariate versus multivariate smart spectrophotometric calibration methods for the simultaneous determination of a quaternary mixture of mosapride, pantoprazole and their degradation products. *Pharmazie* **2013**, *68* (5), 317-326.
9. Lamie, N. T. Simultaneous determination of binary mixture of amlodipine besylate and atenolol based on dual wavelengths, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2015**, *149*, 201-207.
10. Attia, K. A. M.; El-Abassawi, N. M.; Said, R. A. M.; Ramzy, S. Comparative study between three different methods manipulating the ratio spectra applied for the analysis of labetalol hydrochloride in the presence of its oxidative degradation product. *Anal. Chem. Letters*. **2016**, *6* (5), 561-568.
11. Darwish, H.W.; Bakheit, A. H.; Attia, M. I. Three multivariate calibration methods for simultaneous spectrophotometric determination of Olmesartan medoxamil, amlodipine besylate and hydrochlorothiazide in their combined dosage form. *Digest J. Nanomater. Biostruct.* **2013**, *8* (1), 323-333.
12. Naazneen, S.; Sridevi, A. Development of assay method and forced degradation study of valsartan and sacubitril by RP-HPLC in tablet formulation. *Int. J. App. Pharm.* **2017**, *9* (1), 9-15.
13. Chunduri, R.H.B.; Dannana, G. S. Development and validation of a reliable and rapid LC-MS/MS method for simultaneous quantification of sacubitril and valsartan in rat plasma and its application to a pharmacokinetic study. *Biomed. Chromatogr.* **2016**, *30* (9), 1467-1475.
14. Patel, K. H.; Luhar, S. V.; Narkhede, S. B. Simultaneous estimation of sacubitril and valsartan in synthetic mixture by RP-HPLC method. *J. Pharm. Sci. Biosci. Res.* **2016**, *6* (1), 262-269.
15. Metwally, F.H. Simultaneous determination of nifuroxazide and drotaverine hydrochloride in pharmaceutical preparations by bivariate and multivariate spectral analysis. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2008**, *69*, 343-349.
16. López-Martínez, L.; López-de-Alba, P.L.; de-León-Rodríguez, L. M.; Yepez-Murrieta, M. L. Simultaneous determination of binary mixtures of trimethoprim and sulfamethoxazole or sulphamethoxypyridazine by the bivariate calibration spectrophotometric method. *J. Pharm. Biomed. Anal.* **2002**, *30* (1), 77-85.
17. López-de-Alba, P.L.; Wróbel, K.; López-Martínez, L.; Wróbel, K., Yepez-Murrieta, M. L.; Amador-Hernández, J. Application of the bivariate spectrophotometric method for the determination of metronidazole, furazolidone and di-iodohydroxyquinoline in pharmaceutical formulations. *J. Pharm. Biomed. Anal.* **1997**, *16* (2), 349-355.
18. Attia, K. A. M.; El-Abassawi, N. M.; Ramzy, S. Two wavelengths dependant stability indicating spectrophotometric methods for determination of labetalol hydrochloride in the presence of its oxidative degradation product: a comparative study. *Anal. Chem. Lett.* **2015**, *5* (6), 351-363.
19. Massart, D. L.; Vandeginste, B.G.; Deming, S.N.; Michotte, Y.; Kaufman, L. *Chemometric,* Elsevier, Amsterdam, **1988**, p. 124.
20. El-Gindy, A.; Emara, S.; Shaaban, H. Validation and application of chemometrics-assisted spectrophotometry and liquid chromatography for simultaneous determination of two ternary mixtures containing drotaverine hydrochloride. *J. AOAC. Int.* **2010**, *93* (2), 536-548.
21. Dinç, E.; Ustündağ, O. Spectrophotometric quantitative resolution of hydrochlorothiazide and spironolactone in tablets by chemometric analysis methods. *Farmaco.* **2003**, *58* (11), 1151-1161.
22. Wahbi, A. A.; Mabrouk, M. M.; Moneeb, M.S.; Kamal, A. H. Simultaneous determination of the two non-steroidal anti-inflammatory drugs; diflunisal and naproxen in their tablets by chemometric spectrophotometry and HPLC. *Pak. J. Pharm. Sci.* **2009**, *22* (1), 8-17.
23. Mohamed, Ael-M.; Abdelmageed, O. H.; Refaat, I. H. Determination of two antibacterial binary mixtures by chemometrics-assisted spectrophotometry. *J. AOAC. Int.* **2007**, *90* (1), 128-141.
24. Brereton, R. G. Multilevel multifactor designs for multivariate calibration. *Analyst* **1997**, *122*, 1521-1529.
25. Q. B. International Conference on Harmonization (ICH), Federal Register. **1997**, 62.
26. Kramer, R. *Chemometric Techniques for Quantitative Analysis. CRC Press,* **1998**.
27. Haland, D. M.; Thomas, E. V. Partial least-squares methods for spectral analyses. 1. Relation to other quantitative calibration methods and the extraction of qualitative information. *Anal. Chem.* **1988**, *60* (11), 1193-1202.