CHEMOMETRIC ASSISTED SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS DETERMINATION OF AMLODIPINE /CANDESARTAN MIXTURE IN THEIR PURE FORMS AND THEIR PHARMACEUTICAL PREPARATIONS

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ABSTRACT:

Three simple, accurate and precise multivariate calibration models, including classical least square **(CLS)**, principal component regression **(PCR)** and partial least square **(PLS-1)**, have been used for simultaneous determination of the components of recently approved fixed – dose combination tablet containing amlodipine / candesartan mixture in their pure forms and their pharmaceutical preparations, using spectral data in the range of (220nm-400nm). The CLS, PCR and PLS-1 models are useful in spectral analysis because the simultaneous employment of many spectral wavelengths instead of the single wavelength used in derivative spectrophotometry, which greatly improves the precision and predictive abilities of these multivariate calibrations. The developed methods were statistically compared with the reported spectrophotometric method and no significant differences were observed regarding both accuracy and precision, all the developed methods have been validated using external validation set. Moreover, the proposed models were successfully applied to the spectrophotometric determination of amolodipine besylate and candesartane cilexetil in **Unisia®** tablets.

Keywords: Amlodipine; Candesartan; chemometric techniques; PLS-1; PCR; CLS

1. Introduction

Hypertension is a globally common and serious public health problem. Uncontrolled hypertension can increase the risk of developing cardiovascular and cerebrovascular diseases.

Although launching a lot of modern highly effective antihypertensive medications in the last decades, monotherapy has failed to reduce blood pressure levels below the recommended targets in approximately 70% of hypertensive patients. Therefore, most hypertensive patients are prescribed two or more antihypertensive medication of different mode of action either in separate pills or in fixed dose combination. Fixed dose combination (all drugs in one tablet) improved patients' compliance to treatment especially elderly ones [A.F. Rubio-Guerra et al,2018 - B. Kalra et al,2010- A. H. Gradman et al,2011]. The most recommended antihypertensive combinations are dihydropyridine calcium antagonist with either beta-adrenergic blockers, angiotensin-renin axis inhibitors or diuretics [A. de la Sierra et al , 2013].

A fixed dose combinations containing amlodipine besylate **AML** (dihydropyridine calcium antagonist), **Fig. 1 (A)**, together with candesartan cilexetil **CAN** (angiotensin receptor blocker), **Fig. 1 (B)**, have been recently approved for management of elevated blood pressure in certain countries [S Yasuno et al,2012]

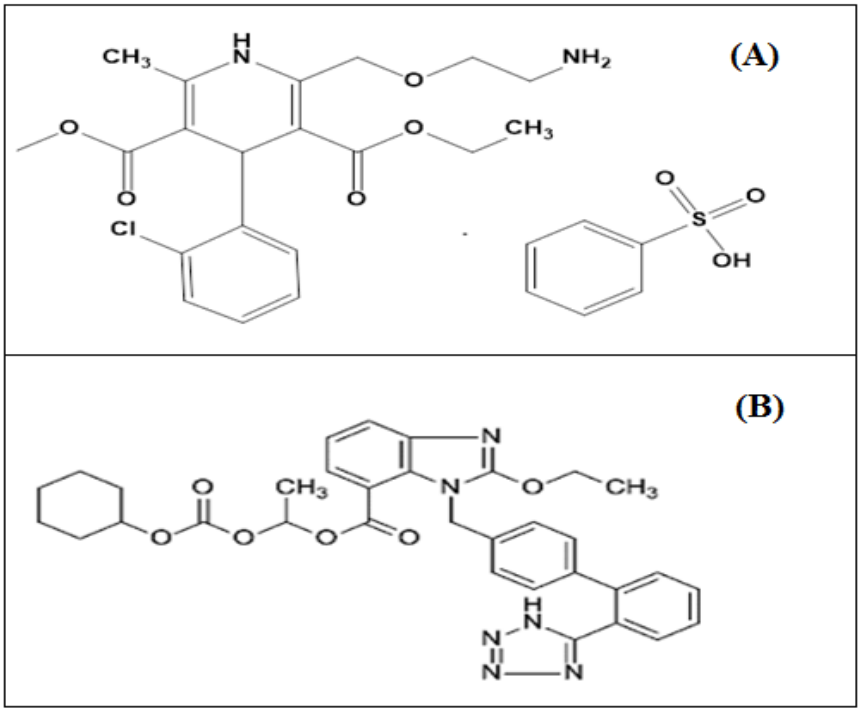

Figure (1): (A) chemical structure of Amlodipine besylate , (B) chemical structure of Candesartan cilexetil

An in-depth look at the literature review of this mixture has shown that so far, few analytical methods have been reported. The literature revealed that HPLC[L. LI et al , 2012] , spectrofluorimetric [H.G. Daabees et al, 2014] and Q-absorbance spectrophotometric techniques [R.B. Badhan et al, 2018- B. Kotecha et al, 2014] have been reported for estimation of amlodipine (AML) and candesartan (CAN) .

In this article, author pleased to introduce for quality control analysts a simple, eco-friendly and validated UV spectrophotometric CLS, PCR and PLS-1 chemometric methods [B.G.M. Vandeginste et al, 1997 - K.R. Beebe et al. 1998] for simultaneous determination of AML/CAN mixture in their pure forms as well as in their pharmaceutical combination pills.

2. Experimental

2.1. Materials and solvents

Powders of both AML (99.60%) and CAN (99.35%) were supplied by Benchmark Health Company, Cairo, Egypt.

Unisia® tablets (Batch No: 056308), a fixed dose combination of candesartan cilexetil 8 mg and amlodipine besylate 5 mg, was gifted by Benchmark Health Company, Cairo, Egypt.

Methanol of HPLC grade was procured from Sigma-Aldrich, Darmstadt, Germany.

2.2. Instrumentation

Shimadzu UV-Visible 1800 Spectrophotometer (Tokyo, Japan), equipped with 10 mm, 3.5 mL, quartz cuvette.

2.3. Software:

UV- Probe personal spectroscopy software version 2.1. (SHIMADZU).

All chemometric methods were implemented in MATLAB R2013b (8.2.0.701).

PLS, PCR, CLS, were carried out by using PLS toolbox software version 2.1.

2.4. Standard solutions:

A freshly prepared standard stock solution of **AML** (85 µg/mL) was made by transferring 8.5 mg of amlodipine powder into 100-mL volumetric flask. The powder was dissolved in 50 mL methanol and the volume was completed to 100 mL with methanol.

A freshly prepared standard stock solution of **CAN** (136 µg/mL) was made by transferring 13.6 mg of candesartan powder into 100-mL volumetric flask. The powder

was dissolved in 50 mL methanol and the volume was completed to 100 mL with methanol.

2.5.Procedure:

Experimental design:

Brereton [K.D. Zissis et al, 1998] constructed multilevel multifactor experimental design applied for the construction of the calibration and validation data sets. A five-levels, two factors experimental designs were used in which 0.8, 0.9, 1, 1.1 or 1.2 ml aliquots of both **AML** (85 µg/mL) and **CAN** (136 µg/mL) from their working standard solutions were combined and diluted to 10 ml with methanol. The concentrations details are given in **(Table 1)**. The absorption spectra of the prepared mixtures were recorded over the wavelength range 220nm - 400 nm with 1 nm interval thus the produced spectral data matrix that has 25 rows representing different samples and 181 columns representing wavelengths (25 x 181). For construction of the CLS, PCR and PLS models, feed the computer with the absorbance and concentration matrices of the training set, using MATLAB® version R2013b (8.2.0.701), together with PLS-Toolbox 2.1. software for the calculations. The concentrations of the calibration and validation data sets were calculated from the corresponding regression equations.

2.6.Application to pharmaceutical formulation

Ten **Unisia®** tablets were accurately weighed and finely powdered. Appropriate weight of powder equivalent to 13.6 mg of CAN and 8.5 mg of AML were transferred into 100-mL volumetric flask and the volume was made up to 50 ml with methanol. The solution was shaken vigorously for 10 minutes then sonicated for 30 min and filtered. The volume was completed to 100 ml with methanol to obtain a solution labeled to contain (136 µg/ mL) of CAN and (85 µg /mL) of AML.

3. Results and discussion

3.1. Spectral characteristics:

Zero - order absorption spectra of CAN and AML as shown in **fig. (2,3)** show severe overlap, which does not permit direct determination of each. So the previously mentioned chemometric methods have been developed to resolve this overlapping and enable determination of each drug in its mixture simultaneously without previous separation

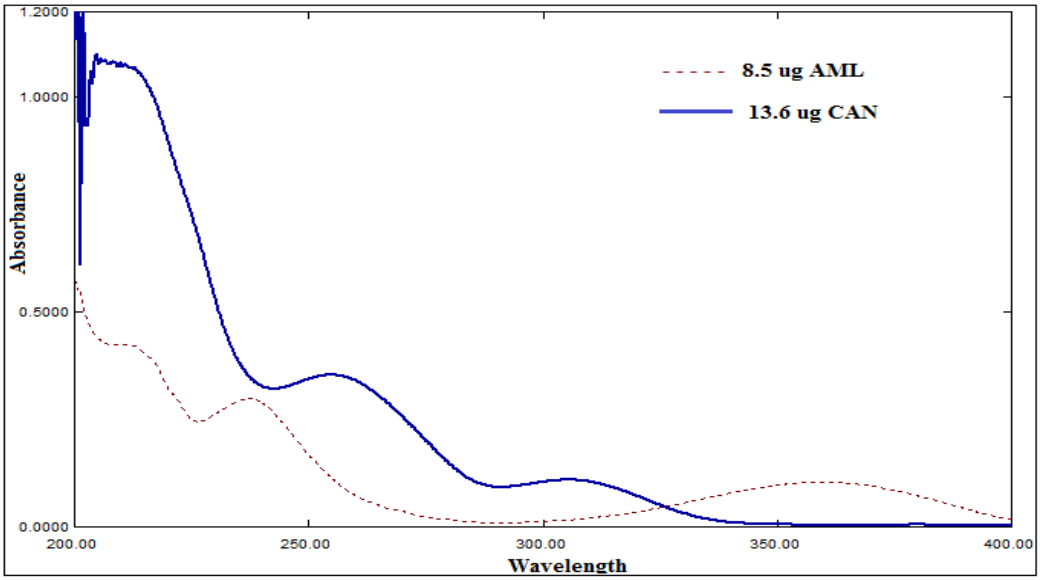

Figure (2): Absorption spectra of Amlodipine and Candesartan

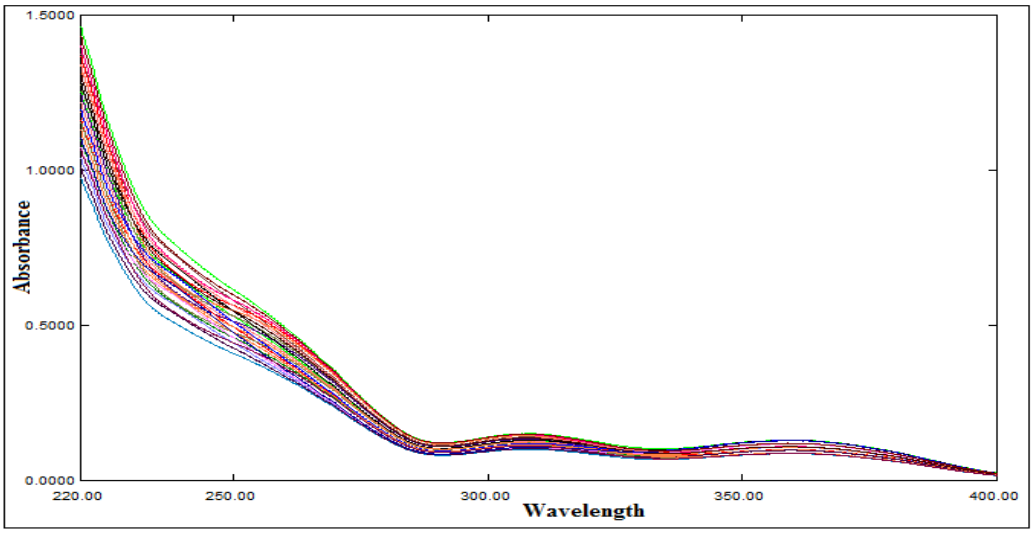

Figure (3): Absorption spectra of 25 mixtures of AML and CAN experimental design

Constructing the calibration set for the mixture of the two components was done to design the described models. The proposed methods were optimized with the aid of five-levels, two factors experimental design [R.G. Brereton,1997] resulting in 25 sample mixtures. These 25 sample mixtures, **table (1)** were split to two groups. The first group was 13 samples which were used to construct a calibration set. Another group of 12 samples were used to compute the predictive benefits of the model. The choice of concentrations was based on the linearity range of each component. Due to selected wavelength range and the used spectral mode enhance the quality of the analysis, the recorded spectral data was pre-processed, this resulted in 181 variables.

The optimum number of latent variables for the PCR and PLS models was a crucial parameter that needed to be carefully optimized keeping away any over fitting of the model. Hence, the leave-one-out cross-validation method was used to find the optimum number of latent variable for the investigated compounds by removing only one analyte at a time and then, the remaining calibration spectra were modeled and the root mean square error of cross-validation (RMSECV) was recalculated upon stepwise addition of different latent variables to the model. The optimum number of latent variables was selected according to Haaland and Thomas's criteria [D.M. Haaland et al, 1988] in which the model with optimum latent variable reveals no significance difference in its cross-validated predicted residual error sum of squares (PRESS) from the model with the minimum cross-validated PRESS. Regarding the PCR model, it was found that 3 latent variables were sufficient for data modelling **fig. 4**. Interestingly, the same number of latent variables were also found to be optimum for the PLS model **fig. (5,6).**

Percent recoveries, mean, standard deviation (SD), root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) of the described models were shown in **tables (2,3)** .

Table (1): Experimental design of concentrations of CAN and AML mixtures used in the chemometric assisted spectrophotometric methods:

Mixture No.	CAN (µg/mL)	AML (µg/mL)
1	13.6	8.5
2	13.6	6.8
3	10.88	6.8
4	10.88	10.2
5	16.32	7.65
6	12.24	10.2
7	16.32	8.5
8	13.6	7.65
9	12.24	7.65
10	12.24	9.35
11	14.96	10.2
12	16.32	9.35
13	14.96	8.5
14	13.6	10.2
15	16.32	10.2
16	16.32	6.8
17	10.88	9.35
18	14.96	6.8
19	10.88	8.5
20	13.6	9.35
21	14.96	9.35
22	14.96	7.65
23	12.24	6.8
24	10.88	7.65
25	12.24	8.5

The shaded rows represent the calibration set.

Table (2): Recovery study of CAN and AML in the calibration set by the chemometric assisted spectrophotometric methods

Calibration mixture	CLS		PCR		PLS-1	
	CAN	AML	CAN	AML	CAN	AML
1	97.88	100.29	100.00	100.05	100.10	100.71
2	99.70	99.07	99.65	99.09	99.75	100.00
3	101.48	99.53	100.05	100.22	100.05	100.00
4	98.38	100.86	99.98	99.91	100.18	99.87
5	101.67	99.56	100.07	100.13	99.98	100.03
6	101.66	99.57	100.01	100.11	100.01	100.23
7	98.08	100.87	99.99	100.12	99.99	99.99
8	101.66	99.56	100.07	100.13	100.13	100.01
9	101.85	99.51	100.01	99.98	100.01	99.97
10	97.93	100.78	99.99	100.04	99.99	100.01
11	101.59	99.49	99.93	100.49	99.13	99.95
12	99.92	99.06	99.77	99.12	99.78	99.70
13	97.54	100.89	100.08	100.07	100.08	99.66
Mean±RSD	99.95±1.73	99.93±0.73	99.97±0.13	99.95±0.42	99.94±0.27	100.01±0.26
RMSEC	0.2286	0.0575	0.0150	0.0293	0.0411	0.0216

Table (3): Recovery study of CAN and AML in the validation set by the chemometric assisted spectrophotometric methods:

Validation mixture	CLS		PCR		PLS-1	
	CAN	AML	CAN	AML	CAN	AML
1	99.76	99.19	99.83	99.45	99.69	99.73
2	101.94	99.59	100.01	100.14	100.01	100.05
3	101.91	100.57	100.10	100.06	100.10	100.24
4	101.55	99.55	100.02	100.16	100.02	100.01
5	101.01	99.50	100.09	100.01	100.09	99.96
6	101.61	99.48	100.07	100.08	100.07	99.94
7	101.77	99.58	100.05	100.09	100.12	100.03
8	100.17	99.03	99.83	99.66	99.83	99.68
9	100.03	99.63	99.74	99.19	99.74	99.25
10	100.96	99.50	100.04	100.03	100.04	99.96
11	101.46	99.54	99.98	100.19	99.78	100.03
12	101.48	99.70	99.62	99.58	99.99	100.16
Mean± RSD	101.14±0.75	99.57±0.37	99.95±0.16	99.89±0.33	99.96±0.15	99.92±0.26
RMSEP	0.1775	0.0453	0.0204	0.0237	0.0220	0.0188

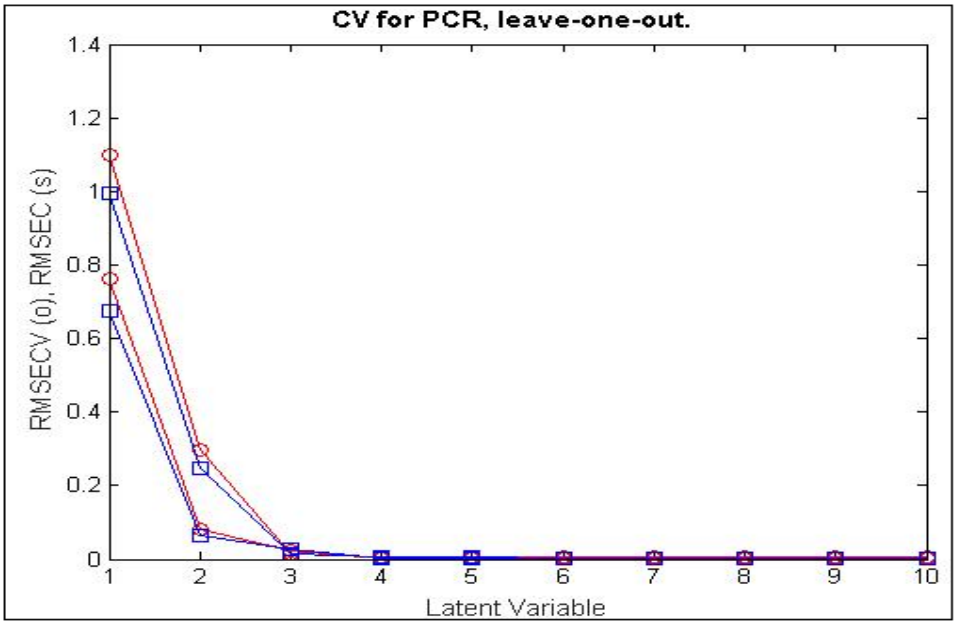

Figure (4): RMSECV plot of the cross validation results of the calibration set as a function of the number of latent variables (LVs) used to construct the PCR model for CAN/AML mixture

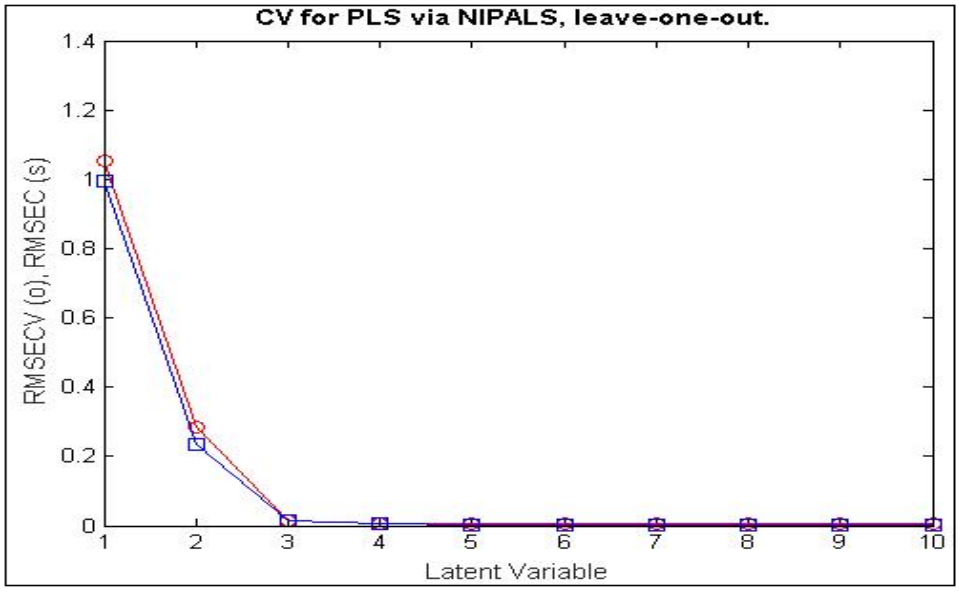

Figure (5): RMSECV plot of the cross validation results of the calibration set as a function of the number of latent variables (LVs) used to construct the PLS model for CAN in CAN/AML mixture.

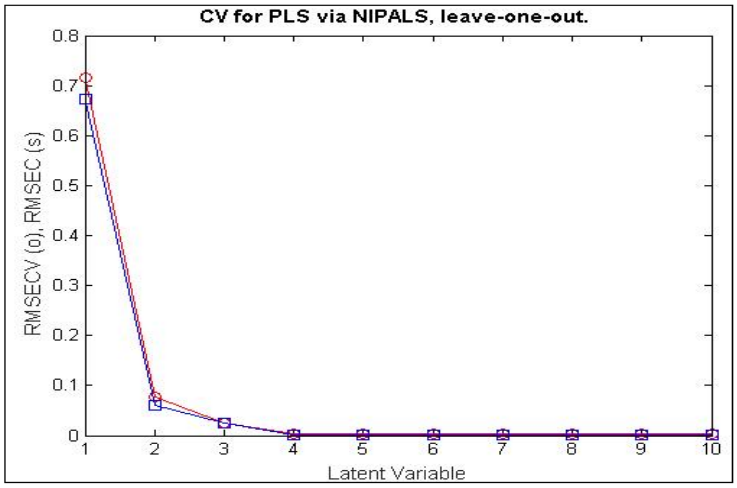

Figure (6): RMSECV plot of the cross validation results of the calibration set as a function of the number of latent variables (LVs) used to construct the PLS model for AML in CAN/AML mixture.

3.2. Pharmaceutical applications

CLS,PCR and PLS models were applied for spectrophotometric quantitative analysis of **AML** and **CAN** in **Unisia®** tablets. The results were in good agreement with the label claims, indicating that there was no interference from excipients and additives. The results were statistically compared with those obtained by the reported method [9]. The accepted values of the statistical tests were obtained as shown in **table (4)**.

Table (4): Determination of AML/CAN in Unisia® tablets by the proposed chemometric assisted spectrophotometric and reported methods:

	Amlodipine				Candesartan			
Parameter	**CLS**	**PCR**	**PLS**	**Reported Method[9]**	**CLS**	**PCR**	**PLS**	**Reported method[9]**
n*	5	5	5	5	5	5	5	5
$\bar{X}$ **	99.52	100.18	100.44	99.85	100.20	100.47	100.28	100.63
SD	0.573	0.631	0.958	0.478	0.477	0.357	0.434	0.726
RSD%	0.576	0.629	0.954	0.479	0.476	0.355	0.433	0.721
Student's t test*	0.987 (2.306)	0.936 (2.306)	1.225 (2.306)	———	1.090 (2.306)	0.425 (2.306)	0.909 (2.306)	———
F value*	1.424 (6.388)	1.725 (6.388)	4.000 (6.388)	———	2.309 (6.388)	4.122 (6.388)	2.791 (6.388)	———

** No. of experiments.*

*** The mean of percent recovery of pharmaceutical preparation*

**** The values in the parenthesis are tabulated values of t and F at p= 0.05 level of significance.*

4. Conclusion

Classical least square **(CLS)**, principal component regression **(PCR)** and partial least square **(PLS-1)** were applied to support spectrophotometric quantitative analysis of amolodipine besylate and Candesartan cilexetil in their dosage form. The models were developed according to established principles. Moreover, the described models were carefully implemented and optimized to allow more accurate quantitative analysis. The models have succeeded in quantifying the investigated drugs in their pharmaceutical preparations.

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استخدام القياس الطيفي بمساعدة القياسات الكيمائية للتحديد المتزامن لمزيج أملوديبين وكانديسارتان في أشكالهما النقية ومستحضراتهما الصيدلانية

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قسم الكيمياء التحليلية الصيدلية بكلية الصيدلة بنين جامعة الازهر القاهرة

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ويتناول البحث استنباط طرائق متعددة المتغير لتعيين المتزامن لمزيج أملوديبين وكانديسارتان في أشكالهما النقية ومستحضراتهما الصيدلانية عن طريق تقنية المربعات الكلاسيكية الصغري وتقنية انحدار العناصر الرئيسية وتقنية المربعات الجزئية الصغري وقد تم استخدام هذه الطريقة لتعيين العقارين ولم يتأثر احدهما بوجود الاخر. كما امتد تطبيق الطرق الى تقدير المركبين فى المستحضر الصيدلى وقد تم إجراء دراسة إحصائية لنتائج الطريقة المقترحة مع نتائج الطريقة المرجعية فوجد أنه لا فرق من حيث الدقة والضبط.

تم استخدام ثلاث طرق للمعايرة متعددة المتغيرات بسيطة ودقيقة، بما في ذلك المربع الصغير الكلاسيكي (CLS) وانحدار المكون الرئيسي (PCR) والمربع الجزئي الأصغر (PLS-1) ، لتحديد مكونات تركيبة الجرعة الثابتة المعتمدة مؤخرًا لقرص يحتوي على خليط أمليوديبين / كانديسارتان في صورتهما النقية ومستحضراتهما الصيدلانية . تم استخدام بيانات طيفية في نطاق (٢٢٠ نانومتر - ٤٠٠ نانومتر). تعد تقنيات CLS و PCR و PLS-1 مفيدة في التحليل الطيفي لأن التضمين المتزامن للعديد من الأطوال الموجية الطيفية بدلاً من الطول الموجي الفردي المستخدم في القياس الطيفي المشتق قد حسن بشكل كبير الدقة والقدرات التنبؤية لهذه المعايرات متعددة المتغيرات. تمت مقارنة الطرق المطورة إحصائيًا بالطريقة المنشورة ولم تُلاحظ فروق ذات دلالة إحصائية فيما يتعلق بالدقة والضبط، وقد تم التحقق من صحة جميع الطرق المطورة. تم تطبيق النماذج المقترحة بنجاح على التحديد الطيفي الضوئي لأموديبين بيسيلات وكانديسارتان سيليكسيتيل في أقراص Unisia®.

الكلمات المفتاحية : لأموديبين ، كانديسارتان ، تقنيات القياس الكيميائي ، PLS-1; PCR; CLS