THREE DIFFERENT CHEMOMETRIC APPROACHES COUPLED WITH UV SPECTROSCOPY FOR ANALYSIS OF AMLODIPINE AND OLMESARTAN IN PRESENCE OF THEIR ACIDIC DEGRADATION PRODUCTS

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ABSTRACT

Amlodipine and olmesartan are two different antihypertensive drugs used in combination in the management of mild to severe hypertension. Degradation of the drug is one of the fatal processes. The potency and efficiency decrease in the presence of degradation products. The univariate spectrophotometric methods cannot provide adequate information for assessment of the presence of the degradation products. In this paper, three different multivariate models were utilized for the assessment of amlodipine and olmesartan in the presence of their degradation products. These methods are principal component regression (PCR), partial least square (PLS), and classical least square (CLS). The linearity range of these methods were 2-10 µg/ml for both drugs. The limits of detection of these methods were from 0.466 to 0.637 µg/ml for amlodipine, and from 0.435 to 0.561 µg/ml for olmesartan while the limits of quantification were from 1.413 to 1.931 µg/ml for amlodipine, and from 1.318 to 1.709 µg/ml for olmesartan.

Keywords: antihypertensive drugs, degradation, amlodipine, olmesartan, PCR, PLS, CLS, Limit of detection, limit of quantitation, multivariate.

Introduction:

The World Health Organization lists hypertension as a leading cause of mortality. Hypertension treatment results in a considerable reduction in cardiovascular diseases and death(Gakidou et al., 2017) The first category of antihypertensives includes those that have an effect on the renin-angiotensin system, either immediately or indirectly, leading to a relaxation of the blood vessels. This class of drugs comprises the angiotensin converting enzyme inhibitors (ACEIs), angiotensin receptors inhibitors, and direct renin blockers, and the weaker one beta blocker. The actions of the other category include an increase in the excretion of water and salt. Medications like calcium channel blockers and diuretics fall into this category (Dahlöf et al., 1991). The use of a combination of these two primary classes has been shown to be more effective than each class used alone in lowering blood pressure(Kalra et al., 2010).

Amlodipine affects Ca^{2+} mobilizations by interacting with the sarcoplasmic reticulum and blocking the voltage-dependent Ca^{2+} inflow. Smooth muscle cells in the heart and the peripheral vasculature are affected(Murdoch & Heel, 1991; Stepien et al., 2002). The chemical structure of amlodipine, benzenesulfonic acid;3-O-ethyl-5-O-methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate, is shown in Figure 1(Wishart et al., 2006).

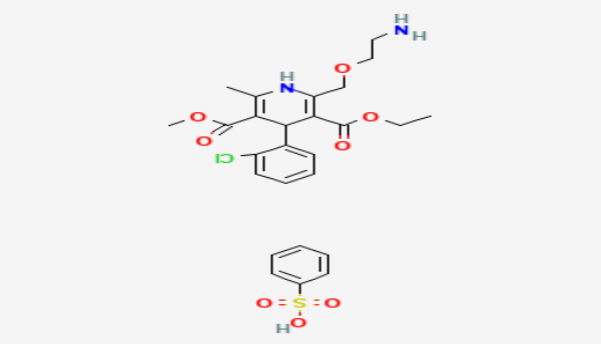

Figure 1 chemical structure of amlodipine besylate.

Olmesartan produce its antihypertensive action through blocking of the binding of angiotensin II protein to its receptors. This, in turn, reduces the protein's ability to cause vasoconstriction, aldosterone synthesis stimulation, cardiac stimulation, and renal reabsorption of sodium (Chrysant et al., 2008). The chemical structure of olmesartan, (5-methyl-2-oxo-1,3-dioxol-4-yl) methyl 5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2*H*-tetrazol-5-yl)phenyl]phenyl]methyl]imidazole-4-carboxylate is shown in figure 2 (Wishart et al., 2006). The combination of amlodipine with olmesartan is characterized by high tolerability and efficacy in the management of hypertension.

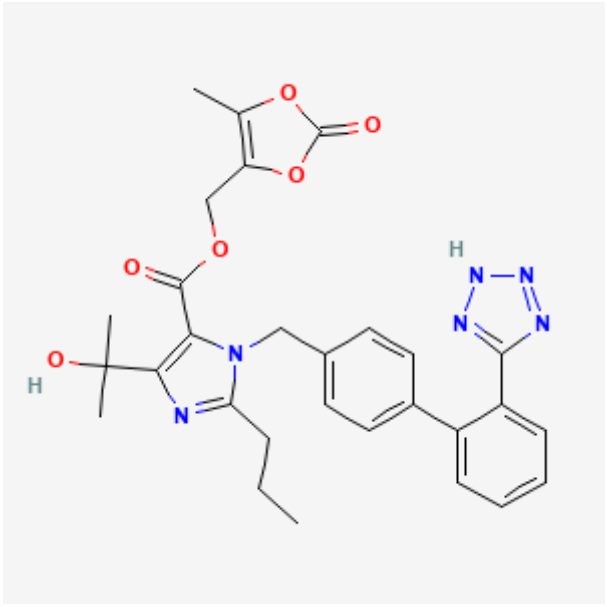

Figure 2 chemical structure of olmesartan medoxmil.

Literature review on amlodipine and olmesartan combined dosage form revealed different spectrophotometric methods (Kardile DP et al., 2010; MehulKumar et al., 2009; Pournima et al., 2011; S. Shah et al., 2016; Sharma & Sharma, 2010; Venkannaa et al., 2015; Wankhede et al., 2009), several RP-HPLC methods (Almeida et al., 2018; Brondi et al., 2017; Chabukswar et al., 2010; E. M. Hassan et al., 2014; Kamble et al., 2010; Kardile DP et al., 2010; Kumar et al., 2019; Patil et al., 2010; Qi et al., 2013; Qutab et al., 2009; S. K. Shah et al., 2012; Siva Rao Bodavula et al., 2020; Wankhede et al., 2009), HPTLC(Kamble et al., 2010; S. Shah et al., 2016), and few spectrofluorometric methods (Darwish & Backeit, 2013) for their determination.

The combination of chemometrics and spectrophotometry is a powerful tool for improving signal-to-noise ratios, enhancing selectivity of determination, optimizing experimental conditions, increasing analytical operation efficiency, and revealing a wealth of scientific insights. As a result, it has been getting a lot of attention from analysts and is being employed for multicomponent determination at the same time (Fang & Liu, 2001). There are different chemometric techniques, from these techniques principal component regression (PCR), partial least square (PLS), and classical least square (CLS) are most used for quantitively resolving of complicated mixtures.

Chemometric models like principal component analysis (PCR) and partial least squares (PLS) depend on relation between matrices of chemical data. Both depend on decomposition of the spectra but the main difference is PLS is dependent on analyte concentration (Garrido Frenich et al., 2006; Ragno et al., 2004). CLS is considered the simplest technique of multivariate algorithm which applies beer's law for analysis(Hadad et al., 2008a).

The main purpose of this study is degradation of both drugs in acid medium and identifying of these acidic degradation products by different aspects, then further analysis of the drugs in presence of their acid degradation by aid of different chemometric methods to ensure specificity and selectivity of the method.

Experimental

2.1.Material and reagents

- Amlodipine and olmesartan medoxomil kindly supplied by Multiapex pharma. The purity of these standards were 99.69±0.67 and 99.57±0.89.
- **Erastapex® Co 5/20** batch no. 13553 (contains 5mg amlodipine and 20mg olmesartan medoxomil), purchased from the local Egyptian market.
- Methanol, HCl, NaOH, chloroform, acetic acid, and formic acid, were purchased from sigma Aldrich (analytical grade).

Instrument

- Spectrophotometer.—SHIMADZU dual-beam UV–visible spectrophotometer (Kyoto/Japan), model UV-1800 PC connected to a compatible IBM and an HP1020 LaserJet printer. The bundled software, UVProbe personal spectroscopy software version 2.43 (SHIMADZU) was used. The spectral band was 1nm and the scanning speed is 2800 nm/min with 0.2 nm interval.
- pHmeter— Jenway® model 35905 (England)
- Sonicator.— Cleanwise® model WUC-A06H (Korea).
- Centrifuge. — TDL-60B bench top (Hunan, China, Mainland).
- MATLAB5 with PLS-Toolbox software version 2.1 was used for PLS and PCR.

Standard solutions

Standard solution of amlodipine and olmesartan

A separate stock solution of each amlodipine and olmesartan was prepared by dissolving 10mg of each standard in 70ml methanol, sonication for 15 min to ensure complete dissolution, then the volume was completed to 100ml with methanol (producing a stock solution 100µg/ml), then preparation of final standard solution by withdrawing appropriate aliquots to 10ml volumetric flask.

standard solution of amlodipine acidic degradation product:

Addition of 15mls of 1M HCl to 50mg of amlodipine with gentle mixing. Then refluxing for 2hr, after that, neutralization of degradation product through using 1M NaOH to pH 7. TLC was performed to ensure complete degradation using chloroform/methanol/acetic acid (86.2:11.5:2.3 by volume) as mobile phase. The degradation solution was complete dried using rotary evaporator, then extraction of degradade by methanol for three times. Further testing the purity of degradade by comparing the IR spectrum, UV spectrum, and mass spectrum of the produced degradade with the reported method (S. A. Hassan et al., 2016).

Standard solution of olmesartan acid degradation:

Addition of 50mls of 1M HCl to 50mg of amlodipine with gentle mixing. Then refluxing for 2hr, afterthat neutralization of degradation product through using 1M NaOH to pH 7. TLC was performed to ensure complete degradation using chloroform/methanol/ formic acid (8:1.5:

0.5 by volume) as mobile phase. The degradation solution was complete dried using rotary evaporator, then extraction of degradade by methanol for three times. . Further testing the purity of degradade by comparing the IR spectrum, UV spectrum, and mass spectrum of the produced degradade with the reported method (Moussa et al., 2010).

Experimental design (calibration, and validation sets)

In order to obtain a suitable calibration set; systematic experimental designs were used. Multilevel partial factorial design was used (Rodionova, 2005) for five concentration levels (l = 5). Mutually orthogonal designs are only possible if the number of concentration levels is a prime number or a power of a prime number. The design requires at least l^2 experiments (l is the number of concentration levels), so 25 experiments were carried to study a mixture. After numbering the levels from −2 (lowest) to 2 (highest) the complete design was obtained using what is often described as a cyclic generator (−2, 1, 2, 1, −2), a repeater of 0 and a difference vector. In this type of design, there is no correlation between any concentrations of the compounds; hence, the correlation coefficient is zero.

A calibration set of 25 synthetic mixtures with different concentrations of each compound were prepared in methanol in range of 2-10 µg/ml for amlodipine, olmesartan, and the acid degradation products as mentioned in Table 1.

The UV absorption spectra were recorded over the wavelength range of 230–390 nm. The data points of the spectra were collected every 1 nm. The computations were made using PLS Toolbox software version 2.1. CLS, PLS and PCR models.

Table 1 Levels and concentration data for the amlodipine and olmesartan in the calibration set.

	level				Concn.µg/ml			
Expermient	Aml	Olm	Aml Deg.	Olm Deg	Aml	Olm	Aml Deg.	Olm Deg
1	0	0	0	0	6	6	6	6
2	0	-2	-2	2	6	2	2	10
3	-2	-2	2	-1	2	2	10	4
4	-2	2	-1	2	2	10	4	10
5	2	-1	2	0	10	4	10	6
6	-1	2	0	-1	4	10	6	4
7	2	0	-1	-1	10	6	4	4
8	0	-1	-1	1	6	4	4	8
9	-1	-1	1	2	4	4	8	10
10	-1	1	2	1	4	8	10	8
11	1	2	1	0	8	10	8	6
12	2	1	0	2	10	8	6	10
13	1	0	2	2	8	6	10	10
14	0	2	2	-2	6	10	10	2
15	2	2	-2	1	10	10	2	8
16	2	-2	1	-2	10	2	8	2
17	-2	1	-2	0	2	8	2	6
18	1	-2	0	1	8	2	6	8
19	-2	0	1	1	2	6	8	8
20	0	1	1	-1	6	8	8	4
21	1	1	-1	-2	8	8	4	2
22	1	-1	-2	-1	8	4	2	4
23	-1	-2	-1	0	4	2	4	6
24	-2	-1	0	-2	2	4	6	2
25	-1	0	-2	-2	4	6	2	2

Application to laboratory prepared mixtures

Different aliquots from amlodipine, olmesartan, amlodipine degradade, and olmesartan degradade were withdrawn and transferred to a 10ml volumetric flask to obtain mixtures containing different ratios of both drugs in concentration range within the linearity range of each drug with their acid degradation product as shown in table 2 then the determination of each drug concentration using the equation of linear regression in each method.

Table 2 Levels and concentration data for the amlodipine and olmesartan in laboratory prepared mixtures

Expermient	level				Concn.µg/ml			
	Aml	Olm	Aml Deg.	Olm Deg	Aml	Olm	Aml Deg.	Olm Deg
1	0	-2	-2	2	6	2	2	10
2	-1	-1	1	2	4	4	8	10
3	2	1	0	2	10	8	6	10
4	-2	0	1	1	2	6	8	8
5	-2	1	-2	0	2	8	2	6
6	-2	2	-2	-2	2	10	4	2

Application to pharmaceutical dosage forms

For **Erastapex Co**®5/20, twenty tablets containing 5mg of amlodipine and 20mg olmesartan were weighted, then crushed to fine powder, then an equivalent weight to one tablet was transferred accurately to 100ml volumetric flask, adding 70ml of methanol for dissolution, shake for 10min and sonicated for 15 min, then the volume was completed to 100ml by methanol. The prepared solution was centrifuged at 5000 rpm for 20 min at 20°C. Then 4 mls of the supernatant was withdrawn into another 10 ml volumetric flask and complete the volume to 10 ml with methanol. Which was further diluted by withdrawing 1ml into another 10ml volumetric flask and complete the volume to 10 ml with methanol. The final obtained solution concentration is 2 µg/ml of amlodipine and 8 µg/ml of olmesartan. The analysis procedure was repeated several times with different ratios of tablet formulation. Determination of the content of amlodipine and olmesartan in the final dosage form was calculated.

Reported method

The reported method used was measuring the amplitude of first derivative at 239.0 and 256.0 nm for amlodipine and olmesartan, respectively(Kardile DP et al., 2010).

Results and discussion

During the various stages of production and storage, many medicinal substances degrade. Hydrolysis and oxidation are two of the most well-known chemical degradation mechanisms for pharmaceuticals(Abdelwahab, 2010), since both amlodipine and olmesartan include ester groups that are susceptible to hydrolytic breakdown following refluxing in acidic settings, they both generated the hydrolytic degradation product in acidic circumstances(S. A. Hassan et al., 2016; Moussa et al., 2010) . Spectrophotometers provide several advantages over other types of analytical instruments, including low running costs, little waste production, cost-effectiveness, expediency, simplicity, and compatibility with solvents suitable for analytical usage. Since it lacked a suitable separation technique for overlapped spectra, direct spectrophotometric techniques could not be employed for the determination of multicomponent formulation(Tambe et al., 2021).the spectra of amlodipine, olmesartan, and their acid

degradation product shows a severe overlapped which prevent direct determination of both drugs as shown in figure 3.

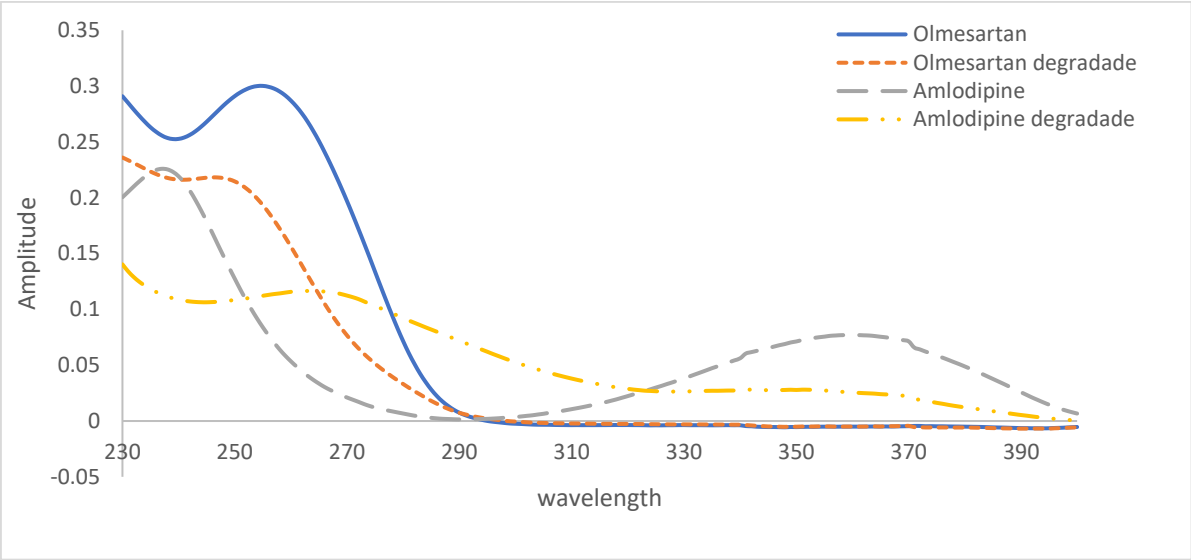

Figure 3 UV spectrum of 6µg/ml of olmesartan, olmesartan degradade, amlodipine, and amlodipine degradade

One major benefit of using multivariate calibration methods on spectral data is that it speeds up the process of resolving complicated systems. The proposed models have a number of benefits, including: the simplicity of the PCR model (only a small number of principal components are required), the accuracy of the PLS model (errors are reduced by collecting absorbance data from multiple locations in the zero-order spectrum), and the novelty of the CLS model (it was the first chemometric method to be developed)(Attia et al., 2018).

Calibration matrix and selection of spectral zones

The main factors affecting on the quality of the analysis is appropriate selection of wavelength. The choice of wavelength range has many parameters, from these is the selection of range where the drug shows information on drug behavior towards UV and excluding the non-informative noisy region. The selected wavelength range was from 230nm to 390nm with 1nm interval.

Selection of the appropriate number of factors.

Calibrations for PCR and PLS need a suitable selection of the number of primary components or factors. As many variables as possible should be taken into and not lead to a case of over fitting to the experimental data. Various the criteria for picking the best possible amount have been worked out. The calibration set was 25 calibration spectra and utilizing cross-validation leaving out one sample at a time method.

Both PLS and PCR calibration were performed on a total of 24 calibration spectra. Then the concentration of the left sample was calculated. Then comparison of the predicted concentrations with the known concentrations of the compounds in each

calibration sample. e. The root mean squares error of cross-validation (RMSECV) was calculated for each method as follows:

$$\text{RMSECV}=\sqrt{\frac{\text{PRESS}}{n}}$$

where n is the number of training samples, PRESS is the sum of difference between predicted and known concentration.

Typically, this is done by calculating the RMSECV and selecting the smallest factor size that achieves that value. However, there are limits to this criteria since there are cases in which the RMSECV doesn't decrease down suddenly but instead flattens out once a certain threshold has been passed. However, it is estimated from a limited number of samples and hence subject to inaccuracy. As a consequence, we employed the Haaland-Thomas devised approach for picking the optimal number of factors, which comprises selecting the model comprising the fewest number of factors that results in a negligible difference between the corresponding RMSECV and the minimal RMSECV(Hadad et al., 2008b). The appropriate number of latent variables were four as shown in figure 4.

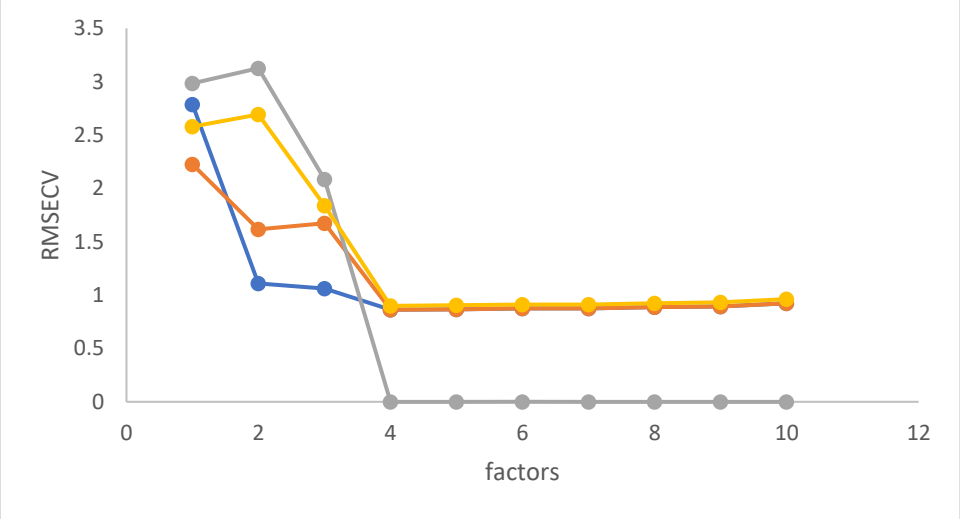

Figure 4 . Plot of RMSECV vs. factor number for a calibration set prediction using cross-validation of PCR model (A) and PLS-1

Two methodologies were used to assess the models' ability for prediction. The first method, a scatter plot of observed and expected concentrations was generated. The CLS, PLS, and PCR optimized models all produced respectable values for the correlation coefficient (r), showing high predictive skills as shown in table 3 and the standard error of prediction was also calculated, demonstrating the accuracy of the models (SEP). In addition, the relative error of prediction (REP) demonstrating the quality of fit of all the calibration data. It was calculated using the following equation:

$$\text{REP (\%)} = \frac{= \text{RMSECV} \times 100}{\bar{c}\text{act}}$$

Where c¯act is the average concentration in the calibration set.

Table 3 Statistical parameters and analytical figures of merit values for simultaneous determination of amlodipine and olmesartan using CLS, PLSand PCR methods

parameter	Amlodipine			Olmesartan		
	CLS	PCR	PLS	CLS	PCR	PLS
Intercept	-0.003	0.015	-0.03	0.0148	0.004	0.002
Slope	0.013	0.044	-0.066	0.044	0.0432	0.013
corelation coffecient	0.9998	0.9996	0.9996	0.9998	0.9997	0.9996
LOD (µg/ml)	0.466	0.564	0.637	0.564	0.435	0.481
LOQ (µg/ml)	1.413	1.709	1.931	1.709	1.318	1.458
SEP	0.934	0.934	0.934	0.922	0.922	0.922
REP	8.62	8.62	8.62	8.64	8.64	8.64

Analysis of pharmaceutical products

The proposed CLS, PLS, and PCR were applied to the simultaneous determination of amlodipine and olmesartan in **Erastapex Co**®5/20 tablet. Satisfactory results were obtained for each compound in good agreement with label claims as shown in Table 3. Moreover, the average recovery of the new methods was statically compared against the reported method (Kardile DP et al., 2010) via the student t-test and f-test, the results of the proposed CLS, PLS, and PCR methods were compared with reported method (Kardile DP et al., 2010)The results were less than the critical values indicating no significant difference. The results were less than the critical values indicating no significant difference between the new proposed methods and reported methods as shown in table 4.

Table 4 Determination of amlodipine and olmesartan in laboratory-prepared mixtures and pharmaceutical product using the proposed methods

	CLS	PCR	PLS
laboratory prepared mixture			
amlodipine (mean ±S.D)	99.63+0.936	99.63+0.936	99.63+0.936
olmesartan (mean ±S.D)	101.04+0.906	101.04+0.916	101.04+0.929
Pharmaceutical dosage form			
amlodipine			
% Recovery (mean ±S.D)	99.286+1.477	99.286+1.477	99.286+1.477
t	0.823	0.823	0.823
F	1.176	1.176	1.176
olmesartan (mean ±S.D)			
% Recovery (mean ±S.D)	99.29+1.05	99.29+1.05	99.29+1.05
t	0.247	0.247	0.247
F	1.188	1.188	1.188

Conclusion

As a matter of general analytical interest, development of new techniques that can accurately and rapidly analyze a huge number of samples is required. Massive volumes of data may be produced using spectrophotometric tools in a very short amount of time for processing; yet, when coupling spectral data with chemometrics methods as CLS, PLS, and PCR may be significantly improved by integrating the two becomes a potent and very handy analytical tool.

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ثلاث اليات كيمومترية مختلفة مقترنة بالكم اطيفي الفوق بنفسجي لتحليل الاملوديبين و الاولمسرتان في وجود ناتجهما للتحليل الحمضي لهم.

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الملخص:

أمليوديبين وأولميسارتان نوعان مختلفان من الأدوية الخافضة للضغط تستخدمان معًا في علاج ارتفاع ضغط الدم الخفيف إلى الشديد. يعتبر تحلل الدواء من العمليات الهامة التي يجب تقييمها حيث تنخفض الفاعلية والكفاءة في وجود منتجات التحلل. لا يمكن أن توفر الطرق أحادية المتغير نتائج كافية للتقييم في وجود منتجات التحلل. في هذا البحث، سيتم استخدام ثلاثة نماذج مختلفة متعددة المتغيرات لتقييم أملوديبين وأولميسارتان في وجود منتجات التحلل الخاصة بهم. هذه الطرق هي انحدار المكون الرئيسي (PCR) ، والمربع الجزئي الصغرى (PLS) ، والمربع الكلاسيكي الأصغر .(CLS) كانت حدود الكشف عن هذه الطرق من 0.466 إلى 0.637 ميكروغرام / مل للأمليوديبين ، ومن 0.435 إلى 0.561 ميكروغرام / مل للأولميسارتان بينما كانت حدود القياس الكمي من 1.413 إلى 1.931 ميكروغرام / مل للأمليوديبين ومن 1.318 إلى 1.709 ميكروغرام. / مل لأولميسارتان.

الكلمات المفتاحية: الأدوية الخافضة للضغط ، التحلل ، أمليوديبين ، أولميسارتان ، PCR، PLS، CLS، حد الكشف ، حد الكم ، متعدد المتغيرات