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Simultaneous spectrophotometric determination of amlodipine and atenolol in pharmaceutical preparations using chemometric techniques

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ABSTRACT

Three simple, accurate and precise multivariate calibration methods, including classical least square (CLS), principal componentregression (PCR) and partial least square (PLS-1), have been used for the simultaneous determination of Atenolol (ATN) and Amlodipine (AML). The CLS, PCR and PLS-1 techniques are useful in spectral analysis because the simultaneous inclusion of many spectral wavelengths instead of the single wavelength used in derivative spectrophotometry has greatly improved the precision and predictive abilities of these multivariate calibrations. The developed methods were statistically compared with a reported method and no significant differences were observed regarding both accuracy and precision, all the developed methods have been validated according to ICH guidelines.

KEYWORDS

Chemometrictechniques;
Amlodipine;
Atenolol;
PLS-1;
PCR;
CLS.

INTRODUCTION

Amlodipine (AML) is 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl- 3,5-pyridine carboxylic acid 3-ethyl5-methyl ester)[1] (Figure 1). It is a dihydropyridine derivative with calcium antagonist activity. It is used in the treatment of hypertension and chronic stable angina pectoris[2]. It inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle[3]. AML is official in British Pharmacopoeia[4] and United State Pharmacopeia[5] where it is determined by reversed phase high performance liquid chromatographic method, Also UV-spectroscopy method[6] is reported. Atenolol (ATN) is chemically 2-[4-{(2RS)-2- hydroxy-3-[(1-methylethyl) amino] propoxy} phenyl] acetamide[1] (Figure 2). It is a β-adrenoreceptor blocking agent primarily used for hypertension, angina pectoris and myocardial infarction. It mainly acts by inhibition of renin release, angiotensin -II (AT-II) and aldosterone production[3]. The British[4] and European Pharmacopoeia[7] describe non-aqueous titration method for the assay of atenolol. Few methods are available for the simultaneous determination of AM and AT in combination; RP-HPLC[8], HPTLC[9] and spectrophotometry[10]. The aim of this work was to develop four, sensitive, accurate, precise, reliable, fast and inexpensive analytical methods for the determination of both drugs without prior separation.

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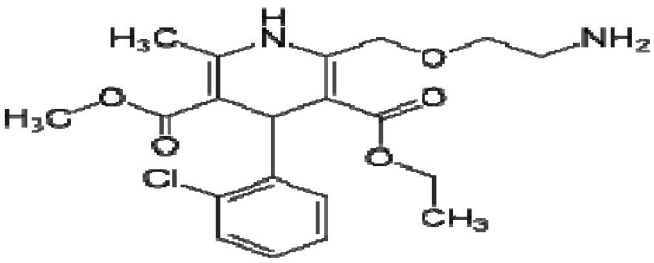

Figure 1 : Structural formula of amlodipine

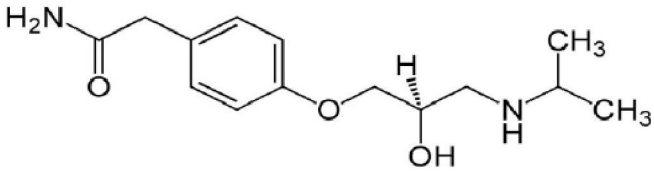

Figure 2 : Structural formula of atenolol

MATERIALS AND METHOD

Apparatus

- Shimadzu UV-Vis. 1650 Spectrophotometer, (Tokyo, Japan), equipped with 10 mm matched quartz cells was used. All measurements were done at medium sensitivity.
- Hot plate, Torrey pines scientific, USA.
- pH meter 3510 (Jenway,U.S.A.).

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.
- Thet-test and F-test were performed using Microsoft_ Excel.
- All calculations were performed using a Quad core CPU, 1.47 GHz, 4.00 GB of RAM under Microsoft Windows 7 ™.

Materials and reagents

All chemicals and reagents used throughout the work were of analytical grade and the water used throughout the procedure was freshly double distilled.

Pure sample

Amlodipine, Atenolol Pure samples were kindly supplied by Epico Pharmaceutical Industry, Cairo, Egypt.

Pharmaceutical preparation

Amlopress-AT 25 tablet dosage forms; labeled to contain 5 mg (AML) / 25 mg (ATN); batch number (A40142) manufactured by Cipla Limited, multinational pharmaceutical and biotechnology company, India. They were procured from Indian market.

Reagents and solvents

- Hydrochloric acid (El-Nasr Company, Egypt) prepared as 1 N aqueous solution
- Sodium hydroxide (El-Nasr Company, Egypt) prepared as 3M aqueous solution.
- Methanol (Sigma–Aldrich, USA).

Standard solutions

AML and ATN standard solutions (each, 0.1mg/ml), were prepared by dissolving 10 mg of AML and ATN separately in distilled water into two100-ml volumetric flasks and then completing to volume with the same solvent.

PROCEDURE

Experimentaldesign

Brereton[11]constructed multilevel multifactor experimentaldesign was applied for the construction of the calibration and validation sets. A five-levels, two factors experimental design was used in which 0.8, 0.9, 1, 1.1 or 1.2 mL aliquots of both intact and degraded form of CFC working solutions were combined and diluted to 10 mL with water. The concentrations details are given in [TABLE 1]. The absorption spectra of the prepared mixtures were recorded over the wavelength range 200-400 nm with 1 nm interval thus the produced spectral data matrix has 25 rows representing different samples and 201 columns representing wavelengths (25 x 201). For construction of the models, to build the CLS, PCR and PLS models, feed the computer with the absorbance and concentration matrices for the training set, use the training set absorbance and concentration matrices using Matlab® version R2013b

TABLE 1 : Concentrations of AML and ATN mixtures used in chemometric methods

No.of Mix	AML (µg/ml)	ATN (µg/ml)
1	5	25
2	5	20
3	4	20
4	4	30
5	6	22.5
6	4.5	30
7	6	25
8	5	22.5
9	4	22.5
10	4.5	27.5
11	5.5	30
12	6	27.5
13	5.5	25
14	5	30
15	6	30
16	6	20
17	4	27.5
18	5.5	20
19	4	25
20	5	27.5
21	5.5	27.5
22	5.5	22.5
23	4.5	20
24	4	22.5
25	4.5	25

(8.2.0.701), together with PLS-Toolbox 2.1. software for the calculations. The concentrations were calculated from the corresponding regression equations.

Analysis of pharmaceutical preparation

Twenty tablets (each tablet labeled to contain 5 mg AML and 25 mg ATN) were weighed and finely powdered. A portion of powder equivalent to one tablet was weighed, dissolved in Water by shaking for about 30 min. The solution was filtered, transferred quantitatively into100-ml volumetric flask and completed to the mark with Water (A). Suitable dilutions were made using water to prepare solutions containing (1-5µg mL^{-1}) for AML and (5-25µg mL^{-1}) for ATN.

RESULTS AND DISCUSSION

Due to the overlapped spectra of the drug and degradate, Figure (3), the previous chemometric methods have been used to analyze this mixture. Various criteria have been developed to select the optimum number[12]. thirteen samples (odd numbers of samples) were chosen and used for calibration and twelve (even numbers of samples) were used for external validation.

For the CLS method, the training set was used for constructing CLS model or (K) matrix (i.e. absorptivity at different wavelengths) but poor predictions were obtained. The results were greatly

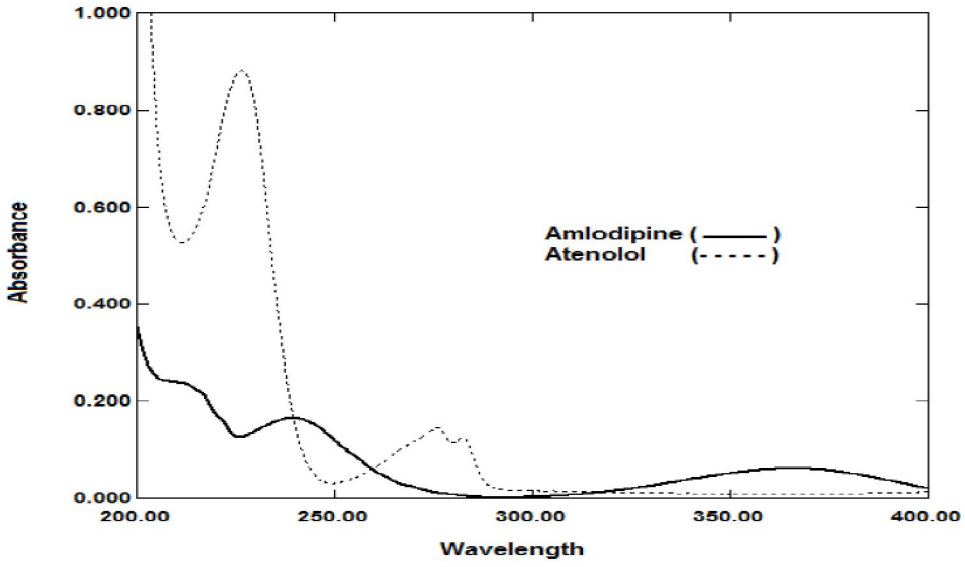

Figure 3 : Absorption spectra of amlodipine (5 µg/ ml) and atenolol (25 µg/ ml)

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improved by using the CLS model with nonzero intercept.

Cross-validation methods leaving out one sample at a time was employed [13]. The predicted concentrations were compared with the known concentrations of the compounds in each calibration sample. The root mean squares error of cross-validation (RMSECV) was calculated for each method for examining the errors in the predicted concentrations. The optimum number of factors was selected by fol-

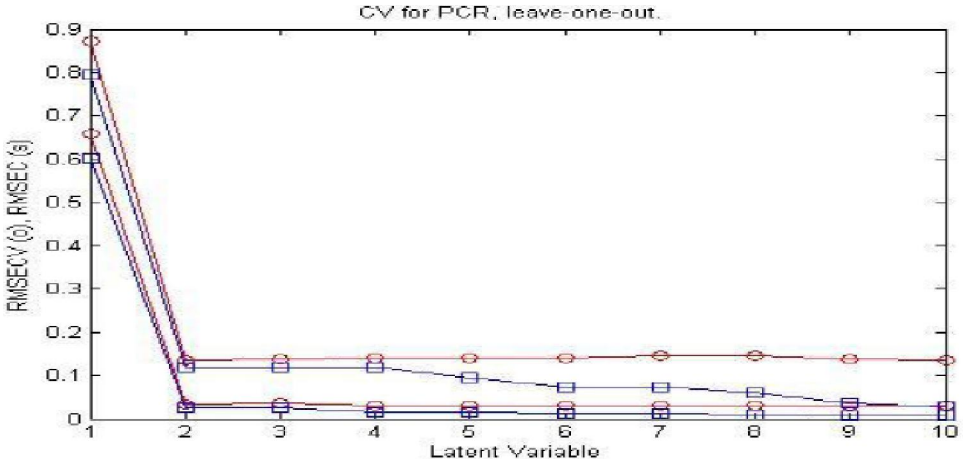

Figure 4 : RMSECV plot of the cross validation results of the calibration set as a function of the number of latent variables (LVs) used to construct the PCR model

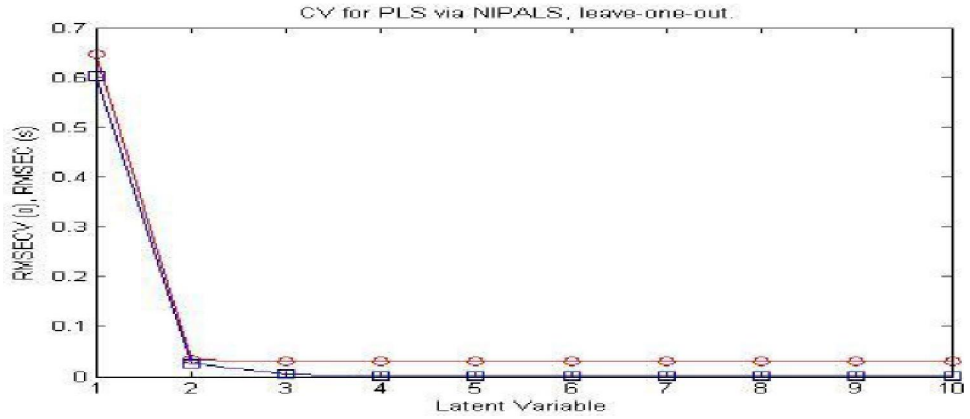

Figure 5 : RMSECV plot of the cross validation results of the calibration set as a function of the number of latent variables (LVs) used to construct the PLS-1 model

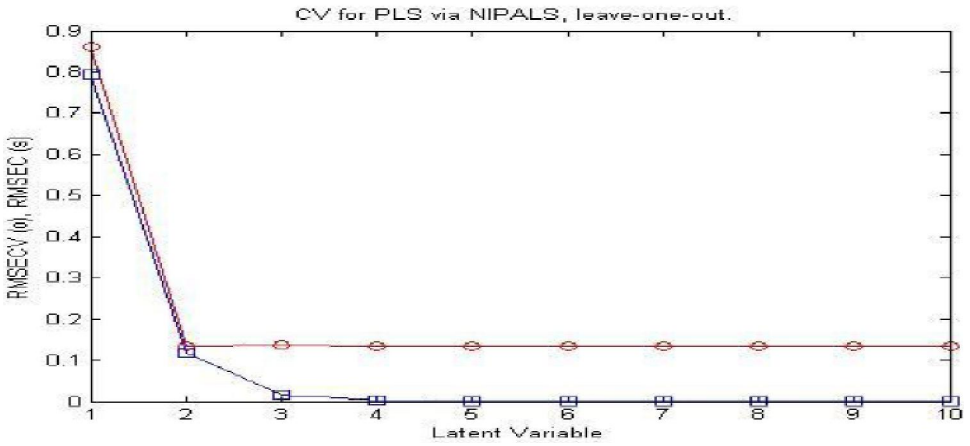

Figure 6 : RMSECV plot of the cross validation results of the calibration set as a function of the number of latent variables (LVs) used to construct the PLS-1 model

TABLE 2 : % of CFC and its degradation product in the validation set by PCR, CLS and PLS methods

Validation mixture	CLS		PCR		PLS-1	
	AML	ATN	AML	ATN	AML in presence of ATN	ATN in presence of AML
1	100.11	101.09	100.10	101.10	100.096	101.10
2	99.83	99.42	99.86	99.40	99.855	99.40
3	99.99	99.37	101.12	99.35	101.118	99.35
4	99.92	101.11	99.91	101.12	99.914	101.12
5	98.12	100.43	98.14	100.41	98.136	100.41
6	100.31	100.65	100.30	100.66	100.298	100.66
7	99.29	99.35	99.30	99.34	99.304	99.34
8	99.75	99.93	99.73	99.46	99.727	99.46
9	99.82	100.06	99.80	100.08	99.804	100.08
10	99.49	99.72	99.49	99.71	99.492	99.71
11	100.71	101.21	100.69	101.22	100.693	101.22
12	98.88	99.28	98.89	99.28	98.885	99.28
Mean±%RSD	99.68±0.682	100.13±0.740	99.77±0.790	100.09±0.772	99.77±0.789	100.09±0.771
RMSEP	0.0339	0.177	0.0339	0.177	0.0361	0.179

TABLE 3 : Determination of AML & ATN in Amlopress-AT 25® Tablets by the proposed and reported methods

Parameter	PCR		CLS		PLS-1		Reported Method[15]***
	AML	ATN	AML	ATN	AML	ATN	
N*	5	5	5	5	5	5	5
$X^?$	101.59	101.92	101.45	101.60	100.14	101.82	101.75
SD	0.8697	0.8529	0.927	0.870	0.296	0.891	1.157
RSD%	0.8560	0.8368	0.914	0.856	0.295	0.875	1.137
t**	0.7475 (2.306)	0.5479 (2.306)	0.4567 (2.306)	1.157 (2.306)	0.4082 (2.306)	0.3522 (2.306)	———
F**	1.448 (6.388)	1.803 (6.388)	1.728 (6.388)	2.675 (6.388)	1.993 (6.388)	3.203 (6.388)	———

*** No. of experimental; ** The values in the parenthesis are tabulated values of t and F at (p= 0.05); *** Simultaneous determination of AML and ATN by using (Dual wavelength technique).**

lowing the criterion of Haaland and Thomas [14].

The selected model was that with the smallest number of factors such that RMSECV for that model was not significantly greater than RMSECV from the model with additional factor. A number of factors were found to be optimum for the mixture of AML and ATN using PCR Figure (4), PLS-1 for AML in presence of ATN Figure (5)PLS-1 for ATN in presence of AML Figure (6).

The percentage recoveries of the validation samples are shown in [TABLE 2] indicated the high predictive abilities of PCR, PLS and CLS models. When results obtained by applying the proposed methods for analysis of AML and ATN compared to those obtained by applying the reported method[15], they showed no significant difference regarding accuracy and precision; and results were given in[TABLE 3].

Application of methods on pharmaceutical preparation

The suggested methods were valid and applicable for the analysis of AML and ATN inAmlopress-AT 25tablets. The recovery percentages forAML and ATN using PCR, PLS and CLS method respectively (average of 5 experiments) [TABLE 3].

CONCLUSION

The developed Chemometric methods have the

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advantages of being simple and not expensive over the chromatographic methods. The procedures applied in each method do not need any sophisticated instruments, critical reactions or any prior separation steps. The proposed methods are found to be sensitive, selective and accurate with no significant difference of the precision compared with the reference method[15]. They could be applied for routine analysis of pure AML and ATN or in their pharmaceutical formulation without interference due to the excipients or the degradation product and could also be easily used in quality control laboratory for its analysis. The methods are also suitable and valid for application in laboratories lacking liquid chromatographic instruments.

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