Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 154 (2016) 58–66

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Advanced stability indicating chemometric methods for quantitation of amlodipine and atorvastatin in their quinary mixture with acidic degradation products

Hany W. Darwish [a,b], Said A. Hassan [a,*], Maissa Y. Salem [a], Badr A. El-Zeany [a]

[a] Department of Analytical Chemistry, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, 11562 Cairo, Egypt
[b] Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

ARTICLE INFO

Article history:
Received 10 February 2015
Received in revised form 2 June 2015
Accepted 4 October 2015
Available online 8 October 2015

Keywords:
Amlodipine
Artificial neural networks
Atorvastatin
Genetic algorithm
Partial Least Squares
Stability indicating

ABSTRACT

Two advanced, accurate and precise chemometric methods are developed for the simultaneous determination of amlodipine besylate (AML) and atorvastatin calcium (ATV) in the presence of their acidic degradation products in tablet dosage forms. The first method was Partial Least Squares (PLS-1) and the second was Artificial Neural Networks (ANN). PLS was compared to ANN models with and without variable selection procedure (genetic algorithm (GA)). For proper analysis, a 5-factor 5-level experimental design was established resulting in 25 mixtures containing different ratios of the interfering species. Fifteen mixtures were used as calibration set and the other ten mixtures were used as validation set to validate the prediction ability of the suggested models. The proposed methods were successfully applied to the analysis of pharmaceutical tablets containing AML and ATV. The methods indicated the ability of the mentioned models to solve the highly overlapped spectra of the quinary mixture, yet using inexpensive and easy to handle instruments like the UV-VIS spectrophotometer.

1. Introduction

Amlodipine (AML) is 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester [1]. AML is a dihydropyridine derivative with calcium antagonist activity, used in the management of hypertension, chronic stable angina pectoris and prinzmetal's variant angina [2]. Atorvastatin (ATV) is [*R*-(*R**,*R**)]-2-(4-Fluorophenyl)-β,δ-dihydroxy–5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrole-1-heptanoic acid [1]. ATV is a selective, competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme that converts 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate, a precursor of the sterols, including cholesterol. It is used to reduce LDL-cholesterol, apolipoprotein B, and triglycerides and to increase ATL-cholesterol in the treatment of hyperlipidemias [3]. Caduet® is the first commercial product that has been launched by Pfizer Ltd. for the simultaneous treatment of hypertension and dyslipidemia [4]. Caduet® contains both AML for the treatment of high blood pressure and ATV for the treatment of hypercholesterolemia. Caduet® tablets are intended for oral administration and are available in several different strength combinations.

Literature survey revealed that AML is official in British Pharmacopoeia [5]. There are reported methods for the determination of AML or ATV in different drug combinations [6–10]. Also different methods have been reported for the simultaneous estimation of AML and ATV in their binary mixture [11–16], and stability indicating HPLC methods [17,18] have been applied for the analysis of this mixture.

Chemometrics is the art of processing data with various numerical techniques in order to extract useful information [19]. It is the application of mathematical and statistical methods to design optimum procedures and to provide maximum chemical information through the analysis of chemical data. Quantitative spectroscopy has been greatly improved by the use of a variety of multivariate statistical methods [20–24]. Multivariate calibrations are useful in spectral analysis because of the simultaneous inclusion of multiple spectral intensities which can greatly improve the precision and applicability of quantitative spectral analysis [25].

The rationales for this manuscript were the simultaneous determination of AML and ATV in the presence of their acidic degradation products, after their separation and characterization, in laboratory prepared mixtures and tablets. To present a comparative study between Partial Least Squares (PLS-1) regression and Artificial Neural Networks (ANN) as multivariate calibrations, and to show the effect of variable selection procedure e.g. genetic algorithm (GA), when preceding these multivariate calibrations, on increasing the predictive power of them.

* Corresponding author.
E-mail addresses: said.hassan@pharma.cu.edu.eg, saidmonem_84@yahoo.com (S.A. Hassan).

http://dx.doi.org/10.1016/j.saa.2015.10.007
1386-1425/

2. Material and methods

2.1. Instruments

- SHIMADZU dual beam UV–visible spectrophotometer (Kyoto/Japan), model 1650 UV-PC, with matched 1-cm quartz cells. The bundled software, UV-Probe personal spectroscopy software version 2.21 (SHIMADZU) is used. The spectral bandwidth is 2.0 nm and wavelength scanning speed is 2800 nm/min with 0.1 nm interval.
- Gas chromatograph coupled to a mass spectrophotometer, Shimadzu Qp-2010 (Japan).
- IR Spectrophotometer: Shimadzu 435 (Kyoto, Japan), sampling was undertaken as potassium bromide disks and NaCl plates.
- pH meter, Jenway, no. 924005-BO3-Q11C.

2.2. Software

All chemometric methods were implemented in Matlab® 7.12.0.635 (R2011a). The t-test and F-test were performed using Microsoft® Excel 2010. All calculations were performed using a Dual CPU, 1.47 GHz, 2.00 GB of RAM under Microsoft Windows 7™.

2.3. Materials and reagents

- Pure amlodipine besylate; kindly supplied by Al-Hekma Pharmaceutical Company, Cairo, Egypt, its purity was certified to be 99.89 ± 0.69.
- Pure atorvastatin calcium; kindly supplied by Al-Delta Pharmaceutical Company, Cairo, Egypt, its purity was certified to be 99.79 ± 0.46.
- Caduet® 5 mg/ 10 mg tablets; labeled to contain 5 mg AML and 10 mg ATV, batch number 1030039 and Caduet® 10 mg/ 10 mg tablets; labeled to contain 10 mg AML and 10 mg ATV, batch number 0795049, manufactured by Pfizer Ltd., Cairo, Egypt.
- Methanol, toluene, chloroform, acetic acid, hydrochloric acid and sodium hydroxide: analytical grade, purchased from El-NASR Pharmaceutical Chemicals Co., Cairo, Egypt.

2.4. Standard solutions

- AML and ATV standard stock solutions; 1.0 mg · mL^{-1} in methanol.
- AML and ATV standard working solutions; 80.0 μg · mL^{-1} in methanol.
- Amlodipine degradation product (AMdeg), atorvastatin degradation product (ATdeg) and aniline (ANL) standard solutions; 100.0 μg · mL^{-1} in methanol.

2.5. Procedures

2.5.1. Preparation and separation of degradation products

2.5.1.1. Amlodipine degradation product. HCl solution (50.0 mL, 1 M) was added to pure AML (50.0 mg) in a flask and then the solution was refluxed for 2 h and cooled. NaOH solution (1 M) was added to the degraded solution till pH about 7.00 and the solution was tested for complete degradation. Complete degradation was tested by TLC using chloroform: methanol: acetic acid (15.0: 2.0: 0.4 by volume) as developing solvent. Then the solution was evaporated slowly in rotavapor just to dryness. The degradation product was extracted from solid NaCl with methanol and then the methanol was evaporated. The extraction was repeated three times to ensure complete extraction of the degradation products from NaCl. The purity of the degradation product was tested by dissolving a small portion in methanol, applying onto TLC plates and developing using the previously mentioned solvent system. The structure of the isolated degradation product was elucidated using IR and mass spectrometry.

2.5.1.2. Atorvastatin degradation products. ATV (50.0 mg) was dissolved in least volume of methanol in a flask, then 50.0 mL of 6 M HCl solution was added and the solution was refluxed for 3 h and cooled. NaOH solution (6 M) was added to the degraded solution till pH about 7.00 and the solution was tested for complete degradation. Complete degradation was tested by TLC using toluene: methanol (7.0: 3.0 v/v) as developing solvent. Then the solution was evaporated slowly in rotavapor just to dryness to obtain the first degradation product (ATdeg). The collected evaporated liquid was heated to get rid of solvents and the second degradation product (ANL) was obtained. The degradation product (ATdeg) was extracted from solid NaCl with methanol and then the methanol was evaporated. The extraction was repeated three times to guarantee complete extraction of the degradation products from NaCl. The purity of the degradation product was tested by dissolving a small portion in methanol, applying onto TLC plates and developing using the previously mentioned solvent system. The structure of the isolated degradation products was elucidated using IR and mass spectrometry.

2.5.1.3. Spectral characteristics of AML, ATV and degradation products. The zero-order (D_0) absorption spectra of 12 μg · mL^{-1} AML, ATV, AMdeg, ATdeg and ANL are recorded against methanol as a blank over the range of 200–400 nm.

2.5.1.4. Experimental design. A 5-level, 5-factor calibration design was performed using 5 concentration levels coded from +2 to −2 for each of the 5 components to be analyzed, including the 2 main drugs and the 3 degradates. The design aims to span the mixture space fairly well; where there are 5 mixtures for each compound at each concentration level, resulting in 25 mixtures [26]. The central level of the design is 10 μg · mL^{-1} for AML and 16 μg · mL^{-1} for ATV.

The concentration levels for each component were based on the linearity range, the ratio of AML and ATV in the dosage form and the fact that the degradation products were involved in levels up to about 30% of the corresponding drugs to cover a wide range of possibilities. Table 1 represents the concentration design matrix. The 2D scores plot for the first two PCs of the concentration matrix confirmed well position of the mixtures in space, orthogonality, symmetry and rotatability [26], (Supp. Mat. Fig. S1). The regions from 200–215 nm were rejected. Fifteen mixtures of this design were used as a calibration set and the other ten mixtures were used as a validation set to test the predictive ability of the developed multivariate models.

2.5.1.5. Application of the PLS-1, GA-PLS and ANN for the simultaneous determination of AML and ATV in Caduet® tablets. Ten tablets of both Caduet® 5(AML)/10(ATV) mg, 10(AML)/10(ATV) mg were accurately weighed and finely powdered. An amount of the powder equivalent to 2 mg ATV was weighed, dissolved in methanol by shaking in ultrasonic bath for about 30 min. The solutions were filtered and transferred quantitatively into two separate 100-mL volumetric flasks. The volume was then completed to the mark with methanol. Necessary dilutions were made to reach concentrations of linear range. Solutions obtained were analyzed by the proposed chemometric methods.

3. Results and discussion

To the best of our knowledge, the stability indicating methods that have been reported for determination of AML and ATV mixture did not separate or identify their degradation products, so the first goal of this paper was to develop a stability indicating methods for determination of AML and ATV after separation and elucidation of the structures of the degradates.