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Three different spectrophotometric methods manipulating ratio spectra for determination of binary mixture of Amlodipine and Atorvastatin

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ABSTRACT

Three simple, specific, accurate and precise spectrophotometric methods manipulating ratio spectra are developed for the simultaneous determination of Amlodipine besylate (AM) and Atorvastatin calcium (AT) in tablet dosage forms. The first method is first derivative of the ratio spectra (^{1}DD), the second is ratio subtraction and the third is the method of mean centering of ratio spectra. The calibration curve is linear over the concentration range of 3–40 and 8–32 μg/ml for AM and AT, respectively. These methods are tested by analyzing synthetic mixtures of the above drugs and they are applied to commercial pharmaceutical preparation of the subjected drugs. Standard deviation is <1.5 in the assay of raw materials and tablets. Methods are validated as per ICH guidelines and accuracy, precision, repeatability and robustness are found to be within the acceptable limit.

1. Introduction

Amlodipine (AM), 2[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine carboxylic acid,3-ethyl,5-methylester (Fig. 1) [1] is a dihydropyridine derivative with calcium antagonist activity. It is used in the management of hypertension, chronic stable angina pectoris and prinzmetal's variant angina [2]. AM inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle [3–5].

Atorvastatin (AT) is chemically described as [R-(R*,R*)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate (Fig. 2) [1]. AT 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 hyperlipidaemias [2–4].

Caduet® is the first commercial product that has been launched by Pfizer Ltd. for the simultaneous treatment of hypertension and dyslipidaemia [6]. Caduet® contains both AM besylate for the treatment of high blood pressure and AT calcium for the treatment of hypercholesterolaemia. Caduet® tablets are intended for oral administration and are available in several different strength combinations including 5(AM)/10(AT) mg, 10(AM)/10(AT) mg.

Literature survey revealed that Amlodipine besylate is official in British Pharmacopoeia [7]. There are many reported methods for the determination of either AM [8–10] or AT [11–13] alone, or in combination with other drugs in pharmaceutical dosage forms [14–20] or individually in biological fluids [21–24]. Different LC methods [25–30], spectrophotometric methods [31–33], chemometric methods [34], capillary electrophoresis [35], HPTLC [36], have been reported for the estimation of AM and AT in their mixture.

In this paper, three different methods manipulating ratio spectra for the simultaneous determination of Amlodipine besylate and Atorvastatin calcium in tablets are described. These methods show very simple and accurate way for the analysis of this binary mixture without the need of sophisticated instruments, expensive solvents or large number of samples. The mathematical explanation of the procedures is illustrated.

2. Experiment

2.1. Apparatus

Spectrophotometer: SHIMADZU dual beam UV–visible spectrophotometer (Kyoto/Japan), model UV-1650 PC connected to IBM compatible and a HP1020 laserjet printer. The bundled software, UV-Probe personal spectroscopy software version 2.21 (SHIMADZU) is used. The spectral band is 2 nm and scanning speed is 2800 nm/min with 0.1 nm interval.

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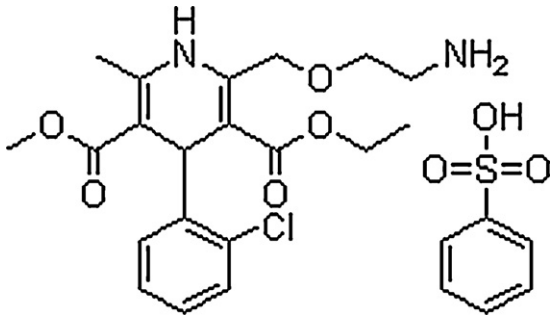

Fig. 1. Structural formula for Amlodipine besylate (MW = 567.05).

2.2. Software

Matlab® version 7, release 14.

2.3. Chemicals and reagents

- *Pure samples:* Pure Amlodipine; kindly supplied by Al-Hekma pharmaceutical Company, Cairo, Egypt, its purity is certified to be 99.89 ± 0.691. Pure Atorvastatin; kindly supplied by Al-Delta pharmaceutical Company, Cairo, Egypt, its purity is certified to be 99.79 ± 0.461.
- *Market samples:* Two Caduet® tablet dosage forms, labeled to contain 5(AM)/10(AT) mg batch number 1030039 and 10(AM)/10(AT) mg batch number 0795049, manufactured by Pfizer Ltd., Cairo, Egypt.
- *Methanol:* Spectroscopy grade is purchased from El-NASR Pharmaceutical Chemicals Co., Abu-Zaabal, Cairo, Egypt.

2.4. Procedures

2.4.1. Standard stock and working solutions

- AM standard stock solution; 1 mg/ml in methanol.
- AT standard stock solution; 1 mg/ml in methanol.
- AM standard working solution; 80 μg/ml in methanol.
- AT standard working solution; 80 μg/ml in methanol.

2.4.2. Spectral characteristics of AM and AT

The zero-order (D_0) absorption spectrum of 20 μg/ml of AM and 20 μg/ml of AT solution is recorded against methanol as a blank over the range of 200–400 nm.

2.4.3. Construction of calibration curves

Aliquots equivalent to 40–400 μg/ml AM and 80–360 μg/ml AT are accurately transferred from their standard working solutions (80 μg/ml) into two separate series of 10-ml volumetric flasks then completed to volume with methanol. The spectra of the prepared standard solutions are scanned from 200 to 400 nm and stored in the computer.

2.4.3.1. For (^{1}DD). For the determination of AM in presence of AT, the stored spectra of AM are divided by the spectrum of 32 μg/ml AT, smoothed with $\Delta\lambda$ = 16 nm, then the first derivative of the ratio spectra (^{1}DD) with $\Delta\lambda$ = 4 nm is obtained. The amplitude of the first derivative peak of (AM/AT) is measured at 341.0 nm. A calibration graph relating the peak amplitude at 341.0 nm to the corresponding concentrations in μg/ml of AM is constructed.

For the determination of AT in presence of AM, the stored spectra of AT are divided by the spectrum of 40 μg/ml AM, then the first derivative of the ratio spectra (^{1}DD) with $\Delta\lambda$ = 4 nm is obtained. The amplitude of the first derivative peak of (AT/AM) is measured at 294.0 nm. A calibration graph relating the peak amplitude at 294.0 nm to the corresponding concentrations in μg/ml of AT is constructed.

2.4.3.2. For ratio subtraction. A calibration curve is constructed relating the absorbance of zero order spectra of AT at 246.6 nm to the corresponding concentrations and the regression equation is computed.

A calibration curve is constructed relating the absorbance of zero order spectra of AM at 359.4 nm, where AT shows no interference, to the corresponding concentrations and the regression equation is computed.

2.4.3.3. For mean centering. The scanned spectra of AM are divided by the spectrum of 32 μg/ml AT and the obtained ratio spectra are smoothed with $\Delta\lambda$ = 16 nm and then mean centered. The same is applied to AT spectra as they are divided by the spectrum of 40 μg/ml AM and are then mean centered. The calibration curves for both AM and AT are constructed by plotting the mean centered values at 357.4 nm and 287.2 nm for AM and AT, respectively, versus the corresponding concentration.

2.4.4. Application of the ^{1}DD, ratio subtraction and mean centering methods for the determination of AM and AT in laboratory-prepared mixtures

Aliquots equivalent to 80.0, 160.0, 240.0, 80.0, 80.0, 160.0 and 240.0 μg of AM are transferred from its standard working solution (80 μg/ml) into a series of 10-ml measuring flasks. Aliquots

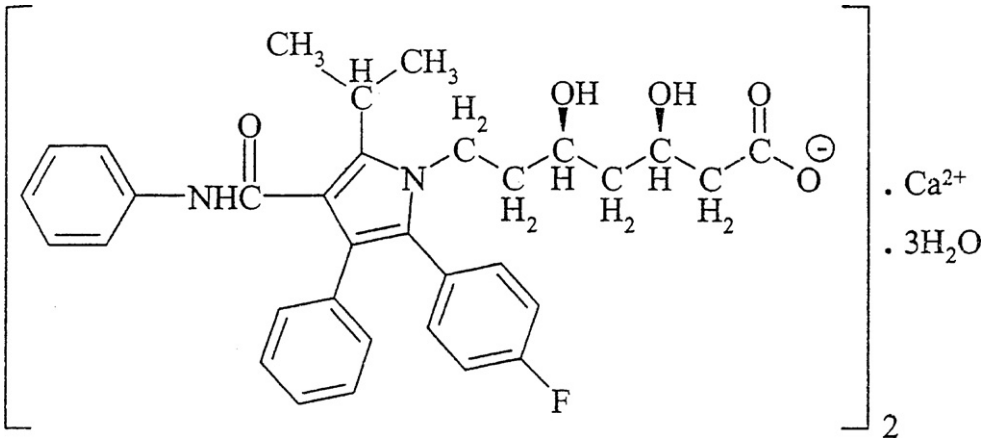

Fig. 2. Structural formula for Atorvastatin calcium trihydrate (MW = 1209.42).