

Simultaneous Determination of Amlodipine and Atorvastatin in Caduet® Tablets Using HPLC

Maha Abdalla Alnowaiser

Department of Chemistry, Faculty of Science,
Princess Nora Bint Abdul Rahman University, Riyadh, Saudi Arabia

Received 2012-10-12, Revised 2013-05-01; Accepted 2013-07-13

ABSTRACT

A simple, selective, sensitive and precise, simultaneous high performance liquid chromatographic analysis of tablets containing amlodipine and atorvastatin was described. Good chromatographic separation was achieved using a Perfectsilr Target ODS-3 (4.6 cm × 250 mm, 5 μm) and a mobile phase consisting of acetonitrile-phosphate buffer pH 4.5 (55:45, v/v) at a flow rate 1ML min⁻¹. The ultraviolet detector was set at wavelength 237 nm. Amlodipine and atorvastatin were measured at 1.071 and 3.765 min, respectively. The linear ranges for amlodipine and atorvastatin were 1-10 and 5-50 μg mL⁻¹, respectively. The recoveries of amlodipine and atorvastatin in pharmaceutical preparation were all greater than 98.5% and their relative standard deviations were less than 2.0%. The limits of detection were 0.19 and 1.25 μg mL⁻¹ for amlodipine and atorvastatin, respectively.

Keywords: HPLC, Cardiovascular, Hyperlipidemia

1. INTRODUCTION

Caduet is a combination of amlodipine (**Fig. 1**) and atorvastatin (**Fig. 2**) that is administered orally. They work together to control the blood pressure in patients with cardiovascular and hyperlipidemia problems.

Amlodipine is a calcium ion influx inhibitor (slow channel blocker or calcium ion antagonist) and inhibits the trans membrane influx of calcium ions into cardiac and vascular smooth muscle (O'Neil, 2006).

Amlodipine was determined in pharmaceutical dosage forms and plasma samples by Chromatographic (Yacoub *et al.*, 2013; Sah and Arora, 2012), spectrophotometric (Vandana *et al.*, 2013; Kazemipour *et al.*, 2009; Li *et al.*, 2002) and electrochemical methods (Gazy, 2004).

Atorvastatin is a competitive inhibitor of HMG-CoA reductase. It is a completely synthetic compound, which is the rate-limiting step in hepatic cholesterol biosynthesis (Elobieta *et al.*, 2012).

Several methods have been reported for the analysis of atorvastatin either in bulk powder,

different dosage forms or in biological fluids. These methods include spectrophotometric (Darwish *et al.*, 2011) and Chromatographic (Shariati *et al.*, 2013; Pili *et al.*, 2011) methods.

2. MATERIALS AND METHODS

2.1. Equipments

Agilent 1200 series, vacuum degasser, thermos tatted column compartment G1316A/G1316B, diode array and multiple wavelength detector SL, quaternary pump (Germany).

2.2. Chemicals

Amlodipine besylate and atorvastatin calcium were kindly supplied by PFIZER Company.

2.3. Pharmaceutical Preparation

Caduet® tablets; B.N. 1127020 (labeled to contain 5 mg amlodipine besylate and 10 mg atorvastatin calcium per each tablet) were kindly supplied from PFIZER Company.

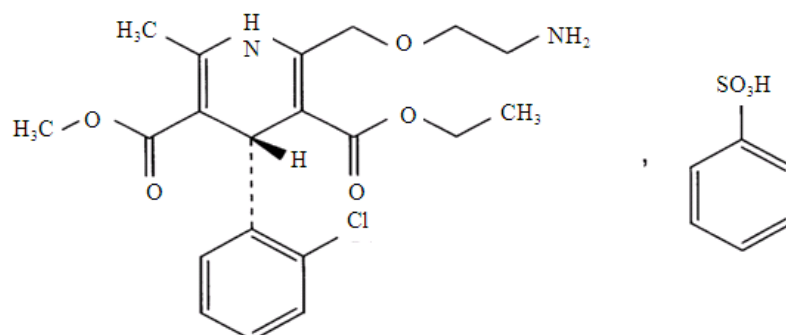


Fig. 1. 3-Ethyl 5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulphonate

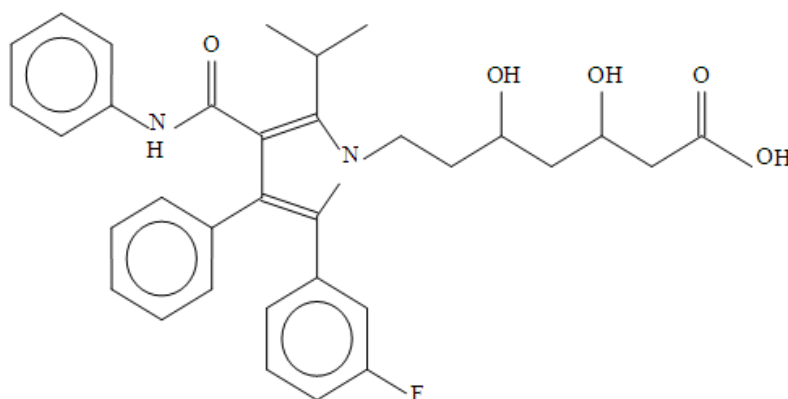


Fig. 2. [R-(R*, R*)]-2-(4-Fluorophenyl)-β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid

2.4. HPLC Procedure

2.4.1. Chromatographic Conditions

The analytical column was a Perfectsilr Target ODS-3 (4.6 cm × 250 mm, 5 μm) and a mobile phase consisting of acetonitrile-phosphate buffer pH 4.5 (55:45, v/v) at a flow rate 1 mL min⁻¹ and at room temperature. The ultraviolet detector was set a wavelength of 237 nm. Solutions and mobile phase were freshly prepared at the time of use.

2.5. Standard Solution Preparation

Stock solutions of amlodipine besylate and atorvastatin calcium were prepared daily by dissolving the appropriate amount of drug standards in mobile phase to yield a final concentration of 5.0 and 10.0 mg mL⁻¹, respectively. Separate stock solutions were prepared for the calibration standards and quality control samples. Further, solutions were obtained by serial dilutions of stock solutions with mobile phase.

2.6. Preparation of Pharmaceutical Dosage Sample

The contents of twenty tablets were weighed and finely powdered and transferred to 100 mL volumetric flasks. Each 10-mL equivalent to 5 mg amlodipine besylate and 10 mg atorvastatin calcium. Working solutions were prepared individually by diluting the stock solutions with mobile phase to obtain concentration range of 1-10 μg mL⁻¹ for amlodipine and 5-50 μg mL⁻¹ for atorvastatin.

3. RESULTS

3.1. Chromatograms of Samples

The aim of this research was to develop a new, simple, accurate, reproducible, sensitive HPLC method for the simultaneous determination of amlodipine besylate and atorvastatin calcium. A satisfactory separation of each drug from pharmaceutical excipients was obtained. To

optimize the appropriate HPLC conditions for separation of the examined drugs, various reversed-phase columns, isocratic and gradient mobile phase systems were tried. The optimum wavelength for detection was 237 nm at which much better detector responses for the three drugs were obtained. The mobile phase was found to be suitable to improve the sharpness and thinness of the amlodipine besylate and atorvastatin calcium. The retention times for the investigated drugs were found to be 1.071 min and 3.765 min, respectively. No pharmaceutical excipients eluted at the retention times of the peaks of interest.

3.2. Calibration and Linearity

Calibration curves were constructed in the ranges of 1-10 and 5-50 $\mu\text{g mL}^{-1}$ for amlodipine besylate and atorvastatin calcium, respectively. The slope, intercept and regression coefficient for each compound were estimated.

3.3. Accuracy

Absolute recoveries of six different authentic concentrations of amlodipine besylate and atorvastatin calcium (**Table 1**) and the studied drugs in tablets (**Table 2**) were determined by assaying the samples as described above. Mean recoveries, standard deviations and the relative standard deviations were calculated by standard method (**Table 1 and 2**).

3.4. Precision

Intra-day precisions were assessed injecting standard solution four to five times during a day (this solution was extracted via the same procedure as the capsules) of each analyte at two different concentrations (a low and a high concentration). The resultant standard deviations were less than 2% for all (**Table 3**). Inter-day precision experiments were done after treatment of the standard solution in the same method of tablets extraction and then analyzed every day over 5 days (**Table 3**). All RSD% were lower than 2%.

Table 3. Reproducibility and precision

Injected amount (μg)	Intra-day (n = 4-5)			Inter-day (n = 5)		
	Observed amount (μg) \pm SD	RSD (%)	Accuracy (%)	Observed amount (μg) \pm SD	RSD (%)	Accuracy (%)
Amlodipine besylate						
5	5.46 \pm 0.87	0.87	100.11	5.01	0.98	100.01
10	9.89 \pm 0.72	0.72	99.12	10.12	1.07	100.12
Atorvastatin calcium						
25	24.88 \pm 0.24	0.97	99.45	25.01 \pm 0.38	1.33	100.10
50	49.32 \pm 0.78	1.13	99.12	50.11 \pm 0.86	0.76	100.01

4. DISCUSSION

4.1. Method Validated

The method was validated with regard to specificity, linearity, Limit of Detection (LOD), Limit of Quantification (LOQ), precision, accuracy and robustness.

Peak areas of amlodipine besylate and atorvastatin calcium of calibration standards were proportional to the concentration in dosage forms over the ranges tested 1-10 and 5-50 $\mu\text{g mL}^{-1}$, respectively. Each concentration was tested in triplicate. The slope values for amlodipine besylate and atorvastatin calcium were calculated with intercept values. The standard deviations of slope were calculated and similarly standard deviations of intercept. The calibration curves were fitted by linear least-square regression and showed correlation coefficients not less than 0.9998.

Table 1. Statistical analysis of the results of authentic amlodipine besylate and atorvastatin calcium

	Amlodipine besylate Proposed method	Atorvastatin calcium Proposed method
X	99.99	100.89
N	6.00	6.00
\pm SD	0.78	0.39
RSD%	0.78	0.39

Table 2. Determination of amlodipine besylate and atorvastatin calcium in Caduet[®] tablets

Amlodipine besylate		Atorvastatin calcium	
Taken $\mu\text{g/ml}$	Recovery (%)	Taken $\mu\text{g/ml}$	Recovery (%)
1	99.20	5	99.20
5	99.30	25	100.10
10	100.20	50	99.70
X	99.80		99.90
\pm SD	0.38		0.57

The LODs and LOQs of thioctic acid, benfotiamine and cyanocobalamin were calculated on the peak area using the following equations: $LOD = 3.3 \times \sigma/S$, $LOQ = 10 \times \sigma/S$, where σ , is the standard deviation of the intercept of regression line of the drugs and S is the slope of the corresponding calibration curve. Determination of authentic samples of amlodipine besylate and atorvastatin calcium and statistical analysis of the results obtained for the proposed method (**Table 1**); show that all the suggested measurements are precise and accurate for all the studied drugs (**Table 1**).

4.2. Application to Pharmaceutical Dosage form

The proposed method were successfully applied for the simultaneous determination of amlodipine besylate and atorvastatin calcium in Caduet tables[®] without interference of the excipients present and without prior separation (**Table 2**). The utility of the method was also verified by applying the standard addition technique.

5. CONCLUSION

The chromatographic method described is adequate for quantitation of amlodipine besylate and atorvastatin calcium in pharmaceutical dosage forms at different concentration levels. It is very simple, accurate and effective and provided no interference peaks for endogenous components and pharmaceutical excipients. Acceptable values of precision and accuracy have been obtained all levels by this method regarding the guidelines for assay validation. The separation of these drugs takes 5 min in one chromatogram, so a large number of samples can be analyzed in a short period of time. The method uses simple mobile phase and is very beneficial for column life. In summary, the method can be successfully applied to samples of pharmaceutical dosage form.

6. REFERENCES

Darwish, H.W., S.A. Hassan, M.Y. Salem and B.A. El-Zeiny, 2011. Three different spectrophotometric methods manipulating ratio spectra for determination of binary mixture of Amlodipine and Atorvastatin. *Spectrochim Acta. Mol. Biomol. Spectrosc*, 83: 140-148. DOI: 10.1016/j.saa.2011.08.005

Elobieta, K., M. Ewa, K. Barbara and P.M. Aleksander, 2012. Development of chromatographic method for determination of drugs reducing cholesterol level. *Acta Poloniae Pharm. Drug Res.*, 69: 139-143.

Gazy, A.A.K., 2004. Determination of amlodipine besylate by adsorptive square-wave anodic stripping voltammetry on glassy carbon electrode in tablets and biological fluids. *Talanta*, 62: 575-582. DOI: 10.1016/j.talanta.2003.08.025.

Kazemipour, M., M. Ansari, A. Mohamadi, H. Beltollah and R. Ahmadi, 2009. Use of adsorptive square-wave anodic stripping voltammetry at carbon paste electrode for the determination of amlodipine besylate in pharmaceutical preparations. *J. Anal. Chem.*, 64: 65-70. DOI: 10.1134/S1061934809010134

Li, B.H., G.L. Yang, D.X. Wang, Z.F. Zhang and Y. Chen, 2002. Chiral separation of enantiomers of synthesized amlodipine and its intermediate by capillary electrophoresis. *Chinese J. Pharm. Anal.*, 20: 338-344. PMID: 12541917.

O'Neil, M.J., 2006. *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*. 14th Edn., John Wiley and Sons, ISBN-10: 091191000X, pp: 2564.

Pilli, N.R., J.K. Inamadugi, R. Mullangi, V.K. Karra and J.R. Vadia, 2011. Simultaneous determination of atorvastatin, amlodipine, ramipril and benazepril in human plasma by LC-MS/MS and its application to a human pharmacokinetic study. *Biomed. Chromatogr*, 25: 439-449. DOI: 10.1002/bmc.1462.

Sah, R. and S. Arora, 2012. Development and validation of a HPLC analytical assay method for amlodipine besylate tablets: A Potent Ca²⁺ channel blocker. *J. Adv. Pharm. Ed. Res.*, 2: 93-100.

Shariati, M., M. Irandoust, T. Amini and M. Shamsipur, 2013. Solving matrix effect, spectral overlapping and nonlinearity by generalized standard addition method coupled with radial basis functions-partial least squares: Simultaneous determination of atorvastatin and amlodipine in urine. *J. Chem.* DOI: 10.1002/cem.2491

Vandana, P.P., J.D. Subhash, S.A. Sachidanand, H. K. Suwarna and D.S. Santosh *et al.*, 2013. New ecofriendly validated spectrophotometric method for the estimation of amlodipine besylate in bulk drug using ninhydrin, *Asian J. Biomed. Pharma. Sci.*, 3: 14-19.

Yacoub, M., A.A. Awwad, M. Alawi and T. Arafat, 2013. Simultaneous determination of amlodipine and atorvastatin with its metabolites; ortho and para hydroxy atorvastatin; in human plasma by LC-MS/MS, *J. Chromatogr. B.*, 917-918: 36-47. PMID: 23369879