

# SIMULTANEOUS DETERMINATION OF AMLODIPINE AND ATORVASTATIN BY CAPILLARY ELECTROPHORESIS FROM FIXED PHARMACEUTICAL FORMULATIONS

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## Abstract

Optimum blood pressure levels through monotherapy may be challenging, especially for patients affected concomitantly by several illnesses. Consequently, fixed-dose which combines several active agents in single pharmaceutical formulations appears to be a golden standard in overcoming the cardiovascular disease. One of these fixed-dose combinations is dihydropyridine calcium channel antagonist amlodipine and the 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitor atorvastatin, which manage two important risk factors simultaneously in patients exposed to the cardiovascular disease and concomitant hypertension and dyslipidaemia. This study presents the development and validation of a simple, rapid, cost-effective capillary electrophoresis method for the simultaneous determination of amlodipine and atorvastatin in tablet formulations. The best results were obtained when using a 50 mM phosphate buffer at a pH of 7.0, the substances being detected in less than 5 minutes, the order of migration being amlodipine, followed by atorvastatin. The optimization of the electrophoretic conditions was based on obtaining improved peak shape, resolution and separation time. The analytical performances of the method were verified by quantifying specific parameters such as linearity, limit of detection, limit of quantitation, precision, accuracy, robustness and selectivity. The applicability of the method was verified by assessing amlodipine and atorvastatin from the original pharmaceutical product Caduet<sup>®</sup>.

## Rezumat

Obținerea unor valori optime ale presiunii arteriale poate fi o sarcină dificilă în monoterapie, în special în cazurile în care pacientul suferă și de alte afecțiuni asociate. În consecință, combinațiile fixe în care regăsim mai multe substanțe active într-o singură formă farmaceutică par să devină o abordare modernă a tratamentului anumitor boli cardiovasculare. Una dintre aceste combinații fixe este aceea dintre derivatul dihidropiridinic antagonist al canalelor de calciu, amlodipina, și inhibitorul de 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductază, atorvastatina, care gestionează simultan doi factori importanți la pacienți cu risc cardiovascular, ce asociază hipertensiune arterială și dislipidemie. Prezentul studiu prezintă dezvoltarea și validarea unei metode simple, rapide și puțin costisitoare de electroforeză capilară pentru determinarea simultană a amlodipinei și atorvastatinei din tablete. Cele mai bune rezultate au fost obținute prin utilizarea unui tampon fosfat de 50 mM la un pH de 7,0; cele două substanțe au fost determinate în mai puțin de 5 minute, ordinea de migrare fiind amlodipină urmată de atorvastatină. Optimizarea condițiilor electroforetice s-a bazat pe îmbunătățirea formei și amplitudinii *peak*-urilor, rezoluției și a timpului de separare. Performanțele analitice ale metodei au fost verificate în ceea ce privește linearitatea, limita de detecție, limita de cuantificare, precizia, acuratețea, robustețea și selectivitatea. Aplicabilitatea metodei a fost verificată prin cuantificarea amlodipinei și atorvastatinei din produsul farmaceutic original, Caduet<sup>®</sup>.

**Keywords:** amlodipine, atorvastatin, fixed-dose combinations, capillary electrophoresis

## Introduction

Fixed-dose combinations are designed to simplify the medication regimen and improving medication compliance which can translate into better clinical outcomes [1].

Hypertension and dyslipidaemia are two of the most commonly co-occurring cardiovascular risk

factors which together cause an increase in coronary heart disease-related events.

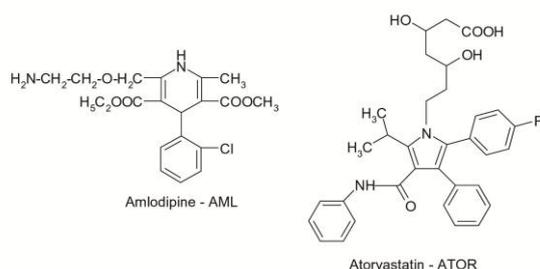
Antihypertensive and lipid-lowering medications substantially reduce the risk of coronary artery disease, stroke, and death in patients with cardiovascular risk factors [2].

Amlodipine ((RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy)-methyl]-4-(2-chlorophenyl)-6-methyl-1,4-di-

hydropyridine-3,5-dicarboxylate) (AML) is a dihydropyridine-type calcium channel blocker used in the management of hypertension and coronary artery disease [3].

Atorvastatin ((3R,5R)-7-[2-(4-Fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid) (ATOR) is a HMG-CoA (3-hydroxy-3-methyl-glutaryl-CoA) reductase inhibitor used primarily as a lipid-lowering agent and for prevention of events associated with cardiovascular disease [3].

The structural characteristics of the two substances are presented in Figure 1.



**Figure 1.**

Chemical structures of the studied analytes

In clinical trials, the fixed-dose combination of AML and ATOR effectively managed two important risk factors simultaneously in hyper-tensive patients at risk of cardiovascular disease or in those with concomitant hypertension and dys-lipidaemia. The combination is bioequivalent to AML and ATOR given alone and does not modify the efficacy of either single agent; compared with the co-administration of each single agent, the convenience of single-pill AML/ATOR has the potential to improve patient adherence and the management of cardiovascular risk [1-5].

Literature survey indicated that several analytical methods have been described for the simultaneous determination of AML and ATOR, including chemometric [5, 6], UV spectrophotometry [7], RP-HPLC [8], HPLC coupled with fluorescence detection [9], HPLC coupled with UV detection [7,10] and HPLC-MS [11].

Since its official use 25 years ago capillary electrophoresis (CE) gained a significant impact in pharmaceutical analysis, its advantages being related to the high selectivity, short analysis time, rapid method development and low consumption of analytes and reagents [4, 12].

CE methods have been successfully developed for the determination of AML and ATOR using a background electrolyte (BGE) composed of phosphate buffer – methanol (80:20 v/v) [13] or plain phosphate buffer [14] at pH 6.5.

The present study describes the development and validation of a simple, rapid, sensitive, cost-effective

CE method for the simultaneous determination of AML and ATOR in tablet formulations.

## Materials and Methods

### Chemicals and Reagents

Pharmaceutical grade samples of amlodipine besylate (Fako Ilaclari A.Ş, Istanbul, Turkey) and atorvastatin calcium (Morepen Laboratories, New Delhi, India) were used in the study.

Phosphoric acid ( $H_3PO_4$ ), disodium hydrogenophosphate ( $Na_2HPO_4$ ), sodium dihydrogenophosphate ( $NaH_2PO_4$ ) were purchased from Merck (Germany), while sodium hydroxide and methanol from LachNer (Czech Republic). All reagents were of analytical purity. Purified water was provided by a Milli-Q Plus water purification system (Millipore, USA).

The pharmaceutical dosage forms used in this study were Caduet® (Pfizer, USA) containing a AML/ATOR ratio of 5/10 and 10/10 mg respectively.

### Instrumentation

The measurements were performed on an Agilent 1600 CE system equipped with a photodiode array (DAD) detector and Chemstation software for data handling. Separations were performed on an uncoated fused-silica capillary with a total length of 48 cm (40 cm effective length), having an internal diameter of 50  $\mu m$  (Agilent, Germany). Buffer pH was measured using a Terminal 740 pH-meter (Inolab, Germany).

### Electrophoretic procedure

Conditioning of the new capillaries was conducted by flushing with 0.1 M NaOH for 60 minutes, water for 30 minutes and with the background electrolyte (BGE) for another 30 minutes. Prior to all runs, the capillary was preconditioned by flushing with 0.1 M NaOH, water, and background electrolyte (BGE) each for 2 minutes.

BGE solutions were prepared dissolving the appropriate amount of buffer constituents in ultrapure water and adjusting the pH if necessary with 1 M  $H_3PO_4$  or 1 M NaOH solutions. Stock solution containing 1 mg/mL of each analyte were prepared in methanol and diluted prior to use with the same solvent to the appropriate concentration. Both BGE and sample solutions were filtered through a 0.45  $\mu m$  pore size membrane filter and sonicated in an ultrasonic bath for 5 minutes prior to use.

In the preliminary analysis we applied some “standard” electrophoretic conditions for a CE analysis: temperature 25°C, applied voltage + 20 kV, injection pressure/time 50 mbar/3 s, sample concentrations 25  $\mu g/mL$ . The samples were introduced in the system at the anodic end of the capillary by hydrodynamic injection. Detection was performed at 210, 230 and 250 nm, and full spectra of the analytes were also stored to facilitate peak identification.

*Preparation of pharmaceutical samples*

Twenty Caduet<sup>®</sup> tablets from the same batch were weighed accurately, average weight was calculated, the tablets were finely powdered in a mortar into a homogenous powder; an amount of powder equivalent to the weight of one tablet was dissolved in 100 mL methanol by sonication for 5 minutes with intermittent shaking. The solution was filtered through a 0.45  $\mu\text{m}$  syringe, centrifuged at 3500 rpm for 10 minutes and diluted with methanol to the appropriate concentration. The same procedure was applied as in the separation from standard solutions.

**Results and Discussion***Preliminary analysis*

Electrophoretic mobility and ionization behaviour of analytes are the key factors driving separations in CE. Knowledge of these basic physicochemical properties of analytes gives valuable information about their nature and makes it easier to choose appropriate experimental conditions for their separation [15].

In order to find the suitable conditions for the separation of AML and ATOR, a series of preliminary experiments were conducted using different buffer compositions at different pH values (2.5 - 11).

AML is a basic compound, due to its amino-ether substituent, and has a pKa value of 9.1, consequently under acidic conditions (pH < 5.0) is positively charged, but as the pH becomes more alkaline, the effective mobility decreases because AML starts to deprotonate [16]. As reported in the literature ATOR has a pKa value of 4.33, and will be completely ionized in anionic forms in neutral and alkaline pH environments [17].

The two analytes can be detected over a pH range between 5 and 11. At a neutral pH AML migrated before the electroosmotic flow (EOF) and ATOR after the EOF, while at a basic pH both analytes migrated after the EOF. The order of migration was AML followed by ATOR, which can be explained by the differences between the own electrophoretic

mobility of the analytes and also by the influence of EOF. A neutral phosphate BGE solution at pH 7.0, was preferred in order to obtain shorter migration times. The neutral BGE was prepared by weighing equal quantities of disodium hydrogenophosphate and sodium dihydrogenophosphate and dissolving the mixture in water.

*Optimization of the analytical conditions*

The effect of BGE concentration was studied by changing the phosphate buffer concentration from 25 to 100 mM at a constant pH of 7.0. High concentration of buffer will result in lower EOF and also in more Joule heating, affecting peak efficiency and migration times. Based on the migration time and the generated current, a 50 mM phosphate buffer was chosen.

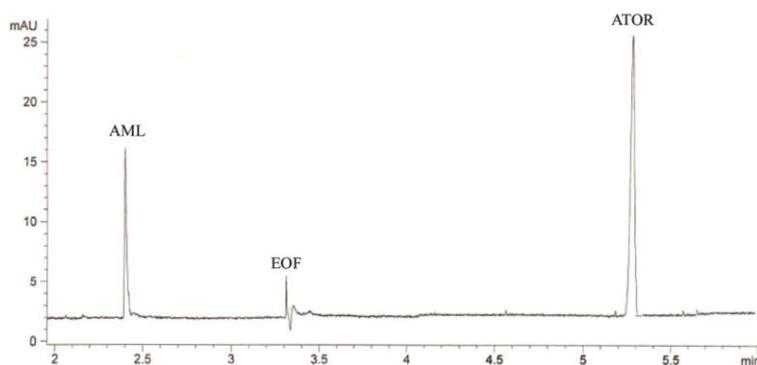
In order to determine the optimal voltage, the influence of applied voltage (15 - 30 kV) on the separation was investigated; voltage higher than 25 kV generated high current intensity; thus 25 kV was selected as optimum.

The influence of capillary temperature (15 - 30°C) was also evaluated; when system temperature increased, migration times decreased; therefore 25°C was chosen as optimum working temperature.

In order to determine the optimum injection parameters, the influence of injection time (1 - 5 s) and injection pressure (30 - 50 mbar) were studied in order to attain low detection limit without affecting the peak shape and reproducibility; an injection pressure of 50 mbar and an injection time of 3 seconds were selected as optimum.

The best results for the simultaneous determination of AML and ATOR were obtained when using 50 mM phosphate buffer at pH 7.0, + 25 kV applied voltage, 25°C system temperature, injection pressure 50 mbar, injection time 3 seconds, UV detection at 210 nm. The two analytes migrated in approximately 5 minutes, the order of migration was AML followed by ATOR.

A typical electropherogram obtained under the optimized analytical conditions is shown in Figure 2.



**Figure 2.**

Simultaneous separation of AML and ATOR by CE (optimized conditions: 50 mM phosphate pH 7.0, voltage 25 kV, temperature 25°C, injection pressure/time 50 mbar/3 s, UV determination 210 nm)

**Analytical performance**

For evaluation of the intra- and inter-day precision, variations were conducted by analysing three concentration levels (25, 50, 100 µg/mL) of standard solutions. The intra-day determination was

performed by analysing six replicates on the same day; while the inter-day determination was conducted over three consecutive days. The results for both migration times and peak area respectively indicated good precision of the method (Table I).

**Table I**

Intra- and inter-day precision for the simultaneous determination of AML and ATOR

Analyte (µg/mL)	RSD (%)			
	Migration time		Peak area	
	AML	ATOR	AML	ATOR
Intra-day precision (n = 6)				
25	0.09	0.11	0.32	0.44
50	0.10	0.13	0.53	0.77
100	0.11	0.14	1.08	1.32
Inter-day precision (n = 18)				
25	0.47	0.55	1.41	1.95
50	0.51	0.54	1.79	2.14
100	0.60	0.66	2.22	2.62

Linearity solutions were prepared from stock solution at six concentration levels and three replicates per concentration. The calibration curves were linear in the studied range (5 - 100 µg/mL for both AML and ATOR) with correlation coefficients above 0.99. The regression equation and correlation coefficients are presented in Table II.

The approach based on the standard deviation of the response and the slope of the calibration plots was used to determine detection (LOD) and quantification limits (LOQ). LOD and LOQ values were estimated as [(standard deviation of repeatability)/(slope of the regression equation)] by multiplying with 3.3 and 10 respectively. The values obtained are given in Table II.

**Table II**

Linearity parameters for the simultaneous determination of AML and ATOR

Analyte	Regression equation	Correlation coefficient	LOD (µg/mL)	LOQ (µg/mL)
AML	$y = 0.123x + 7.542$	0.998	0.64	2.10
ATOR	$y = 0.042x + 1.419$	0.997	0.47	1.53

To demonstrate the robustness of the method, minor changes in the experimental conditions have been made; as pH of the buffer was varied in the range ± 0.5 pH units, separation temperature in the range ± 2°C while applied voltage in the range ± 2 kV. None of the modifications caused significant changes in the resolution between the drugs with RSD (%) for migration times and peak areas under 2%.

The accuracy study was performed by weighing an appropriate amount of Caduet® tablet powder and spiking it with a known amount of the standard compounds; the resulted mixtures were analysed in triplicates. The good recoveries suggested the high accuracy of the proposed method (Table III).

**Table III**

Accuracy results for the determination of AML and ATOR spiked to tablet

Amount (µg/mL)	Recovery (%) ± SD	
	AML	ATOR
25	99.32 ± 1.43	102.08 ± 1.65
50	99.21 ± 1.10	103.32 ± 0.86
100	101.12 ± 0.45	100.26 ± 1.21

**Analysis of pharmaceutical formulations**

The optimized method was successfully applied for the simultaneous determination of AML and ATOR in the co-formulated original tablet, Caduet®. Satisfactory results were obtained for each compound as the found amounts were in good agreement with label claims (Table IV).

**Table IV**

Assay results of AML and ATOR simultaneous determination in pharmaceutical formulations

Pharmaceutical preparation	Declared amount (mg)		Found amount (mg)		RSD (%)	
	AML	ATOR	AML	ATOR	AML	ATOR
Caduet® 10/5	5	10	4.90	9.84	0.86	0.72
Caduet® 10/10	10	10	9.90	9.95	0.77	0.85

The results of the assay indicate that the method is selective for the analysis of both AML and ATOR

without interference from the excipients used to formulate the analysed pharmaceutical preparation.

## Conclusions

A simple, rapid, reagent-saving and inexpensive capillary zone electrophoresis (CZE) method for the simultaneous determination of AML and ATOR was successfully developed; under the optimized conditions baseline separation of AML and ATOR was obtained in less than 5 minutes. Good analytical performance with regards to linearity, reproducibility and accuracy was achieved. The developed method can be therefore used in the preliminary analysis of the studied substances.

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