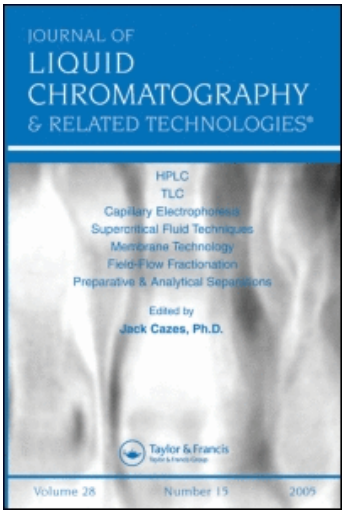

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Development of Capillary Electrophoresis Technique for Simultaneous Measurement of Amlodipine and Atorvastatin from Their Combination Drug Formulations

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Abstract: A simple, accurate, precise, and sensitive capillary electrophoresis (CE) technique coupled to a diode array detector (DAD) has been developed for the separation and simultaneous determination of amlodipine (AM) and atorvastatin (AT) from their combination formulations. The proposed method utilized fused silica capillary (50 cm × 75 μm ID) and background electrolyte (BGE) composed of phosphate buffer (pH 6.5, 25 mM)-methanol, (80:20, v/v). The separation was achieved at 15 KV applied voltage and 25°C. Losartan was chosen as the internal standard to guarantee a high level of quantitative performance. The two drugs were subjected to thermal, photolytic, hydrolytic, and oxidative stress conditions and the stressed samples were analyzed by the proposed method. The method has shown adequate separation for AM and AT from its main degradation products (UK-55-410) & (PD 0162910-00), respectively, which demonstrated the specificity of the assay. The described method was linear over the range of 1 – 50 μg/mL ($r = 0.9998$) for both drugs ($2.4 \times 10^{-6} - 1.2 \times 10^{-4}$ M for AM and $1.8 \times 10^{-6} - 8.6 \times 10^{-5}$ M for AT). Intra- and inter-day RSD ($n = 6$) was ≤2.2%. The limits of detection for AM and AT were 0.5 μg/mL. The percentage recoveries ($n = 6$) of the two drugs from their tablet formulations were 99.97 ± 1.84 and 100.96 ± 1.12, respectively. Degradation products produced as a result of stress studies did not interfere with the detection of AM and AT and the assay can thus be considered stability indicating.

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Keywords: Amlodipine, Atorvastatin, Capillary electrophoresis, Diode array detector

INTRODUCTION

Amlodepine (AM), 2-[(2-Ainoethoxy)-methoxy]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester is a dihydropyridine derivative with calcium antagonist activity.[1] It is used in the management of hypertension, chronic stable angina pectoris, and prinzmetal variant angina.[2] It inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscles.[3,4] It is used as a monobenzensulphonate (besilate) salt. Atorvastatin (AT), is chemically described as (βR, δR)-2-(4-fluorophenyl)-β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1 H-pyrrole-1-heptanoic acid.[1] It is a selective, competitive inhibitor of a 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-COA) reductase, the rate limiting enzyme that converts 3-hydroxy-3-methylgluteryl-coenzyme A to evaluate, a precursor of the sterols, including cholesterol. It is used to reduce LDL-cholesterol, apolipoprotein B, and triglycerides, and to increase HDL- cholesterol in the treatment of hyperlipidaemias.[2,4] It is administered as the calcium salt of the active hydroxyl acid. The combination therapy of AM and AT in pharmaceuticals (Caduet® tablets) is the first commercial product that has treated two different conditions, high blood pressure and high cholesterol, in a one dosage form. Caduet® tablets are intended for oral administration and are available in several different strength combinations including 5(AM)/10(AT) mg, 5(AM)/20(AT) mg, 5(AM)/40(AT) mg, 10(AM)/10(AT) mg, 10(AM)/20(AT) mg, and 10(AM)/40(AT) mg.

Capillary electrophoresis (CE), a versatile technique of high speed, high efficiency, low consumption of run buffer, and small sample size, is a major trend in analytical science, and numerous publications have appeared on the stability indicating assay of some drug substances and pharmaceuticals in recent years.[5–7]

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and enables recommendation of storage conditions, retest periods, and shelf lives to be established. The two main aspects of the drug product that play an important role in shelf life determination are the assay of active drug, and degradation products generated, during the stability study. The assay of drug product in stability test samples needs to be determined using the stability indicating

method, as recommended by the International Conference on Harmonization (ICH) guidelines[8] and USP-26.[9] Although stability indicating methods have been reported for the assay of various drugs in drug products, most of them describe assay procedures for drug products containing only one active drug substance. Only few stability indicating methods are reported for the assay of combination drug products containing two or more active drug substances. The objective of this work was to develop a simple, precise, and rapid analytical CE procedure, which would serve as a stability indicating assay method for the combination drug product of AT and AM.

Both the drugs AT and AM are not official with USP 26.[8] EP 2002[10] describes an HPLC method for the determination of AM, but does not involve simultaneous determination of AT. Detailed survey of literature for AT revealed several methods based on different techniques, *viz.* HPLC[11–13] and LC-MS[14–16] for its determination in plasma and serum; HPLC[17] for its determination in human serum and pharmaceutical formulations; HPLC;[18,19] HPTLC[20] for its determination in pharmaceuticals. Similarly, a survey of literature for AM revealed methods based on spectrophotometry,[21] RP-HPLC using fluorescence detection,[22] HPLC-tandem mass spectrometry,[23,24] RP-HPLC using UV detection,[25,26] HPLC[27–31] in combination with other drugs. Flow injection analysis using UV-detection,[32] HPTLC,[33,34] stability indicating HPLC,[35] and stability indicating HPLC[36] in combination with benazepril hydrochloride have been reported. Spectrophotometric[37] and HPLC[38] methods have been reported for simultaneous determination of AT and AM, but these methods lack stability indicating nature. Only two HPLC methods[39,40] were reported for stability indicating methods for both drugs in the presence of their degradation products. None of the reported methods described capillary electrophoresis method for the simultaneous determination of the two drugs in the presence of their degradation products.

This manuscript describes the development and validation of a capillary electrophoresis method for simultaneous determination of AT and AM in the presence of their degradation products as per ICH guidelines. To establish the stability indicating nature of the method, forced degradation of drug substances and drug product was performed under stress conditions (thermal, photolytic, acid/base hydrolytic, and oxidative), and stressed samples were analyzed by the proposed method. The proposed CE-DAD method was able to separate both drugs from degradation products generated during forced degradation studies. The method was validated as per ICH guidelines[41] and its updated international convention.[42] The linearity of response, accuracy, and intermediate precision of the described method has been checked.

EXPERIMENTAL

Electrophoretic Instrumentation and Conditions

The employed CE system consisted of a Beckman P/ACE MDQ instrument (Beckman Coulter, Inc. Fullerton, CA, USA) equipped with a photodiode array detector (PDA) and a data handling system comprised of an IBM personal computer and P/ACE system MDQ software. Detection was performed at 210 nm. A deactivated fused silica capillary was obtained from Agilant Technology (Fullerton, CA, USA) and had the following dimensions 67 cm total length, 50 cm effective length, 75 μm ID. The temperature of the capillary and the samples was maintained at 25°C. The background electrolyte solution (BGE) consisted of phosphate buffer–methanol (80:20, v/v), pH 6.5. Samples were injected into the capillary by pressure at the anodic side at 0.8 psi for 10 s. The electrophoresis was carried out by applying high voltage to the capillary, with the cathode being at the detector end. The capillary was washed between run with a sequence of rinses: 0.1 M sodium hydroxide (2 min), deionized water (2 min), then equilibrated with the running buffer (4 min), to ensure reproducibility of the assay. Before sample injection, the capillary was conditioned with 0.1 M sodium hydroxide (5 min), deionized water (5 min), and running buffer electrolyte (10 min).

Chemicals and Reagents

Amlodipine besilate and its main degradation product (UK-55-410) 3-ethyl 5-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate, atorvastatin calcium and its main degradation product (PD 0162910-00) were kindly supplied by Pfizer labs (Division of Pfizer Inc, NY 10017, USA). Combination product of amlodipine and atorvastatin (label claim: 5/10, 5/20, 5/40, 10/10, 10/20, 10/40 mg/tablet for each drug, respectively), Caduet® tablets, (lot. 0280K05A, 0617K05A, 0282K05A, 0284K05A, 0285K05A, 0286K05A), were purchased from the local market. Methanol, disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid, hydrogen peroxide, orthophosphoric acid, acetonitrile were obtained from Merck (Darmastud, Germany). All chemicals were analytical grade and used without further purification. Deionized water was used throughout the experiments.

Preparation of Stock and Standard Solutions

Stock solutions containing 1 mg/mL of AM besilate and AT calcium were prepared in methanol on a free base basis and corrected for salt

and purity. The internal standard (IS) losartan was prepared in methanol to give a concentration of 1 mg/mL and was further diluted with methanol to get the working solution 400 μg/mL. The solutions were stable for at least three weeks if kept in the refrigerator. Aliquots of the standard stock solutions of AT and AM were transferred into 20 mL volumetric flasks, 1.0 mL of IS solution (400 μg/mL) was added to each flask, then completed to the mark with water to yield final concentrations 2, 5, 10, 20, 30, 40, and 50 μg/mL for AT (maintaining the AM concentration at a constant level 25 μg/mL) and concentration of 2, 5, 10, 20, 30, 40, and 50 μg/mL for AM (maintaining the AT concentration at a constant level 25 μg/mL). Triplicate injections of each concentration were performed. The peak- area ratio of each concentration to the IS against the corresponding standard concentration were plotted, to obtain the calibration graphs. Alternatively, the corresponding regression equations was derived.

Analysis of Authentic Mixture

Stock solutions of AM besilate and AT calcium (equivalent to 1 mg/mL of free base) were prepared in methanol. Aliquots of the standard stock solutions of AT and AM were transferred into 20 mL volumetric flasks, 1.0 mL of IS solution (400 μg/mL) was added to each flask, then completed to the mark with water to yield different concentrations ratios (1:1, 1:2, 1:4, 1:8, w/w), respectively. Triplicate injections for each solution were made. The peak area ratio of each concentration to the IS was calculated. The concentration of each drug is obtained using the calibration curve or the corresponding regression equations.

Preparation of Tablets Solutions

Twenty Caduet® tablets were weighed, their mean weight determined, and powdered. An accurately weighed portion equivalent to one tablet content was transferred into each of ten 100 mL volumetric flask containing 20 mL water. The solution was shaken for 10 min, sonicated for 5 min, then made up to volume with methanol and mixed well, centrifuged at 3000 rpm for 10 min. Two mL aliquots of the solution was transferred to a 20 mL volumetric flask, 1 mL of IS solution (400 ug/mL) was added to each flask and made up to volume with water, to yield concentrations for each of the two drugs in the range of linearity previously described. The solutions were filtered through a

Millipore membrane filter (0.2 μm) from Nihon, Millipore (Yonezawa, Japan) before injection.

Stress Decomposition Studies

Forced degradation studies of bulk drug and drug formulation included appropriate solid state and solution state stress conditions in accordance with the ICH regulatory guidance.[35,36] Stress decomposition studies were performed initially with AT and AM working concentration of 500 μg/mL in methanol. Acid hydrolysis was performed by mixing 1 mL of AT and AM working solution in two separate 5 mL volumetric flasks with 1 mL of 0.1 mol/L and 0.5 mol/L HCl solutions, respectively, and the mixtures were kept at room temperature for 2 hours. The study in alkaline condition was carried out in a similar manner with 0.1 mol/L and 0.5 mol/L NaOH for 2 hours. For study in neutral condition, the drug was held in methanol solution at room temperature for 24 hours. Oxidative studies were carried out by mixing 1 mL of AT and AM working solution in three separate 5 mL volumetric flasks with 1 mL of 3% (v/v) H_2O_2, 15% (v/v) H_2O_2, and 30% (v/v) H_2O_2 and the resultant solutions were after 1, 2, 4, 8, 16, and 24 hr at ambient temperature. Photolytic and thermo degradation studies were performed with bulk drug powder (1 mm thick layer in a Petri plate) and in methanol solution (1 mL of AT and AM working solution), which were exposed to sunlight during two weeks and to 80°C for one week, respectively. As a control, the parallel set of samples was kept in dark at refrigerator temperature. The same procedure concerning photolytic and thermal degradations was repeated with AT and AM tablet formulations. Prior to CE-DAD analysis, samples were withdrawn at an appropriate time, neutralized (in case of acid and alkali hydrolysis), and the solutions were diluted with methanol to attain the predicted concentration of non-degradated AT and AM of 25 μg/mL. Several control samples were prepared for comparison with the stressed samples. First of all, the drugs solutions stored under normal conditions were analyzed. The chromatograms of the blank solutions, consisting of stress agents and without the drug and the zero time drug solutions together with stress agents, were inspected in order to mark the peaks corresponding to stress agents and to distinguish them from the potential drugs degradation products. The stressed samples were detected under different wavelengths in order to ensure that no additional degradation products were formed with different extinction values than the parent drugs. After recording UV spectrum (200–400 nm) of the drugs and the representative samples from each stress condition, the detection wavelength of 210 nm was finally selected.

RESULTS AND DISCUSSION

Optimization of the Chromatographic Conditions

Effect of pH of the BGE

The pH value is always a critical selectivity parameter for the determination of acidic and basic compounds. The initial study to electrolyte optimization was based on the effective mobility (μ_{eff}) curve of the two drugs and IS versus pH, (Figure 1). As it can be seen in Figure 1, it is not possible to carry out the separation in pH lower than 3.5. As for the pH interval of 2.5–5.5, AT has been found to be present in interaction with internal capillary wall. Therefore, the pH in intervals of 6–10.5 was chosen for the preliminary study. Three buffers were selected, namely phosphate, sodium tetra borate, and acetate, aiming to investigate the separation behavior of the standard mixture containing AM, AT, its main degradation products and IS. Each buffer was studied in three selected concentrations (5, 10, and 25 mM) under constant instrumentation conditions (voltage, injection time, temperature, wave length, etc.). A + 15 kV voltage was applied to the buffer in order to avoid current trouble, such as bubble formation which would result in current interruption. The incomplete separation or deformation peak in all electrolyte systems approached can be verified for the standard mixture of combination drug and their degradation products. Phosphate buffer, 25 mM (pH 6.5) has a reasonable resolution, signal intensity and analysis time, in comparison to the other electrolyte system, (Table 1). A second set of preliminary experiments was carried out using additives in the electrolyte system such as, sodium dodecyle sulphate (SDS) and cyclodextrain (CD). The addition of SDS and CD in the running buffer

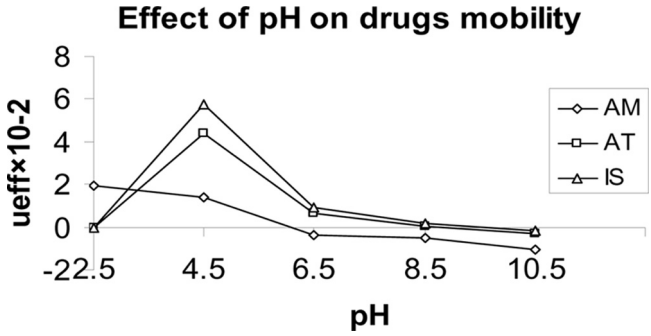

Figure 1. Effective mobility curve in function of the pH for resolution of AM, AT, and IS.

Table 1. Effect of various parameters on separation of AM and AT

	Migration time (min)		
Parameter	Rs	AM	AT
Buffer concentration (Mm)			
5	1.85	4.10	6.33
15	2.80	4.64	7.29
25	4.26	5.14	8.38
35	4.30	5.20	8.59
50	4.29	6.02	10.43
pH			
2.5	–	25.30	0.00
4.5	–	20.90	43.90
6.5	6.04	7.80	15.50
8.5	5.5	6.80	11.00
10.5	–	2.70	8.500
Voltage (kV)			
15	7.55	7.80	15.50
20	7.10	5.60	11.00
25	6.50	5.30	10.20
30	6.23	3.30	6.50
Temperature°C			
15	8.00	8.30	15.70
25	9.60	6.50	12.10
35	7.40	4.50	9.800
45	5.0	4.03	7.03
55	4.03	3.20	5.60
Organic modifier			
0	4.70	7.30	13.40
5	5.20	7.90	15.10
15	5.70	8.20	15.70
25	6.10	8.30	16.20
35	6.30	8.30	16.90
Pressure			
0.2	–	8.60	15.30
0.4	10.89	8.41	14.88
0.6	7.14	8.42	15.10
0.8	6.41	8.10	14.10
1.0	6.52	8.20	14.32

over the critical micelar concentration (CMC) promotes spontaneous aggregation of the surfactant molecules forming micelle in the running buffer, causing change of the apparent mobility of the analytes due to hydrophobic interaction,[37] so the buffer containing SDS and CD did

not present relevant improvement in comparison to the experiment carried out in their absence.

Effect of Buffer Composition and Concentration

Buffer concentration has a significant effect on the separation performance through its influence on the EOF and the current produced in the capillary. Different BGEs have been tested, the best results considering selectivity, reproducibility, baseline separation, and current performance was obtained with phosphate buffer, pH 6.5. Keeping other parameters constant (pH 6.5, 25 kV, 25°C), the buffer concentration varied from 15 to 55 mM were examined. Increasing in migration times as well as current was observed when the buffer concentration increased. The resolution of the two studied drugs increased as the buffer concentration increased, but no appreciable improvements were observed at buffer concentrations more than 25 mM. Therefore, 25 mM phosphate buffer, pH 6.5 was selected as optimal (Table 1).

Effect of Concentration of the Organic Modifier

There is a relationship between the EOF velocity and the organic modifier concentration, due to viscosity modulation of EOF by the organic modifier.[38] Additionally, it is possible to influence the partitioning equilibrium by adding organic solvents. The use of organic modifier increases the migration window and guarantees better resolution of late migrating compounds. The increased amount of organic modifier (methanol) from 5–30% caused a general increase in the migration times of the two drugs. At the same time, an increase in the Rs factor was observed, especially for AM and its degradant, without significant improvement resolution of the two drugs at concentrations more than 20%, (Table1).

Effect of Applied Voltage

Applied voltage can affect the efficiency of analysis since resolution of compounds is directly proportional to the applied voltage.[39] In order to obtain optimum between AM and AT by the proposed method, several runs were performed with gradual increases in the applied voltage. It was found that, an increase in efficiency (Rs factor) following an increase in applied voltage from 15 to 30 kV. Further increases in applied voltage resulted in decreased efficiencies, due to that the capillary becomes less effective in heat dissipation after a certain voltage level, where excessive Joule heat is generated.[40] As heat is produced inside the capillary, the viscosity of the buffer decreased. An applied voltage of 15 kV was thus selected for further analysis, (Table 1).

Effect of Cartridge Temperature

Capillary temperature control is extremely important for reproducibility of the assay. When current passes along a capillary, part of the electrical energy is converted into Joule heating. Temperature changes the viscosity of the buffer and therefore the migration velocity of the analytes affecting the migration times, and consequently the resolution of analytes.[41] To control or minimize the effects of Joule heating, temperature can be controlled with fan blown air or by a recirculating liquid, with the capillary mounted in a cartridge. The Beckman P/ACE MDQ equipment used for this study uses a circulating coolant containing perfluoro compounds, to maintain the temperature inside the capillary cartridge. The migration time decreased with an increase in temperature from 15–60°C, which was attributed to decreased viscosity of the BGE at higher temperature. A convenient operational temperature of 25°C was selected as an optimal temperature (Table 1).

Separation Performance

In order to significantly reduce the injection related imprecision to ensure better reproducibility and greater control over the sample amount injected, the quantitative analysis is generally preferred. Losartan was used as an internal standard to guarantee a high level of quantitative performance. Figure 2 shows the electropherogram obtained from the

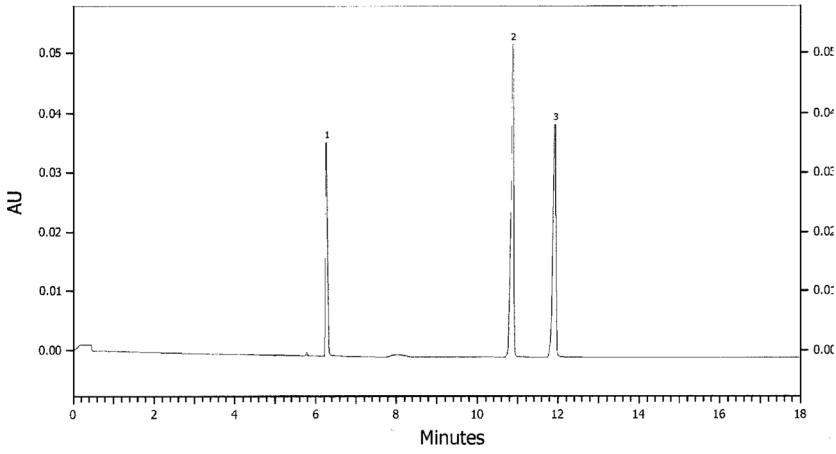

Figure 2. Electropherogram corresponding to standard solution of (1) 25 μg/mL of AM, (2) 40 μg/mL of AT and (3) 400 μg/mL of losartan (IS).

standard solution of combination drugs analysed using optimized conditions. The migration times of AM, AT, and IS were found to be 6.4, 10.8, and 11.8 min., respectively. These migration times did not vary to any considerable degree during and in between analyses (% RSD less than 1.0 for the migration time of each peak).

Linearity, Precision and Accuracy

The linearity of the detector responses for both AM and AT were determined by plotting peak area ratios of the drug to the internal standard versus concentrations. The analytical data for the calibration graphs are listed in Table 2. The calibration curves were linear in the range of $1-50\,\mu g/mL$ for AM and AT, with correlation coefficient (r) of more than 0.999. A typical calibration curve has the regression equation of $y=0.0119x+0.0046$ for AM and $y=0.0329x-0.0245$ for AT. A summary of the accuracy and precision results is given in Table 3. The precision and accuracy of the method were determined by using sample stock solutions spiked with standard drug solutions containing 0.125%, 0.5% and 1% of the labeled amount of both drugs (5.0 mg AM and 10 mg AT) (Table 3). The data indicate that intra-day precision and accuracy ($n=6$) as expressed by percentage RSD and percentage error were $0.56-2.16\%$ and $0.16-0.96\%$, respectively, for AM and AT. The inter-day precision and accuracy ($n=6$) expressed by percentage RSD and percentage error were 0.43–1.70% and $-0.04-0.04\%$, respectively, for AM and AT. The detailed analytical data are shown in Table 3.

Table 2. Validation parameters for the determination of standard solution of amlodipine and atorvastatin by the proposed method

Parameter	Amlodipine	Atorvastatin
Concentration range (μg/mL)	1–50	1–50
Intercept (a)	0.0046	−0.0245
Slope(b)	0.0119	0.0329
Correlation coefficient(r)	0.9996	0.9998
S_a	0.0141	0.0201
S_b	3.155×10^{-4}	4.464×10^{-4}
$S_{x/y}$	8.867×10^{-3}	7.867×10^{-3}
LOD(μg/mL)	0.5	0.5
LOQ(μg/mL)	1.0	1.0

S_a = standard deviation of intercept.
S_b = standard deviation of the slop.
$S_{x/y}$ = standard deviation of the residual.

Table 3. Accuracy and precision data for amlodipine and atorvastatin in tablet formulations

Analyte	Conc. added ($\mu g\,mL^{-1}$)	Conc. found ($\mu g\,mL^{-1}$) Mean ± SD (n = 6)	Error(%)	RSD(%)
Intra-day				
Amlodipine	6.25	6.23 ± 0.05	−0.32	0.80
	25	24.96 ± 0.54	−0.16	2.16
	50	50.11 ± 1.07	0.22	2.14
Atorvastatin	12.5	12.51 ± 0.13	0.08	1.04
	50	50.48 ± 0.51	0.96	1.01
	100	100.14 ± 0.56	0.14	0.56
Inter-day				
Amlodipine	6.25	6.24 ± .057	−0.16	0.91
	25	24.9 ± 0.40	−0.40	1.61
	50	49.88 ± 0.83	−0.24	1.70
Atorvastatin	12.5	12.47 ± 0.07	−0.24	0.54
	50	49.98 ± 0.42	−0.04	0.84
	100	100.04 ± 0.43	0.04	0.43

Limit of Detection and Limit of Quantitation

The LOD was 0.5 μg/mL for AM and AT. The LOQ of each calibration graph was one μg/mL for each drug. The good linearity of the calibration graphs and the negligible scatter of experimental points are evident by the values of the correlation coefficient and standard deviation.[36]

Selectivity

Selectivity is described as the ability of the method to discriminate the analytes from all potential interfering substances. Selectivity of method was investigated by both peak purity and spiking experiments with pure standard compounds. Peak purity was evaluated by use of a diode array detector and the P/ACE system MDQ soft wear. Otherwise, there are no peaks detected at the retention time of individual drug and of internal standard at the level of LOQ or less. Excipients commonly coformulated with the studied drug such as magnesium stearate, cellulose, starch, calcium hydrogen phosphate, colloidal silicon dioxide, and coloring agents, did not interfere with the determination of AM and AT, indicating the high selectivity of the proposed method.

Table 4. Application of the proposed method to the analysis of AM and AT in tablet formulations

Ratio AM/AT	Quantity taken μg/mL AM/AT	AM		AT	
		Found	Recovery(%)	Found	Recovery(%)
1:1	10:10	10.12 ± 1.11	101.21 ± 0.71	10.22 ± 1.8	102.2 ± 0.83
1:2	12.5:25	12.56 ± 0.78	100.48 ± 0.83	24.92 ± 0.05	99.68 ± 0.83
1:4	5:20	4.925 ± 0.25	99.70 ± 0.41	20.6 ± 0.98	101.70 ± 0.49
1:8	5:40	4.853 ± 0.06	97.10 ± 0.98	40.26 ± 0.35	100.53 ± 0.57
Avarage		99.97 ± 1.84		100.96 ± 1.12	

Application to Tablet Formulations

The applicability of the proposed method was examined by analyzing commercial Caduet tablets. The obtained results of the test solution are shown in Table 4. All the obtained data fully met the criteria from the ICH regulative in every observed segment of the method validation.

Forced Degradation Study

Degradation was not observed with exposure of the AT solution to day light for two weeks whereas, the chromatograms obtained from the photodegradation revealed that the peak area of AM was reduced proportionally with time. Figure 3 shows the semi logarithmic plots of concentration of AM ($\log a/a-x$) versus time (t) for the degraded sample of AM. This indicates a first order degradation behavior with the rate constant (K) of 0.0283 day^{-1} and a correlation coefficient of 0.996.

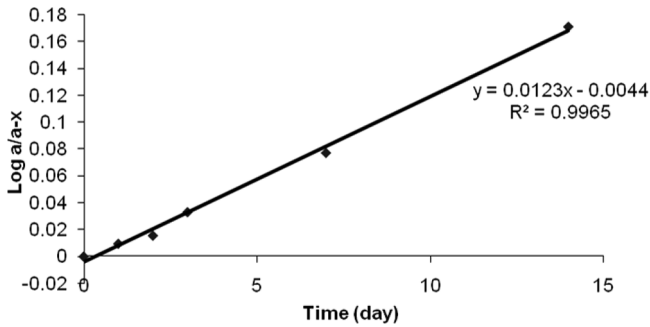

Figure 3. Semi logarithmic plot of amlodipine concentration versus time for photodegradation sample.

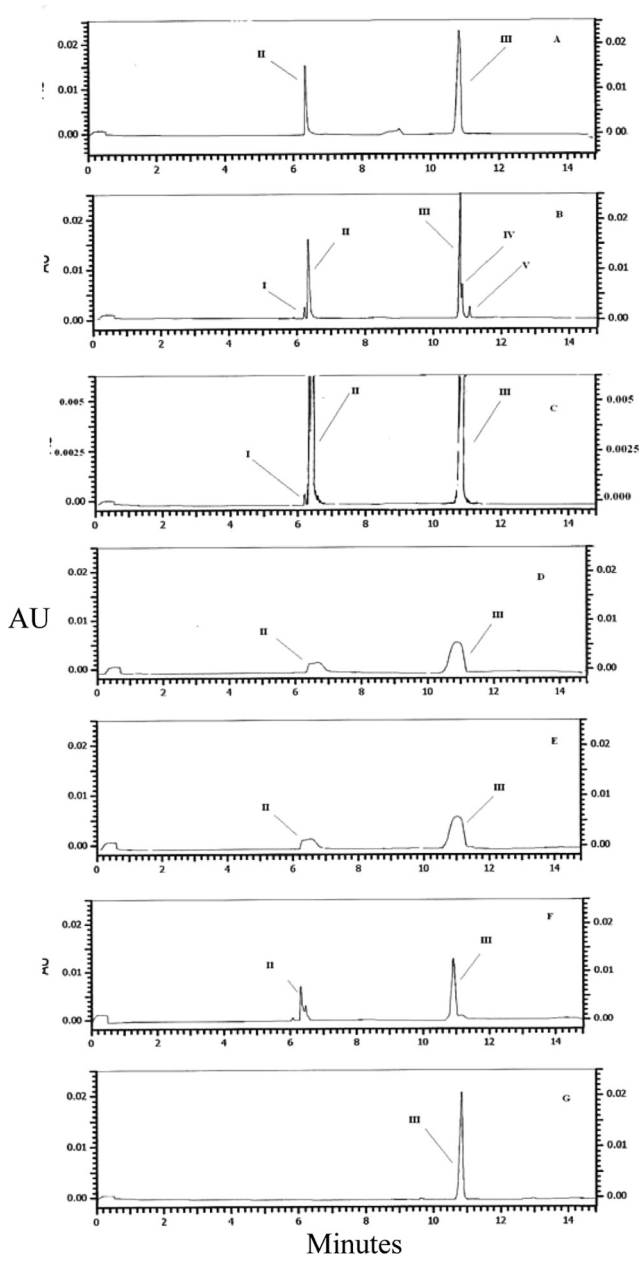

Figure 4. Typical CE electropherograms of: (a) API solution mixture, (b) API spiked with degradation products, (c) photolytic decomposition after 14 days, (d) acid hydrolysis, (e) alkali hydrolysis, (f) oxidative degraded API solution, (g) effect of dry temperature after one week, where (I) AM degradant, (II) AM, (III) AT, (IV) and (V) AT degradante.

The first order disappearance rate constant was calculated using the following formula: $\text{Log}[a/a-x] = \log[a] - k_t/2.303$. Where [a] is the initial concentration of AM at $t = 0$ and [a/a−x] is concentration at time t. The half life time ($t_{1/2}$) was calculated according to the following formula $t_{1/2} = 0.693/k$ and it was found to be 24.43 days, the compound eluted at

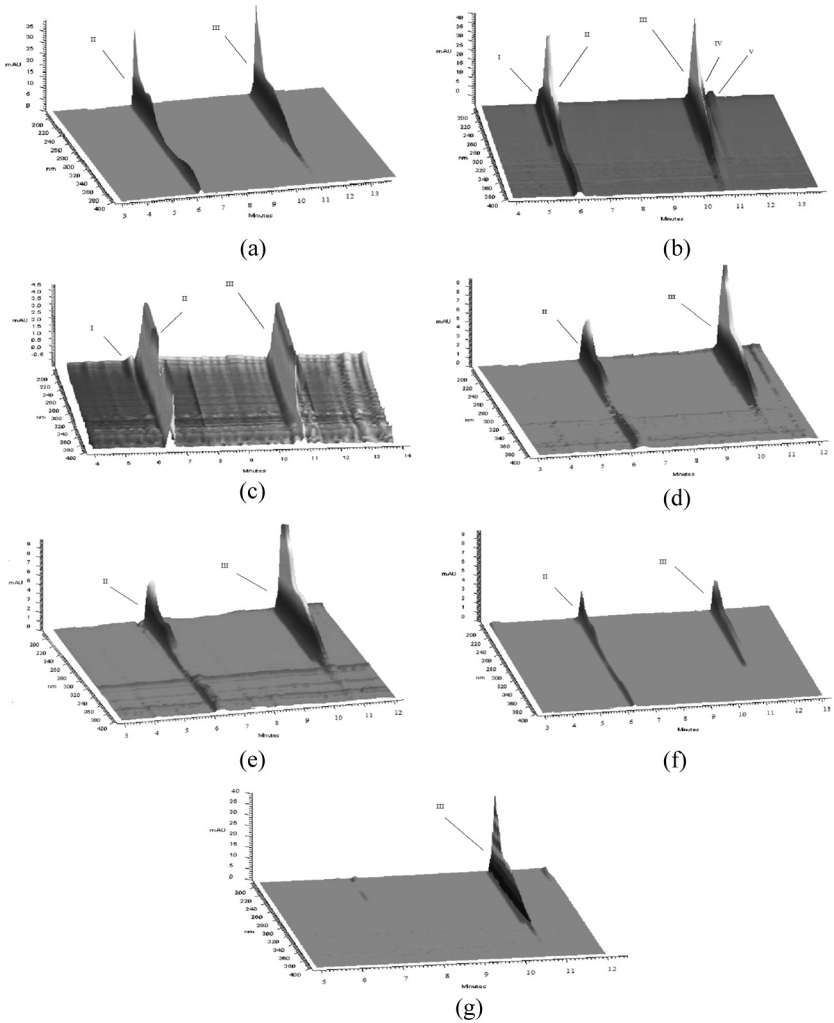

Figure 5. Three-dimensional (absorbance vs. time vs. wavelength) electropherograms of: (a) API solution mixture, (b) API spiked with degradation products, (c) photolytic decomposition after 14 days, (d) acid hydrolysis, (e) alkali hydrolysis, (f) oxidative degraded API solution, (g) effect of dry temperature after one week, where (I) AM degradante, (II) AM, (III) AT, (IV) and (V) AT degradante.

6.2 min. Figures 4C, 5C is the main degradation product of AM, UK-55-410 {Ethyl 5-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinecarboxylate}, lacking any therapeutic effect.[42] The CE-DAD analyses revealed the significant formation of one major degradation product. Its retention time was shorter compared to AM indicating different polarity. Acid and base hydrolysis of AM and AT solution was started with 0.5 M hydrochloric acid and 0.5 M sodium hydroxide separately for 2 hrs. Both drugs showed extensive degradation under these hydrolytic conditions and almost complete degradation of both drugs occurred. Thus, in later experiments, solutions for acid and base degradation studies were prepared in methanol and 0.1 M hydrochloric acid or 0.1 M sodium hydroxide. The degradation of AM is ascribed to the acid or alkaline hydrolysis of the acetyl groups of AM.[43,44] On the other hand, the hydrolysis of the lacton and lactonization of the β-hydroxy acid in the AT molecule can take place at the same time (Figures 4D, 5D and 4E, 5E).[45] AM and AT showed to be very unstable towards oxidation. Mild degradation was observed while the sample was exposed to 3% (v/v) H_2O_2. The degradation was also accelerated by increasing H_2O_2 concentration to 15% (v/v) where the concentration of AM decreased by 83.5% and 75.2% for AT (Figures 4F, 5F). AM and AT were found to be relatively stable following exposure to dry heat (80°C) for 2 hour but there is a significant decrease in the peak area of AM during the study interval (completely decomposed after one week). On the other hand, AT was less affected by heat (22% decomposed) (Figures 4G, 5G). The forced degradation behavior of the two drugs in both bulk and tablet formulations appeared to be similar since no additional products were noticed suggesting, thereby that there was no significant interaction between the drug and the excipient.

CONCLUSION

The proposed CE-DAD method is considered as the first method proven to be capable to direct separation of AM and AT from their combination products. The data generated from the performed forced degradation studies enabled the evaluation of AM and AT stability under a variety of ICH recommended conditions. Such data is valuable for the safety and potency assessment of the drug products. The developed CE-DAD method proved to be able to measure the drugs in the presence of degradation products and related organic impurities expected to be present in the formulations. In that manner, the proposed chromatographic procedure confirmed its applicability as a stability indicating method.

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