Ali Reza Fakhari[1]
Saeed Nojavan[1]
Soheila Haghgoo[2]
Ali Mohammadi[3,4]

[1]Department of Chemistry, Faculty of Sciences, Shahid Beheshti University, Tehran, I.R. Iran
[2]Food and Drug Laboratory Research Center, Ministry of Health and Medical Education, I.R. Iran
[3]Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences, I.R. Iran
[4]Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, I.R. Iran

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Research Article

Development of a stability-indicating CE assay for the determination of amlodipine enantiomers in commercial tablets

A simple, accurate, precise and sensitive method using CD for separation and stability indicating assay of enantiomers of amlodipine in the commercial tablets has been established. Several types of CD were evaluated and best results were obtained using a fused-silica capillary with phosphate running buffer (100 mM, pH 3.0) containing 5 mM hydroxypropyl-α-CD. The method has shown adequate separation for amlodipine enantiomers from its degradation products. The drug was subjected to oxidation, hydrolysis, photolysis and heat to apply stress conditions. The range of quantitation for both enantiomers was 5–150 μg/mL. Intra- and inter-day RSD ($n = 6$) was $<4\%$. The limit of quantification that produced the requisite precision and accuracy was found to be 5 μg/mL for both enantiomers. The LOD for both enantiomers was found to be 0.5 μg/mL. Degradation products produced as a result of stress studies did not interfere with the detection of enantiomers and the assay can thus be considered stability indicating.

Keywords:
Amlodipine / CD / Enantioseparation / Stability indicating
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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) is a dihydropyridine derivative with calcium antagonist activity [1, 2]. AM inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle [3–5]. Similar to other dihydropyridine-type calcium channel blockers, AM is currently used as the racemate. The R-(+) and S-(−) enantiomers of AM have different biological activities. It has been reported that the pharmacokinetic behavior of S-(−)-AM shows that it has significant vasodilating effect [6–8]. Therefore, the development of stereoselective analysis method is of great importance. For this purpose, CE is still proving to be a highly effective tool [9–12]. CD-modified CE has been found to have a high resolving power [13, 14] and is therefore an effective tool for the enantioseparation and the evaluation of the enantiomeric excess [15–19]. Most CE methods for separation of the enantiomers of chiral drugs have used CD derivatives as chiral selectors [20–23]. Gilar and Holzgrabe reported the use of CD and their derivatives as chiral additives for separation of enantiomers of dihydropyridine calcium channel blockers [24, 25]. The separation of AM enantiomers was also investigated by using both native and derivatized CD [26, 27]. A method for the separation of the enantiomers of AM in serum by CE using hydroxypropyl (HP)-β-CD as the selector has also been reported [28].

The presence of degradants and impurities in pharmaceutical formulations may affect their efficacy and safety. This may cause a change to the chemical, pharmacological and toxicological properties of drugs. Drug stability is considered to be the secure way of ensuring the delivery of therapeutic values to patients [29–31]. Therefore, the adoption of stability-indicating method is always required to control the quality of pharmaceutical during and after preparation in industries and dispensaries.

In literature, HPLC methods have been reported for the indication of AM stability [32–34]. CE has not been used previously for this purpose. CE has many advantages over HPLC with respect to separation efficiency, consumption of reagents and samples, analysis time and simplicity of instrumentation [35]. Nevertheless, HPLC is still the dominant technique in pharmaceutical analysis, but the extensive use of CE will generate complementary and alternative methods. On the other hand, the main component and structurally related degradants and impurities in pharmaceutical formulations have similar chemical properties and thus make resolution difficult. However, the greater separation efficiency of CE makes this type of pharmaceutical analysis possible [36].

Correspondence: Professor Ali Reza Fakhari, Department of Chemistry, Faculty of Sciences, Shahid Beheshti University, Evin, G. C., Tehran, Iran
E-mail: a-zavareh@sbu.ac.ir
Fax: +98-21-22431661

Abbreviations: AM, amlodipine; **API,** active pharmaceutical ingredient; **HP,** hydroxypropyl

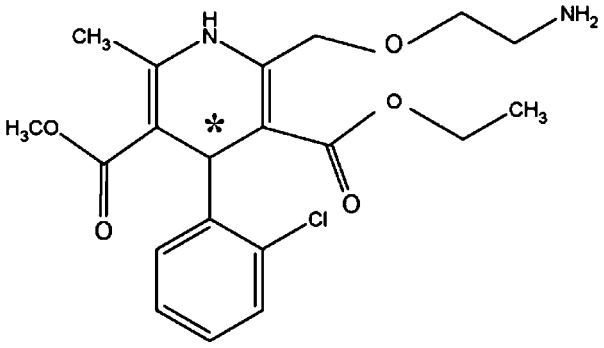

Figure 1. Chemical structure of AM. The asterisk denotes the chiral center.

The objective of this study is to use CE as a stability-indicating assay of the AM enantiomers in commercial tablets. The use of native and substituted CD as chiral selectors was investigated and the electrophoretic conditions for the assay were optimized.

2 Materials and methods

2.1 Materials

AM besylate (99.90%) was obtained from Arya pharmaceutical company (Tehran, Iran) and was used without further purification. β-CD, HP-β-CD, α-CD and HP-α-CD were purchased from Fluka (Buchs, Switzerland). Analytical grade H_3PO_4, $NaH_2PO_4.2H_2O$, Na_2HPO_4, NaOH and HCl were purchased from Merck (Darmstadt, Germany). Amlopers®5 and AM Arya®5 tablets were obtained from Cipla (Mumbai, India) and Arya pharmaceutical company, respectively. Methanol and hydrogen peroxide were obtained from Merck. HPLC grade water was obtained through a Milli-Q® system (Millipore, Milford, MA, USA) and was used to prepare all solutions. All CD used were CE grade (purity > 95%). Phosphate buffer, pH 3.0, was prepared by mixing appropriate amounts of H_3PO_4 and NaH_2PO_4 solution.

2.2 CE equipment

CE was carried out using a Lumex Capel 105 (Ohiolumex, Twinsburg, Russia) equipped with a UV detector with filter operated at 237 nm. The electrophoretic experiments were performed in an uncoated fused-silica capillary (Ohiolumex) 57 cm × 50 μm id (49 cm effective length). Throughout the studies CE was performed at 20°C, at a constant potential of 20 kV. The current increased until approximately 100 μA after power application. Before use, the capillary was conditioned for 20 min with 0.5 M HCl, 5 min with water, 30 min with 0.5 M NaOH and 5 min with water. Additionally, the capillary was washed for 2 min with 0.5 M NaOH, 1 min with water and 2 min with the running buffer with positive pressure applied at the injection end before each run. Acquisition of electropherograms was computer-controlled by Chrom&Spec software version 1.5. The analytes were injected at the anodic end by applying a pressure of 30 mbar × 6 s.

2.3 Preparation of stock and standard solutions

Stock solution of AM (1000 μg/mL) was prepared in methanol–water (20:80). The stock solution was protected from light using aluminum foil and stored for 3 wk at 4°C with no evidence of decomposition. Aliquots of the standard stock solution of AM were transferred using A-grade bulb pipettes into 100 mL volumetric flasks and the solutions were made up to volume with methanol–water (20:80) to yield final concentrations of 1, 5, 10, 20, 30, 50, 100, 150 and 200 μg/mL for each enantiomer.

2.4 Preparation of tablets for assay

Twenty AM tablets were weighed, crushed and mixed in a mortar and pastel for 20 min. A portion of powder equivalent to the weight of one tablet was accurately weighed into each of nine 25 mL A-grade volumetric flasks and 5 mL of HPLC-grade methanol was added to each flask. The volumetric flasks were sonicated (Beijing Branson Ultrasound Co., China) at room temperature for 20 min to effect complete dissolution of the AM and the solutions were then made up to volume with HPLC-grade water. Aliquots of the solution were filtered through a 0.45 μm Chrom Tech Nylon-66 filter and 5 mL of the filtered solution was transferred to a 10 mL A-grade volumetric flask and made up to volume with water, to yield concentration of the each enantiomer in the range of linearity.

2.5 Choice of the chiral selector

Several types of CD (native and derivatized forms) in different concentrations were tested in order to obtain separation of AM enantiomers. A standard solution of racemic AM (50 μg/mL) in methanol–water (20:80 v/v) was injected into the capillary. The CD and its concentrations that were examined were α-CD (1–20 mM), β-CD (1–10 mM), HP-α-CD (1–20 mM) and HP-β-CD (1–20 mM).

2.6 Effect of CD concentration on resolution

Resolution of enantiomers was compared using various concentrations of CD in the run buffer. Phosphate buffers (100 mM, pH 3.0, prepared by mixing appropriate amounts of H_3PO_4 and NaH_2PO_4 solution) containing 1, 2, 4, 6, 10 and 20 mM of HP-α-CD, CE of racemic AM (50 μg/mL) in

methanol–water (20:80 v/v) was performed. Resolution factors (R_s) for the enantiomers were calculated and the optimal CD concentration for the analysis was determined.

2.7 Effect of buffer concentration on the efficiency

The effect of buffer concentration on the separation efficiency of AM enantiomers was assessed by performing the electrophoretic separation using phosphate buffer, pH 3.0, at 25, 50, 100 and 120 mM, (different buffer concentrations were prepared by mixing appropriate amounts of H_3PO_4 and NaH_2PO_4 solution) with 5 mM HP-α-CD as the chiral selector.

2.8 Effect of voltage on the efficiency

The effect of the voltage on the efficiency of separation of AM enantiomers by CE was examined over the 12–22 kV range. Resolution factors (R_s) for the enantiomers were calculated and the optimal voltage for the analysis was determined.

2.9 Effect of cartridge temperature on resolution

The temperature inside the capillary cartridge and sample tray may affect separation in CE because it affects solute mobility and the EOF. At the operating pH of 3.0 EOF is minimal. Therefore, temperature will solely affect electrophoretic mobility. In order to determine the optimum temperature for the resolution of AM enantiomers, electrophoretic runs at different cartridge temperatures (15, 20, 25 and 30°C) were performed and the separation of the enantiomers examined.

2.10 Forced degradation studies of active pharmaceutical ingredient (API) and tablets

Forced degradation of drug and its product was carried out under thermolytic, photolytic, acid/base hydrolytic and oxidative stress conditions. Thermal and photodegradation of drug substance and drug product was carried out in both solid and solution states. Solutions were prepared by dissolving appropriate amount of drug or its product in 2 mL of methanol and later diluted with different solutions (to prepare stress conditions) mentioned below to achieve a concentration of 1000 μg/mL of racemic AM. After the degradation, these solutions were diluted with HPLC-grade water to achieve a concentration of 50 μg/mL of each AM enantiomer.

Solutions for acid hydrolysis study of drug substance and its product (1000 μg/mL) were prepared in 0.08 M hydrochloric acid in a mixture of methanol–water (20:80 v/v) and analyzed after 48 h.

During the initial forced degradation experiments, it was observed that basic hydrolysis was a fast reaction for the drug and almost complete degradation of the drug occurred when 0.5 M sodium hydroxide solution was used. Thus, in the later experiment, base hydrolysis of drug substance and drug product (1000 μg/mL) in solution state was conducted using 0.08 M sodium hydroxide in a mixture of methanol–water (20:80 v/v) for a period of 2 h.

Solutions for use in oxidation studies of drug substance and drug product (1000 μg/mL) were prepared in 2.0% hydrogen peroxide in a mixture of methanol–water (20:80 v/v) and the resultant solutions were analyzed after 48 h.

Solution for neutral degradation studies of drug substance and drug product (1000 μg/mL) was prepared in methanol and water (20:80 v/v) and the resultant solution was heated on a water bath at 90°C for 6 h prior to analysis.

Tablets and API were exposed to dry heat of 100°C in a convention oven for 48 h. The tablets and API powders were removed from the oven and 20 tablets were crushed and mixed and then an aliquot of powder equivalent to the weight of one tablet and API powder were then prepared for analysis as previously described.

Tablets and API powder and solutions of AM were prepared and exposed to light to determine the effects of irradiation on the stability of the drug in solution and in the solid state. Approximately 100 mg of API was spread on a glass dish in a layer that was less than 3 mm in thickness. A solution of API (1000 μg/mL) was prepared in methanol and HPLC-grade water (20:80 v/v). All samples for photostability testing were irradiated for 24 h with UV radiation (Thermolab, India) having peak intensities at 254 nm.

3 Results and discussion

3.1 Method development and optimization

3.1.1 Choice of the chiral selector

Several types of CD were initially tested in high and low concentrations in the run buffers, and resolution of AM enantiomers was examined. β-CD at 5 and 10 mM did not produce resolution of the enantiomers as can be observed for a concentration of 10 mM (see Fig. 2A). This could be due to the large size of the cavity of the CD (β-CD has seven glucose units) which did not allow stereoselective interaction with AM. HP-β-CD at concentrations of 5, 10 and 20 mM was examined and baseline separation of the enantiomers as two peaks was observed for a concentration of 10 mM (see Fig. 2B). Resolution of AM enantiomers has also been recently reported by Wang *et al.* [28] using a bare silica capillary column and a run buffer consisting of 75 mM sodium phosphate, pH 2.5, and 15 mM HP-β-CD. Substitution at the secondary hydroxyl rim on the surface of the CD can dramatically affect selectivity of the separation, as it will provide additional interaction points with the analyte [37–39]. An increase in the concentration to 20 mM resulted

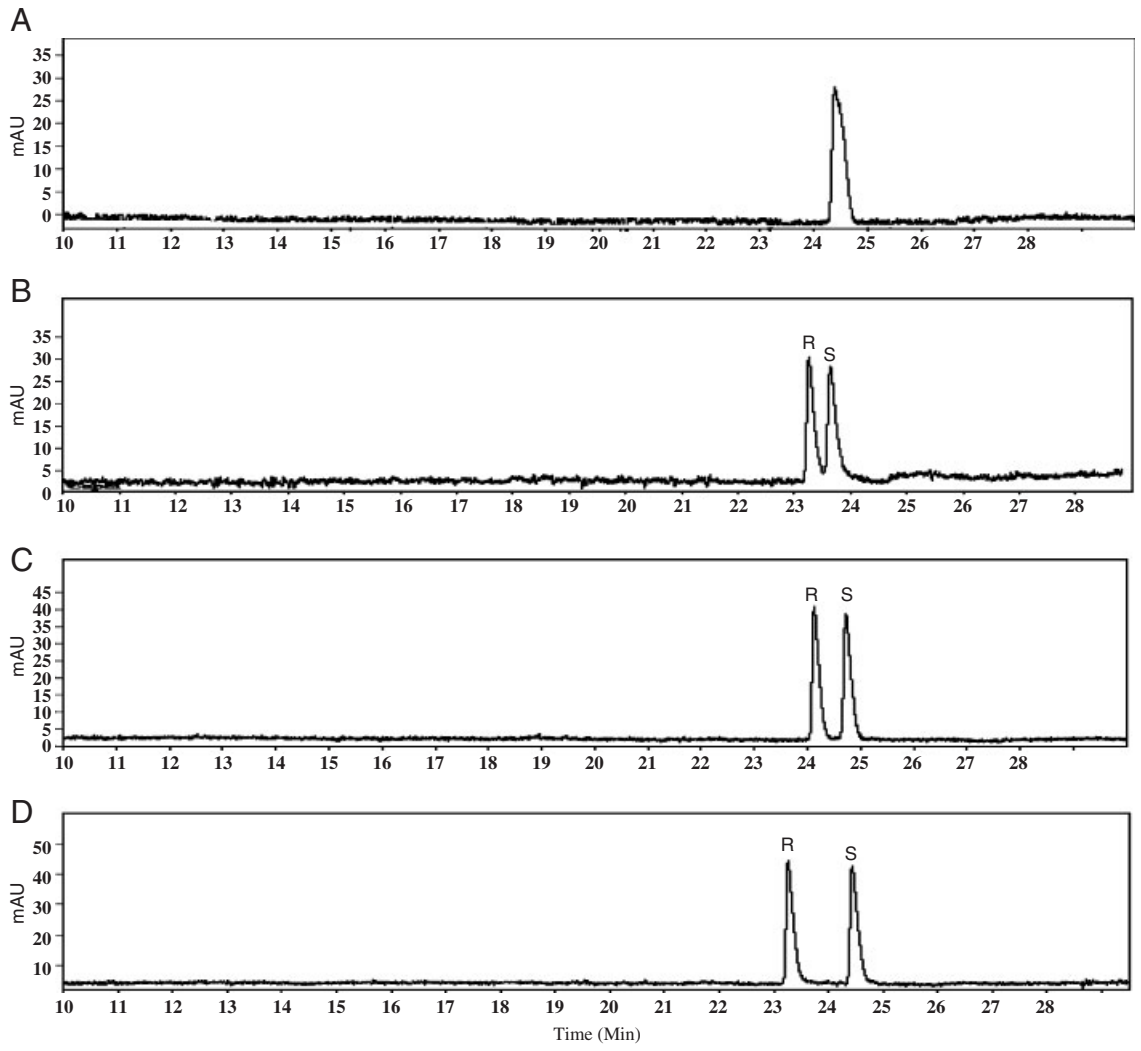

Figure 2. Influence of the chiral selector type on the chiral separation of AM. (A) β-CD (10 mM), (B) HP-β-CD (10 mM), (C) α-CD (5 mM) and (D) HP-α-CD (5 mM). Experimental condition: capillary column: 57 cm (49 cm effective length) × 50 μm id; detection: 237 nm; applied voltage: 20 kV; temperature: 20°C; injection: 6 s (at a pressure of 30 mbar); separation solution: 100 mM phosphate buffer, pH 3.0 containing specified amount of chiral selector.

in even less separation of the enantiomers. Good resolution was obtained with 5 mM α-CD, as can be observed for a concentration of 5 mM (see Fig. 2C). This CD has a smaller cavity, as it contains only six glucose units in its structure, apparently providing better interaction of the enantiomers of AM. Optimal results were obtained using an α-CD substituted with HP groups. HP-α-CD, at 5 mM in the run buffer, produced very good separation of the enantiomers, as can be observed for a concentration of 5 mM (see Fig. 2D). It has been postulated that the substitution of the secondary hydroxyl groups on the CD rim with HP groups provides a less restricted hydroxyl group and at appropriate length for hydrophilic interactions with the hydroxyl group close to the chiral center for propranolol [40]. The authors observed improved resolution for propranolol enantiomers when changing the chiral selector from unsubstituted β-CD to HP-β CD. The same explanation could be valid for AM where the same effect of the type of CD on resolution was obtained and in which the positions of the interacting groups are the same.

3.1.2 Effect of CD concentration on resolution

The optimum concentration of HP-á-CD for the separation of AM enantiomers was determined. As the HP-α-CD concentration increased from 1 to 20 mM, an increase in the separation of AM enantiomers was observed. Maximum separation of the enantiomer peaks was obtained at 5 mM HP-α-CD in the run buffer. However, at CD concentrations higher than the optimum, enantioselective interactions appear to be overwhelmed by non-specific hydrophobic interactions and enantiomer resolution is precluded [41]. The migration time increased with an increase in CD

concentration. This is due to longer residence time of the drug in the complex form as well as an increase in the viscosity of the buffer with a reduction in the mobility of the analytes. This has also been observed by other investigators for propranolol [42, 43] and clenbuterol [44]. Table 1 represents the resolution factors (R_s) calculated for each electropherogram. The sufficient and suitable resolution was obtained using 5 mM HP-α-CD.

3.1.3 Effect of voltage on efficiency

In CE, voltage plays a major role in determining analysis times. It can also affect the efficiency of analysis since efficiency is directly proportional to voltage [45]. The separation efficiency (N) of the CE system was estimated using the following equation:

$$N = 5.54\ (t/w_{0.5})^2 \quad (1)$$

where t is the migration time of the maximum of the peak and $w_{0.5}$ is the width of the peak at 50% peak height.

In order to determine the optimum separation voltage for the analysis of AM enantiomer by CE, several runs were performed with gradual increases in the applied voltage. Table 2 presents the number of theoretical plates (N), migration time and resolution *versus* voltage applied for the AM enantiomer peaks. There was an increase in efficiency following an increase in applied voltage from 12 to 22 kV, with a maximum of 117 263 theoretical plates obtained at 20 kV. Further increases in voltage resulted in decreased efficiencies. The explanation for this is that the capillary becomes less effective in heat dissipation after a certain voltage level, where excessive Joule heat is generated [46]. As heat is produced inside the capillary, the viscosity of the buffer decreases, and sample diffusion resulting in peak broadening becomes significant. An applied voltage of 20 kV was thus selected for further analyses.

Table 1. Effect of HP-α-CD concentration on the migration time (min) and resolution of amlodipine enantiomers

Concentration (mM)	$t_{R-(+)}$	$t_{S-(-)}$	Resolution
1	20.60	21.07	2.28
5	22.84	23.94	4.57
10	25.58	26.95	5.39
20	31.60	32.95	4.05

Table 2. Effect of voltage on the migration time (min) and resolution of amlodipine enantiomers

Voltage	$t_{S-(-)}$	$t_{R-(+)}$	Resolution	Number of theoretical plates
12	43.99	46.37	4.38	64 742
15	33.09	34.81	4.64	83 259
18	25.65	26.88	4.56	94 135
20	21.81	22.82	4.35	117 263
22	19.31	20.18	4.35	98 956

3.1.4 Effect of buffer concentration on efficiency

A series of phosphate buffer concentrations (25, 50, 100, 120 mM, pH 3.0) that was prepared by mixing appropriate amounts of H_3PO_4 and NaH_2PO_4 solution were evaluated for the effect on efficiency of enantiomer resolution and migration times. Buffer concentrations from 25 to 100 mM resulted in similar resolution for AM enantiomers with a gradual decrease in migration times. Efficiency and peak areas increased as the buffer ionic strength increased. This could be explained by a reduction in drug-wall interactions, which can cause peak broadening and lower mass recovery. However, the use of buffer concentrations higher than 100 mM greatly decreased enantiomer resolution probably due to high current generated inside the 50 μm id capillary. Therefore, the use of high buffer concentrations would require using a capillary with smaller internal diameter. Based on these observations an optimum buffer concentration of 100 mM was selected for further analyses.

3.1.5 Effect of cartridge temperature on resolution

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. Changing of temperature can change the viscosity of the buffer and then mobility of analytes that can affect the migration times and consequently the resolution of the analytes [44]. To control or minimize the effects of Joule heating, temperature can be controlled with fan-blown air or by recirculating water, with the capillary mounted in a cartridge. The Lumex Capel 105 equipment used for this study uses a circulating coolant containing water to maintain the constant temperature inside the capillary cartridge. The resolution of AM enantiomers decreased slightly with an increase in temperature from 15 to 30°C. This data are summarized in Table 3. Temperature may also influence the kinetics of the inclusion complex with the CD. For example, the stability constant of the CD inclusion complex would decrease by increasing the temperature, thus resulting in a decrease in resolution [47]. Migration time decreased as the cartridge temperature increased (15–30°C). However, at temperatures lower than 20°C, it was noticed that the CE instrument was not as efficient in controlling

Table 3. Effect of temperature on the migration time (min) and resolution of amlodipine enantiomers

Temperature (°C)	$t_{S-(-)}$	$t_{R-(+)}$	Resolution
15	25.55	26.88	4.34
20	21.81	22.82	4.35
25	19.64	20.42	3.90
30	17.33	17.93	3.45

the temperature and that equilibration time was rather long. A convenient operational temperature of 20°C was thus selected for the analyses.

3.2 Validation of the method

The method was validated with respect to several parameters including linearity, LOD, limit of quantification, precision, accuracy, selectivity, ruggedness and robustness and recovery [48–50]. The order of migration of enantiomers of AM was such that R-(+)-enantiomer eluted before S-(−)-enantiomer [51, 52].

3.2.1 Linearity

The equation of the calibration plots was established by linear regression of peak area (γ) against enantiomer concentration (x, μg/mL) for both R-(+) and S-(−)-enantiomer standard solutions. The plots were linear in the range 5–150 μg/mL for both enantiomers ($n = 7$). Typically, the regression equations were $Y = 310.5X + 3.84$ ($R^2 = 0.9994$) for R-(+)-AM and $Y = 325.2X + 7.32$ ($R^2 = 0.9985$) for S-(−)-AM.

3.2.2 LOD and limit of quantification

The LOD and limit of quantification were determined as analyte concentrations giving rise to signal-to-noise ratios of 3 and 10, respectively. The limit of quantification for both enantiomers was found to be 5 μg/mL. The LOD for both enantiomers was found to be 0.5 μg/mL.

3.2.3 Precision

The inter- and intra-day precision data are summarized in Table 4 and were assessed by using standard solutions prepared to produce solutions of three different concentrations for both enantiomers. Repeatability or intra-day precision was investigated by injecting six replicate of each concentration. Inter-day precision was assessed by injecting the same four samples over three consecutive days. Intermediate precision was studied by performing six injections of freshly prepared mixtures, 24 and 48 h later than the first series under the same experimental and instrumental conditions, but by different analysts. In this case, the obtained data ($n = 18$) for S and R enantiomers were, respectively, 1.4 and 2.1% for their migration times, 1.6 and 2.0% for their corrected peaks areas, 3.7% for R_S between enantiomers and 1.1% for the ratio peak areas of enantiomers (R/S).

3.2.4 Accuracy

Accuracy data for the assay of each AM enantiomer are summarized in Table 5. Accuracy was determined by interpolation of replicate ($n = 6$) peak areas of three accuracy standards of different concentrations, from calibration curve that had been prepared as previously described. In each case, the percent relevant error and accuracy was calculated and found to be less than 5.0% for each of the enantiomers.

3.2.5 Specificity

The results of stress testing indicated a high degree of specificity of this method for both enantiomers. Typically electropherograms obtained following the assay of untreated tablet powder samples and stressed tablet samples are shown in Fig. 3. These electrophoregrams are shown such that the obtained eventual by-products in stress condition do not interfere with any AM enantiomers. Also stress condition reagents such as acid, base and hydrogen peroxide are ineffective on enantiomers resolution.

3.2.6 Recovery

A known amount of each standard powder was added to samples of tablet powders, which was then mixed, extracted and subsequently diluted to yield a starting concentration of 65 μg/mL for both enantiomers. These samples were prepared and analyzed as previously described. The observed concentrations of R-(+)-AM and S-(−)-AM were found to be 65.17 ± 0.94 μg/mL (mean ± SD) and 65.07 ± 0.89 μg/mL, respectively. The resultant %RSD for these studies was found to be 1.44% for R-(+)-AM and 1.36% for S-(−)-AM with a corresponding percentage recovery value of 100.26 and 100.11%, respectively.

Table 4. Intra- and inter-assay precision data ($n = 6$)

Enantiomer	Actual concentration (μg/mL)	Measured concentration (μg/mL), RSD (%)	
		Inter-day	Intra-day
R-(+)	10	10.0, 2.2	9.9, 1.7
	20	20.1, 2.5	20.1, 2.3
	30	30.2, 3.1	29.7, 2.7
S-(−)	10	10.1, 2.8	10.0, 1.8
	20	20.0, 2.9	20.2, 2.4
	30	30.1, 2.4	29.7, 3.2

Table 5. Accuracy data ($n = 6$)

Enantiomer	Actual concentration (μg/mL)	Interpolated concentration (mean ± SD)	RSD	RE
R-(+)	10	10.12 ± 0.22	2.17	1.2
	20	19.76 ± 0.47	2.37	1.2
	30	31.17 ± 0.74	2.37	3.9
R-(−)	10	10.18 ± 0.17	1.67	1.8
	20	20.87 ± 0.34	1.63	4.4
	30	30.96 ± 0.23	0.74	3.2

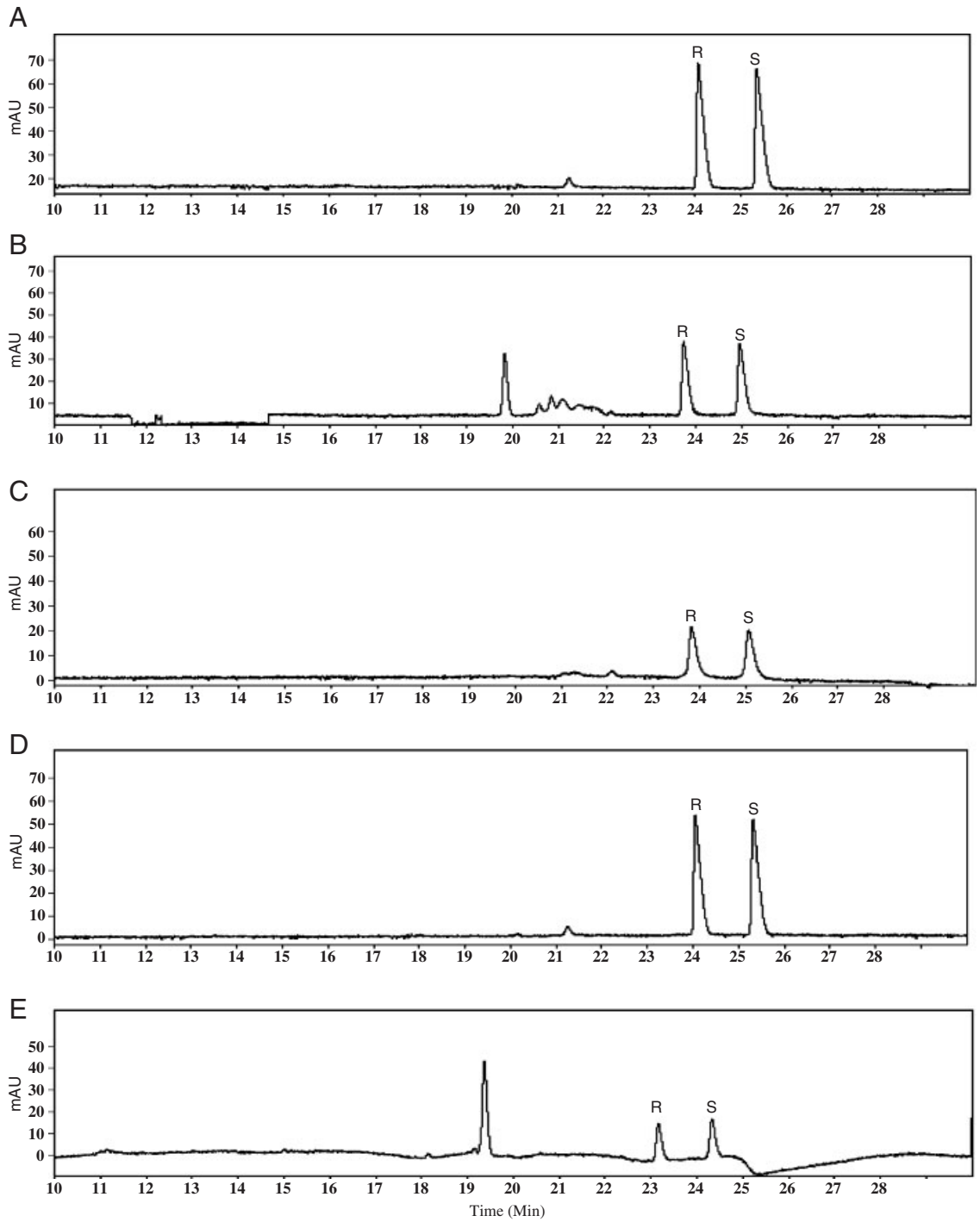

Figure 3. Typical CE electropherograms of: (A) untreated API; (B) oxidative degraded API; (C) base hydrolysis-degraded API; (D) acid hydrolysis-degraded API; (E) photodegraded API. Experimental condition: capillary column: 57 cm (49 cm effective length) × 50 μm id; detection: 237 nm; applied voltage: 20 kV; temperature: 20°C; injection: 6 s (at a pressure of 30 mbar); separation solution: 100 mM phosphate buffer, pH 3.0 containing 5 mM of HP-α-CD.

3.2.7 Robustness and ruggedness

The robustness of the method was investigated under a variety of conditions. Parameters investigated were the HP-α-CD concentration (4 and 6 mM), phosphate buffer concentration (90 and 110 mM) and temperature (23 and 27°C). The applied voltage was adjusted under each condition to minimize Joule heating. The effect that varying each parameter had on the resolution of the two isomers was studied. Concentrations of HP-α-CD less than 10%

resulted in a loss of resolution and concentrations greater than 10% did not improve resolution. A concentration of at least 10 mM phosphate buffer was necessary for sample buffering. Concentrations of phosphate buffer greater than 10 mM resulted in improved resolution, but also resulted in a loss of sensitivity. Use of a temperature less than 20°C resulted in improved resolution, but also resulted in an increased runtime. In summary, there is a fine interplay between the parameters in order to achieve the maximum resolution and sensitivity, while minimizing Joule heating and runtime. Therefore, only minor adjustment of each parameter (±5%) is recommended.

The ruggedness of the method assessed by comparison of the intra- and inter-day assays of AM enantiomers has been performed by two analysts. The %RSD values for intra- and inter-day assays of AM enantiomers in Arya® tablets performed in the same laboratory by two analysts did not exceed 2.8%, indicating the ruggedness of the method.

3.3 Stability studies

The degradation products of the parent compound were found to be similar for both the tablets and API powders assessed. All compounds of the 1,4-dihydropyridine class are susceptible to photolytic decomposition and AM is no exception and undergoes oxidation when exposed to light [53–55] resulting in the formation of a pyridine analogue, lacking any therapeutic effect [56]. AM was found to be photosensitive in both the solid state and in the solution. In spite of testing in the solid state, this drug was found to be less stable under photolytic stress conditions for both pure API and tablets when stored in methanol and water (20:80% v/v), resulting in 67.4% for R-(+) and 69.4% for S-(−)-AM decomposition of pure API, respectively, and 62% for R-(+) and 65% for S-(−)-AM decomposition of tablets, respectively. AM API and the pharmaceuticals tablets resulted sufficiently stable, with a decrease of AM enantiomer of 6.3% for R-(+) and 5.7% for S-(-)-AM of API, respectively, and 5% for R-(+) and 4% for S-(−)-AM of pharmaceutical tablets, respectively, after 48 h of light exposure in a photostability chamber. These drugs were found to be more stable under acidic and neutral degradation conditions rather than under alkali stress condition. The percent of decomposition for AM API was 18.2% for R-(+), 19.7% for S-(−)-AM under acidic condition and 2.3% for R-(+), 2.1% for S-(−)-AM under neutral condition and 85.3% for R-(+), 87.1% for S-(−)-AM under alkali condition. The degradation of molecules is ascribed to the acid or alkaline hydrolyses of the acetyl groups of AM [57, 58]. Also all compounds of the 1,4-dihydropyridine class are susceptible to oxidative decomposition and undergoes oxidation when exposed to hydrogen peroxide [53–55] resulting in the formation of a pyridine analogue same as exposed to light, lacking any therapeutic effect [56]. AM was more unstable under oxidative stress condition and the percent of decomposition for AM API was 74.8% for R-(+) and 77.5% for S-(−)-AM. The complete stability test data are summarized in Table 6.

The stability of stock solutions was determined by quantitation of each enantiomer in solution in comparison with the freshly prepared standard solution. No significant changes (<1%) were observed for the responses for the stock solutions analyzed, relative to freshly prepared standards. The stock solution stability data are summarized in Table 7.

3.4 Assay

The optimized and validated method was applied to the determination of AM enantiomers in commercially tablets. Figure 4 shows two typical CE electropherogram obtained following the assay of Arya tablets (A) and from a standard solution (B). The results of the assay ($n = 6$) undertaken yielded 98.2% (% RSD = 2.1) and 99.1% (% RSD = 2.3) of label claim for R-(+) and S-(−)-AM, respectively. The observed concentrations of R-(+) and S-(−)-AM were found to be 2.45 ± 0.18 mg/tablet (mean ± SD) and 2.48 ± 0.16 mg/tablet, respectively. The migration times of R-(+) and S-(−)-

Table 6. Results of forced degradation study samples using proposed method, Indicating percentage degradation of amlodipine enantiomers

Stability condition/duration/state	(%) Degradation	
	R-(+)-amlodipine	S-(−)-amlodipine
Thermal/100°C/48 h/solid	1.7	1.9
Photo/UV/24 h/solid	6.3	5.7
Acidic/0.1 M HCl/48 h/solution	18.2	19.7
Alkaline/0.1 M NaOH/2 h/solution	85.3	87.1
Oxidative/2.5% H_2O_2/48 h/solution	74.8	77.5
Photo/UV/24 h/solution	67.4	69.4
Thermal/neutral/90°C/6 h/solution	2.3	2.1

Table 7. Stability of stock solutions stored at 4°C for three weeks ($n = 6$)

Enantiomer	Actual concentration (μg mL^{-1})	Interpolated concentration (μg mL^{-1}), RSD (%)	
		Standard solution	Stock solution
R-(+)	10	10.03, 2.14	9.91, 1.58
	20	20.11, 1.76	20.24, 1.67
	30	29.78, 2.48	30.04, 2.47
S-(−)	10	10.15, 3.18	10.07, 3.46
	20	19.94, 1.42	20.17, 2.11
	30	30.14, 1.89	29.76, 2.64

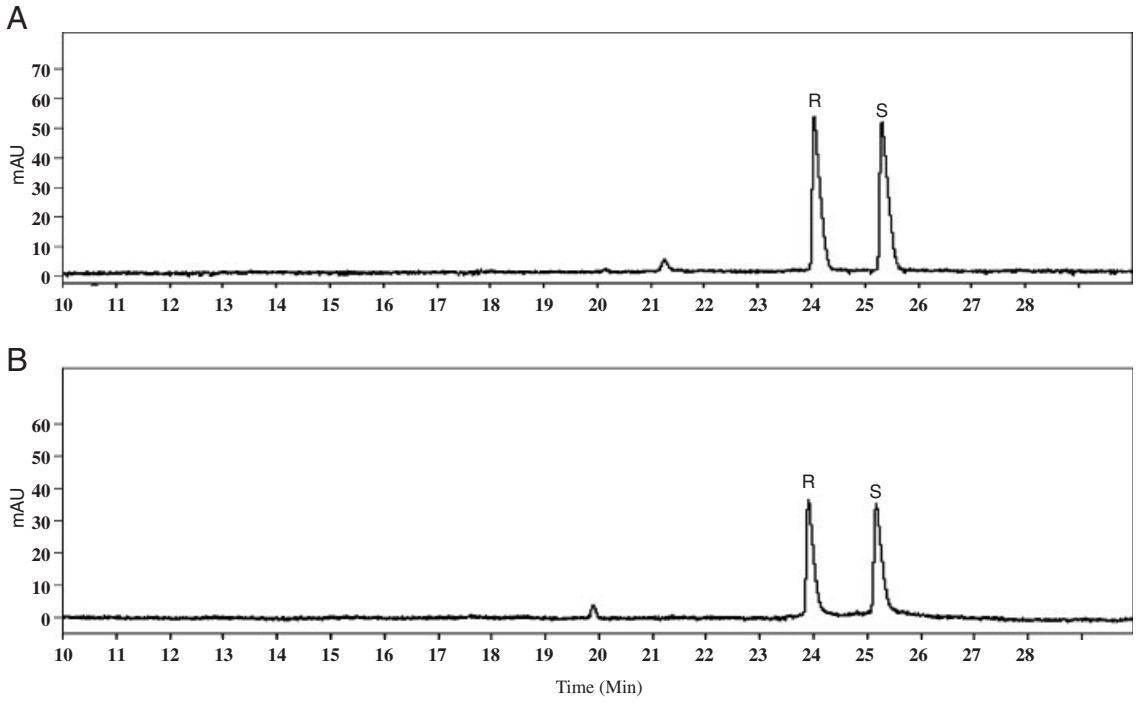

Figure 4. Resultant CE electropherograms obtained following the analysis of a standard solution containing racemic AM (A) and Arya® tablets (B). Experimental condition: capillary column: 57 cm (49 cm effective length) × 50 μm id; detection: 237 nm; applied voltage: 20 kV; temperature: 20°C; injection: 6 s (at a pressure of 30 mbar); separation solution: 100 mM phosphate buffer, pH 3.0 containing 5 mM of HP-α-CD.

AM were 24.09 and 25.34 min, respectively. The small peaks at 21.17 and 19.86 min in Fig. 4A and B, respectively, are due to impurity that exists in AM working standard and tablets. The results of the assay indicated that the method is selective for the analysis of both enantiomers without interference from the excipients used to formulate and produce these tablets.

4 Conclusion

In conclusion, an efficient stereoselective CE method was developed and validated for the separation and stability indicating of AM enantiomers in API and dosage forms. The results of stress testing undertaken according to the International Conference on Harmonization guidelines reveal that the method is selective and stability indicating. The method uses a simple sodium phosphate buffer and HP-α-CD as the chiral selector added to the run buffer. The assay produced good separation of the enantiomers with sharp peaks in a relative short analysis time and has the ability to separate the drug from degradation products and excipients found in the tablets dosage forms. Method development was fast and inexpensive since only minor quantities of the chiral selectors (5 mM) were required for the preliminary tests. This study showed that CE can be a very valuable alternative to HPLC using chiral stationary phases or chiral derivatizing reagents.

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