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Optimization and validation of a micellar electrokinetic chromatographic method for the analysis of several angiotensin-II-receptor antagonists

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Abstract

We have optimized a micellar electrokinetic capillary chromatographic method for the separation of six angiotensin-II-receptor antagonists (ARA-IIs): candesartan, eprosartan mesylate, irbesartan, losartan potassium, telmisartan, and valsartan. A face-centred central composite design was applied to study the effect of the pH, the molarity of the running buffer, and the concentration of the micelle-forming agent on the separation properties. A combination of the studied parameters permitted the separation of the six ARA-IIs, which was best carried out using a 55-m*M* sodium phosphate buffer solution (pH 6.5) containing 15 m*M* of sodium dodecyl sulfate. The same system can also be applied for the quantitative determination of these compounds, but only for the more stable ARA-IIs (candesartan, eprosartan mesylate, losartan potassium, and valsartan). Some system parameters (linearity, precision, and accuracy) were validated.
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Keywords: Experimental design; Optimization; Validation; Pharmaceutical analysis; Angiotensin receptor antagonists

1. Introduction

Angiotensin-II-receptor antagonists (ARA-IIs) are safe and effective agents for the treatment of hypertension and heart failure, either alone, or in conjunction with diuretics. They have been proposed as an alternative to the more traditional angiotensin-converting enzyme (ACE) inhibitors, because they selectively block the angiotensin type 1 (AT_1) receptor, which is responsible for vasoconstriction, and for salt and water retention. The angiotensin type 2 (AT_2) receptor, which is thought to have cardio-protective and inhibitory effects on growth, is left unaffected by ARA-IIs [1–4]. There are six ARA-IIs available on the market: candesartan (C), eprosartan mesylate (E), irbesartan (I), losartan potassium, (L) telmisartan (T), and valsartan (V). Candesartan, irbesartan, losartan potassium, and valsartan contain a biphenyltetrazole moiety, whereas telmisartan contains a structurally related biphenylcarboxylic acid moiety. The structure of eprosartan differs from that of the other ARA-II compounds. Candesartan is orally administered as the pro-drug candesartan cilexetil, and is completely converted to the active

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compound candesartan during absorption from the gastrointestinal tract. Losartan potassium is also converted into a more active drug during metabolism in the liver. However, losartan potassium is not a classic pro-drug, because it possesses significant ARA activity on its own. All the other ARA-IIs are active on their own, and do not require to be metabolized into active molecules [4–6].

Until now, high-performance liquid chromatography (HPLC) has been the major technique used to determine the concentration of different ARA-IIs, but studies have been limited to the determination of a single component [7–21]. One study has reported the determination of five ARA-IIs using HPLC [22]. Capillary electrophoresis (CE) offers an alternative technique. Although analysis by means of CE has been carried out for losartan potassium [23], the literature shows that no single selective method is able to separate and quantify ARA-IIs. In a previous investigation, we optimized a capillary zone electrophoretic method to separate and identify six ARA-IIs: candesartan, eprosartan mesylate, irbesartan, losartan potassium, telmisartan, and valsartan [24]. The same system was also applied to quantitatively determine the concentration of these compounds, but only for the more soluble ARA-II compounds (eprosartan mesylate, irbesartan, losartan potassium, and telmisartan) [24].

The introduction of micellar electrokinetic capillary chromatography (MEKC) has overcome the difficulty of separating neutral analytes using CE, and has increased the selectivity in the separation of charged molecules. Compounds having the same charges and similar structures often migrate at almost the same velocity in CE, whereas differences in their distribution constants in the micellar phase lead to baseline separations [25]. Many examples demonstrating an improved resolution using MEKC when compared to CE have been published [26].

The aim of the present study was to develop a selective MEKC method that was capable of separating and quantifying six ARA-II compounds. Statistical experimental design was used to optimize the method [27,28]. After preliminary investigations to adjust the experimental domain under study, a face-centred central composite design was applied to study the impact of three parameters on the retention of these compounds [29,30]. The parameters studied were: pH, the molarity of the running buffer, and the concentration of the micelle-forming agent. The usefulness of the system for the quantitative determination of these compounds in pharmaceutical formulations was then investigated, and the most important parameters for quantitative analysis were validated.

2. Experimental

2.1. Instrumentation and electrophoretic procedure

Experiments were performed on a Waters Quanta 4000 (Millipore, Milford, USA). A fused-silica capillary was used, 30 cm (22.5 cm to the detector)×75 μm I.D. Hydrostatic injections were performed by lifting the sample vial ~10 cm above the height of the buffer vial for 2 s. For detection, the absorbance was measured by means of an on-line fixed-wavelength UV detector with a zinc discharge lamp and a 214-nm filter. The experiments were performed at 10 kV at room temperature (20±2 °C). Data were collected on a Hewlett-Packard Integrator (HP 3396 Series II, Avondale, USA), which was also used for calculating the areas under the peaks. The pH measurements were performed on a calibrated Metrohm 744 pH Meter (Herisau, Switzerland).

2.2. Reagents

Sodium dihydrogenphosphate monohydrate and disodium hydrogenphosphate dihydrate (both analytical-reagent grade) were obtained from Merck (Darmstadt, Germany). Sodium dodecyl sulfate (SDS) was obtained from Sigma (St. Louis, MO, USA), and sodium hydroxide from UCB (Leuven, Belgium). Candesartan was obtained from AstraZeneca (Mölndal, Sweden), eprosartan mesylate from Solvay (Weesp, The Netherlands), irbesartan from Sanofi-Synthelabo (Gentilly Cedex, France), losartan potassium from Merck Sharp & Dohme (Rahway, NJ, USA), telmisartan from Boehringer Ingelheim (Ingelheim, Germany) and valsartan from Novartis (Basel, Switzerland).

The commercially available drugs Teveten (Solvay), Cozaar (MSD), and Diovane (Novartis) were used for quantitative determinations.

All solutions were prepared with distilled water obtained from deionized water.

2.3. Running buffers

During the development of the method, sodium phosphate buffers with different pH values and molarities were used. In the pH range 6.0–7.5, a mixture of a disodium hydrogenphosphate solution and a sodium dihydrogenphosphate solution was used. Running buffer solutions were prepared at different SDS concentrations (10–40 m*M*).

2.4. Internal standard solutions

For quantitative determination of the ARA-IIs, a different ARA-II compound was always used as the internal standard, with the selection made based on the substance to be examined. Although each ARA-II can be combined, candesartan was the most frequently chosen internal standard because of its high solubility. An appropriate mass of the compound (Table 1) was dissolved in 10 ml of 0.1 *M* NaOH, and diluted to 100 ml with water.

2.5. Choice of solvent

The running buffer cannot be used as a solvent for the preparation of the reference and sample solutions because of the poor solubility of the ARA-II compounds. Therefore, 0.1 *M* NaOH was added to dissolve the active substances, and the solutions were then diluted with water. In this medium however, irbesartan has stability problems after a period of 4 h. Therefore, irbesartan was not amenable to quantitative determination. Owing to the instability of irbesartan, its degradation product was also included in the experimental design to improve the selectivity of the separation.

2.6. Reference solutions for the experimental design

Reference solutions of the six compounds were prepared by dissolving ~3 mg of the corresponding reference substance in 1 ml of 0.1 *M* NaOH, and diluting the solution to a volume of 10 ml with water.

2.7. Reference solutions for quantitative determination

Reference solutions were prepared by accurately weighing an appropriate mass of the corresponding reference substance, dissolving it in 10 ml of 0.1 *M* NaOH, and diluting the solution to a volume of 100.0 ml with water. An appropriate volume of each solution was then mixed with 10.0 ml of the internal standard solution, and diluted to an appropriate concentration with 0.01 *M* NaOH (Table 2).

2.8. Sample preparations for the quantitative determination

A minimum of 20 tablets (or the contents of a minimum of 20 capsules) of each compound were

Table 1
Sample preparation for the quantitative determination

	Average mass (mg)	Sample solution (mg powder/100 ml)	Internal standard solution (mg/ml) (candesartan)	Diluted sample solution (mg active substance/ml)
Eprosartan mesylate (Teveten) 735.82 mg, tablets	987.0	~85	±0.22	~0.25
Losartan potassium (Cozaar) 50 mg tablets	152.9	~150	±0.28	~0.20
Valsartan (Diovane) 160 mg, capsules	267.2	~65	±0.22	~0.16

Table 2
Reference solutions for the quantitative determination

Reference substance	Reference solution (mg/100 ml)	Diluted reference solution (mg/ml)
Eprosartan mesylate	~63	~0.25
Losartan potassium	~50	~0.20
Valsartan	~40	~0.16

weighed, ground, and mixed. The requisite mass of powder was mixed with 10 ml of 0.1 *M* NaOH, and diluted to a volume of 100.0 ml with water. A suitable volume of the filtrate was mixed with 10.0 ml of the appropriate internal standard solution, and diluted to the required concentration with 0.01 *M* NaOH (Table 1).

All samples and buffers were filtered by passing them through 0.45-μm membrane filters (Millipore, Bedford, USA).

2.9. Experimental set-up and analysis of results

The set-up of the design and the statistical analysis of the response variables were carried out using STATGRAPHICS Plus v. 4.1 (STSC, Rockville, MD, USA) statistical graphics software package.

3. Results and discussion

Until now, the literature has shown no selective capillary electrophoretic method or micellar electrokinetic chromatographic method that is able to separate and quantify ARA-IIs. Therefore, MEKC was investigated as a separation method, with an experimental design applied to optimize the separation conditions.

3.1. Screening phase

Several parameters were considered for screening. From preliminary results, it was found that the factors that most affected the response migration time were the pH value, the molarity of the running buffer, and the concentration of the micelle-forming agent. The pH of the separation buffer plays an important role, because it affects the observable migration velocity of the solutes by changing the effective electrophoretic mobility of the solutes by affecting the degree of dissociation (or protonation), and by changing the velocity of the electroosmotic flow (EOF) by affecting the zeta potential at the capillary walls. Different concentrations of the running buffer were tested to optimize the separation. Since SDS is widely used in the MEKC technique, the usefulness of this additive was also evaluated. Selection of the experimental domain was made from prior experience and knowledge of the separation system. The voltage was also initially considered, but it was found to have less influence on the selectivity of the separation, and so was kept constant at 10 kV.

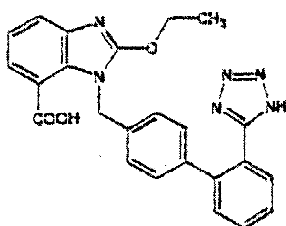
3.1.1. Selection of the pH

Owing to the amphoteric character of the ARA-II compounds (Fig. 1), their retention is greatly influenced by the pH value of the solution, which determines whether these compounds are negatively or positively charged. This offers the possibility of using either an acidic or an alkaline running buffer. Since candesartan and valsartan have low solubility in an acidic medium [24], this medium was abandoned for these two compounds and a medium having a higher pH value was used in the investigation. Between pH 6.0 and 7.5, telmisartan, eprosartan, valsartan, and candesartan could be baseline separated, while irbesartan and losartan potassium co-eluted. Under these conditions, separation took more than 20 min, and the compounds possessed poor peak shapes because of the long migration time. From pH 7.5 and up, the six ARA-IIs could be divided into two groups for separation: telmisartan, irbesartan, and losartan potassium in one group, and eprosartan, valsartan, and candesartan in the other group. In the first group, no baseline separation between the three ARA-IIs could be achieved, while in the second group, eprosartan and valsartan co-eluted, and telmisartan had poor peak symmetry.

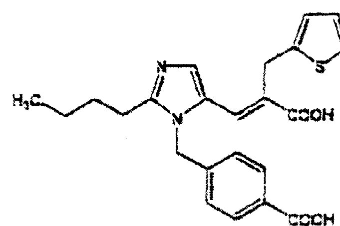
The best selectivity was obtained between pH 6.0 and 7.5, and the addition of SDS was therefore studied for solution pH values of 6.0, 6.75, and 7.5.

3.1.2. Concentration of the running buffer

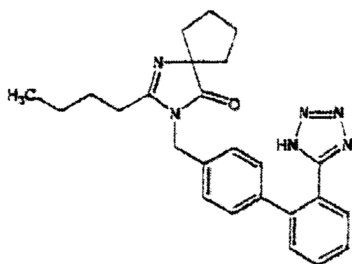
In earlier investigations, the molarity of the sodium phosphate buffers varied in the range 20–80 m*M*. When the concentration of the electrolyte increased, the selectivity of the separation improved,



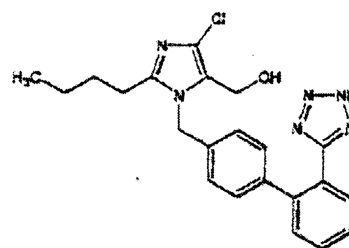
Candesartan (C)



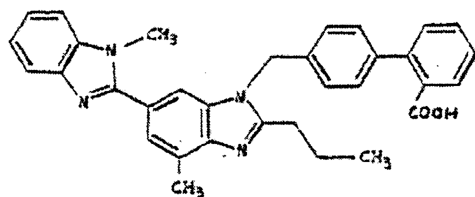
Eprosartan (E)



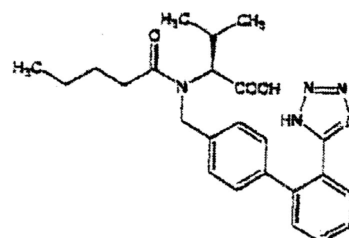
Irbesartan (I)



Losartan (L)



Telmisartan (T)



Valsartan (V)

Fig. 1. Chemical structures of the angiotensin-II-receptor antagonists (ARA-II).

and the migration times increased. At concentrations above 80 m*M*, high currents were generated. To obtain an optimum balance in ionic strength, the concentration of the running buffer was tested at three levels: 40, 60, and 80 m*M*.

3.1.3. Concentration of SDS

Since the simple capillary zone electrophoretic (CZE) method cannot separate the ARA-IIs studied, we investigated the MEKC method as well. SDS is one of the most popular surfactants. Its critical micelle concentration (CMC) is ~8 m*M* in pure water at ambient temperature [31]. When SDS is employed at concentrations above its CMC, then the SDS micelles (negatively charged) migrate towards the positive electrode under electrophoresis. The EOF is in the direction of the negative electrode, and it is stronger than the electrophoretic migration of the SDS micelle for pH higher than 5, and so under these conditions, the SDS micelles migrate towards the negative electrode [25]. An SDS concentration of at least 10 m*M* is necessary for separations to work in the MEKC method, and a SDS concentration above 40 m*M* leads to baseline fluctuations and loss of resolution. Therefore, we used SDS at three different concentrations (10, 25, and 40 m*M*) for optimization purposes.

3.2. Response surface design

To establish the influence of the three parameters and their interaction on the ease of separation, a face-centred central composite experimental design was applied. This design requires 14 runs. The experimental matrix included two extra experiments at the central level of the design to obtain an estimate of the experimental variance. Thus, the entire design required 16 runs. The parameter settings in the design are given in Table 3, while the design is reproduced in Table 4. The individual runs of the design were carried out in a randomized sequence. Randomization offers some assurance that uncontrolled variation of factors, other than those studied, did not influence the estimations. Replicate measurements ($n=3$) were performed to verify that retention times were stable, and that the capillary was well equilibrated after tuning to new electrophoretic conditions.

The measured responses were the relative migration times of candesartan ($t_{r,C}$), eprosartan ($t_{r,E}$), irbesartan ($t_{r,I}$) and its degradation product ($t_{r,Ia}$), losartan ($t_{r,L}$), telmisartan ($t_{r,T}$), and valsartan ($t_{r,V}$). The migration time of a benzyl alcohol marker was used as a reference. Table 5 shows a compilation of the measured relative migration times for each design run.

Table 4
Face centred central composite design

Run	pH	Molarity of the the running buffer (m*M*)	SDS (m*M*)
1	0	0	0
2	−1	−1	−1
3	+1	−1	+1
4	−1	+1	−1
5	+1	0	0
6	0	+1	0
7	+1	+1	+1
8	−1	+1	+1
9	−1	0	0
10	0	0	−1
11	+1	−1	−1
12	+1	+1	−1
13	0	0	+1
14	−1	−1	+1
15	0	0	0
16	0	−1	0

Table 3
Parameter settings in the design

Parameter	Low value (−1)	Medium value (0)	High value (+1)
pH	6.0	6.75	7.5
Molarity of the running buffer (m*M*)	40	60	80
SDS (m*M*)	10	25	40

Table 5
Measured response variables: relative migration times (t_r) in minutes ($n=2$ consecutive measurements)

Run	$t_{r,C}$	$t_{r,E}$	$t_{r,I}$	$t_{r,Ia}$	$t_{r,L}$	$t_{r,T}$	$t_{r,V}$
1	1.87	1.60	2.28	1.44	1.94	4.76	1.82
2	1.92	1.47	2.32	1.47	1.92	3.73	1.88
3	1.63	1.56	2.09	1.34	1.79	3.75	1.59
4	2.08	1.56	2.52	1.56	2.13	7.63	2.06
5	1.89	1.82	2.13	1.42	1.82	4.59	1.82
6	1.99	1.69	2.51	1.52	2.06	6.76	1.93
7	1.88	1.80	2.93	1.57	2.20	8.34	1.85
8	1.97	1.63	–[a]	2.02	3.39	9.75	1.91
9	1.88	1.53	3.07	1.69	2.49	4.69	1.86
10	2.04	1.72	1.79	1.39	1.64	4.03	1.96
11	2.03	1.92	1.66	1.35	1.55	3.38	1.95
12	2.28	2.18	1.88	1.45	1.69	4.62	2.18
13	1.70	1.50	2.45	1.47	2.04	4.44	1.66
14	1.60	1.46	2.63	1.67	2.27	3.09	1.58
15	1.89	1.63	2.24	1.45	1.89	4.82	1.83
16	1.71	1.48	1.97	1.35	1.74	3.36	1.66

[a] Bad measurement: value omitted.

3.2.1. Regression modelling

The following model was determined for each response:

$$y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

where y is the measured response (relative migration time) for each compound; b_0 is the intercept; b_i are the regression coefficients; and X_i are the values of the independent electrophoretic variables (X_1=pH, X_2=molarity of the running buffer, and X_3= concentration of SDS). The modelling was performed after scaling the X_1, X_2 and X_3 variables in the $[-1,+1]$ interval.

Initially, the significant and non-significant factors, and their interactions, were distinguished for each response, but afterwards, only the significant factors were included in the model. This significance is based upon the critical t-values for $\alpha=0.05$ in the respective standardized Pareto charts. Further, the coefficients of the significant factors and the significant interactions were calculated once more to determine a new model for each response.

To obtain a good separation of compounds, an adequate difference in (relative) migration time was needed. The minimal time difference, or the time difference of the two worst separated peaks ($\Delta t_{r,min}$), was especially important. Therefore, we were interested in the domain(s) where $\Delta t_{r,min}$ was at a maximum.

First, the measured migration times for each ARA-II compound were modelled. Then, the responses were predicted for all possible, different experimental conditions in the studied domain. Subsequently, the relative migration times of the compounds were sorted for each situation, and the difference in the relative migration times of the successive pairs of peaks ($t_{r,i}$) was calculated, and the appropriate $\Delta t_{r,min}$ value was selected. Finally, all the $\Delta t_{r,min}$ values were plotted as a function of the molarity of the buffer and the SDS concentration, and the region(s) where $\Delta t_{r,min}$ was maximal were investigated.

From preliminary results, it was found that a baseline separation of the ARA-IIs can be expected with a predicted value of $\Delta t_{r,min}=0.08$. To distinguish the regions with this value, contour plots of $\Delta t_{r,min}$ as a function of the molarity of the buffer and the SDS concentration were created in the pH range 6.0–7.5. Only four pH values seemed to meet this requirement: pH 6.5, 6.75, 7.0, and 7.25. At pH 6.0, 6.25, and 7.5, the $\Delta t_{r,min}$ value was lower than 0.08, and so inadequate separation could be expected. The robustness of the selected regions was also evaluated: those that were too narrow and those that were not robust enough were not retained as optimal separation conditions, because small differences in experimental conditions can lead to inadequate separations. By employing this additional condition, only three pH values remained: pH 6.5, 6.75, and 7.25.

Not only the value of $\Delta t_{r,min}$ is important, but the total analysis time also plays a role. The region with an optimum balance between $\Delta t_{r,min}$ and the analysis time must be determined to obtain a baseline separation within an acceptable analysis time for the different ARA-IIs. The maximal total analysis time was set at 20 min, corresponding to a maximum relative migration time of five. At the three pH values under consideration (see above), the relevant contour plot of the longest migration time (t_{max}) as a function of the molarity of the buffer and the SDS concentration was created to verify which regions with a $\Delta t_{r,min}=0.08$ also meet the t_{max} requirement. The contour plots were compared, and five conditions were identified (Table 6). These five statisti-

Table 6
The final five statistical predicted optimal conditions

pH	SDS (mM)	Molarity of the running buffer (mM)
6.5	15	52–66
6.75	10	60–72
6.75	18	64–67
6.75	30–32	54–60
6.75	32	48–56
7.25	10–12	56–75

cal predicted optimal conditions were confirmed experimentally. In three of the conditions, the telmisartan peak shape was particularly poor, while in the fourth, valsartan and candesartan were not baseline separated. Only one condition (55 mM sodium phosphate buffer (pH 6.5) containing 15 mM SDS) led to an adequate separation of the ARA-IIs. Moreover, the peak symmetry for all the ARA-IIs was acceptable (Fig. 2), and consequently, this condition was the one that was finally selected.

3.2.2. Quantitative determination in pharmaceutical formulations

The same 55 mM sodium phosphate buffer (pH 6.5) containing 15 mM SDS solution may be applied for the quantitative determination of candesartan, eprosartan mesylate, losartan potassium, and valsartan in tablets and capsules (Figs. 3–5). Using different placebo mixtures, it was demonstrated that the following excipients do not adversely affect the results: microcrystalline cellulose, lactose, pregelatinized starch, pregelatinized maize starch, magnesium stearate, hydroxypropylcellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, polyvidone, sodium lauryl sulfate, crospovidone, and titanium dioxide.

As already mentioned, stability problems occur for irbesartan after 4 h, and therefore, irbesartan was not amenable for quantitative determination. As the migration time of telmisartan is long, the optimum conditions investigated in this study are not appropriate to quantify this compound. Another optimization study using selective capillary zone electrophoretic separation of the same ARA-II compounds as this study has been reported [24], in which better conditions for the quantification of irbesartan and telmisartan were found when quantified using a 60-mM sodium phosphate buffer solution (pH 2.5).

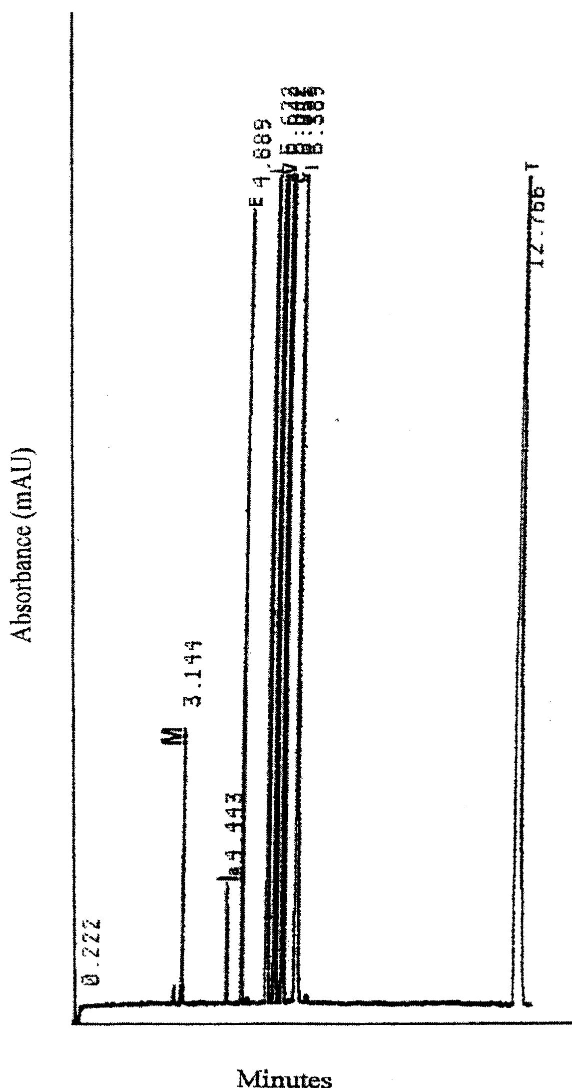


Fig. 2. Electropherogram of a mixture of six ARA-IIs using a fused-silica capillary 30 cm (22.5 cm to the detector)×75 μm I.D., and 55 mM sodium phosphate buffer (pH 6.5) containing 15 mM SDS as the running buffer. The applied voltage is 10 kV and detection is at 214 nm. M is the marker (formamide) and I_a is the degradation product of irbesartan.

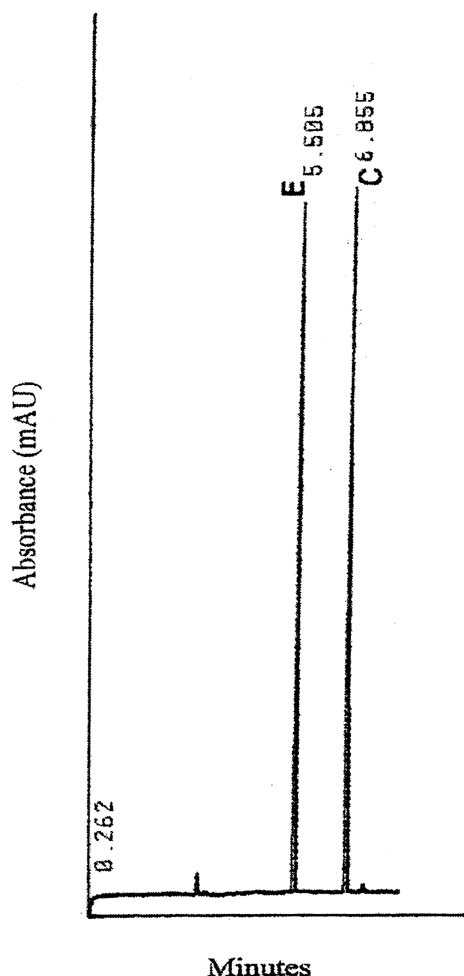


Fig. 3. Electropherogram of the quantitative determination of eprosartan mesylate (Teveten) on a fused-silica capillary 30 cm (22.5 cm to the detector)×75 μm I.D. Conditions: 55 m*M* sodium phosphate buffer (pH 6.5) containing 15 m*M* SDS as the running buffer; applied voltage, 10 kV; detection at 214 nm.

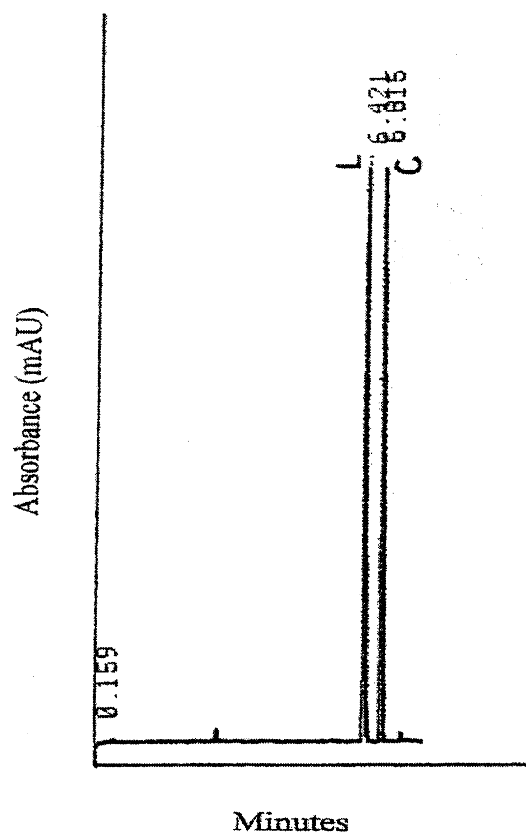


Fig. 4. Electropherogram of the quantitative determination of Losartan potassium (Cozaar) on a fused-silica capillary 30 cm (22.5 cm to the detector)×75 μm I.D. Conditions: 55 m*M* sodium phosphate buffer (pH 6.5) containing 15 m*M* SDS as the running buffer; applied voltage, 10 kV; detection at 214 nm.

3.2.3. Validation of the method

3.2.3.1. Linearity

The detector responses were found to be linear for the different components in the concentration range studied, as shown in Table 7. The amount of the internal standard was adjusted according to the concentration range used. Regression analysis data for the calibration curves were calculated using the peak areas.

3.2.3.2. Precision

The precision (repeatability) was determined by the analysis of ten replicate samples under the same operating conditions, carried out by the same analyst, and on the same day. The mean value of the concentration and the relative standard deviation are summarized in Table 8.

Error produced by the equipment, the accuracy of electrophoretic separation, and the relative standard

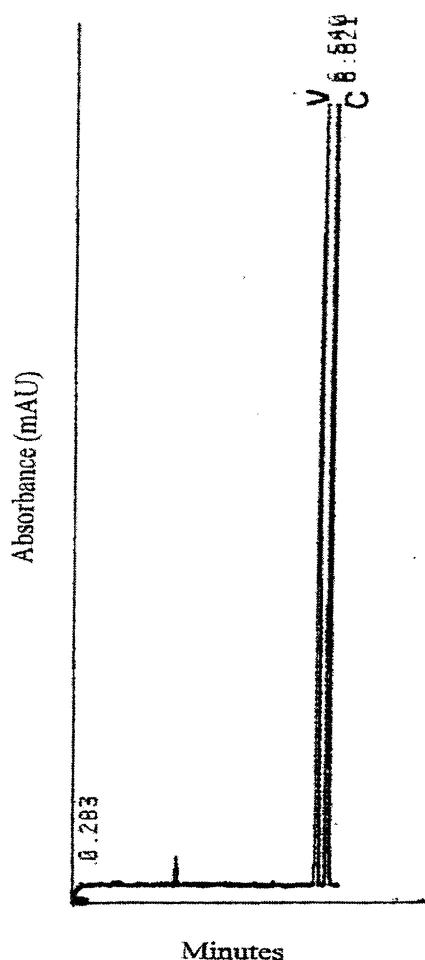


Fig. 5. Electropherogram of the quantitative determination of valsartan (Diovane) on a fused-silica capillary 30 cm (22.5 cm to the detector)×75 μm I.D. Conditions: 55 m*M* sodium phosphate buffer (pH 6.5) containing 15 m*M* SDS as the running buffer; applied voltage, 10 kV; detection at 214 nm.

deviations of estimations were determined by performing ten consecutive injections of the same sample (Table 9).

Table 7
Linearity

	Concentration range (mg/ml)	Correlation coefficient (r^2)
Eprosartan mesylate	0.07–0.35	0.9998
Losartan potassium	0.06–0.30	0.9998
Valsartan	0.04–0.22	0.9999

3.2.3.3. Accuracy

The accuracy of the method was determined by investigating the recovery of each component at three levels, ranging from 80 to 120% of the theoretical concentration, from placebo mixtures spiked with the active substance (Table 10).

The above results demonstrate that micellar electrokinetic capillary chromatographic separation of the six ARA-IIs and the degradation product of irbesartan can be achieved using a 55-m*M* sodium phosphate buffer solution (pH 6.5) containing 15 m*M* SDS. Even when applying the optimized conditions, however, it was impossible to quantify all six compounds because of the instability of irbesartan and the long migration time of telmisartan. This system can only be applied for the quantitative determination of candesartan, eprosartan mesylate, losartan potassium, and valsartan in pharmaceutical formulations.

To quantify the six ARA-IIs, a combination of the CZE and MEKC methods is therefore necessary (Table 11). The best conditions found in this study can be used initially as a method to identify the six ARA-IIs, and to quantify four of them. The two other compounds then have to be quantified by the other method. The optimization of a selective CZE separation of the same ARA-IIs [24] found better conditions for the quantification of irbesartan and telmisartan, as discussed above, using a 60-m*M* sodium phosphate buffer solution (pH 2.5). As all the ARA-IIs and the degradation product of irbesartan can be identified, the MEKC method is the preferred identifying and quantifying method; then the sequential application of both the CZE and MEKC methods allows for the quantification of all compounds under consideration.

4. Conclusions

Our results demonstrate that the micellar electrokinetic capillary chromatographic separation of six angiotensin-II-receptor antagonists (candesartan, eprosartan mesylate, irbesartan, losartan potassium, telmisartan, and valsartan) and the degradation product of irbesartan can be achieved using a 55-m*M* sodium phosphate buffer solution (pH 6.5) con-

Table 8
Precision (repeatability) of the total analysis of ten replicate samples

Substance to be examined	Theoretical amount (mg/tablet)	Amount found (mg)	Relative standard deviation (%) ($n=10$)
Eprosartan mesylate (Teveten)	735.82	742.29±9.30 or 100.9%	1.25
Losartan potassium (Cozaar)	50	49.15±0.71 or 98.30%	1.44
Valsartan (Diovane)	160	158.79±0.96 or 99.24%	0.60

Table 9
Repeatability of ten consecutive injections of the same sample

Sample solution	Concentration of the sample solution (mg/ml)	Relative standard deviation (%, $n=10$)
Eprosartan mesylate	~0.25	0.59
Losartan potassium	~0.20	1.16
Valsartan	~0.16	0.66

Table 10
Accuracy

	Recovery (%)		
	Placebo+80% ($n=3$)	Placebo+100% ($n=3$)	Placebo+120% ($n=3$)
Eprosartan mesylate	100.7±0.2	99.8±0.3	99.3±0.4
Losartan potassium	101.0±1.0	100.1±0.5	101.5±1.5
Valsartan	102.0±0.5	101.9±0.6	100.2±0.5

taining 15 m*M* SDS. This system can be successfully applied to the identification of these compounds. The same system can also be applied to the quantitative determination of the more stable ARA-IIs (candesartan, eprosartan mesylate, losartan potassium, and valsartan). The possibility of simultaneous quantification and identification of the active ingredient in the finished product is therefore very attractive from the analytical viewpoint.

Table 11
Overview of the two identifying methods and their usefulness to quantify the ARA-IIs

	CZE method[a]	MEKC method[b]
Candesartan	–	+
Eprosartan mesylate	+	+
Irbesartan	+	–
Losartan potassium	+	+
Telmisartan	+	–
Valsartan	–	+

[a] 60 m*M* sodium phosphate buffer (pH 2.5) [24].
[b] 55 m*M* sodium phosphate buffer (pH 6.5) containing 15 m*M* SDS.

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