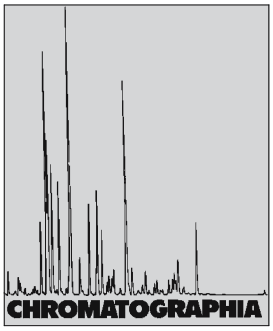
Development of a CZE Method for the Determination of Olmesartan Medoxomil in Tablets



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Abstract

A CZE method was developed and validated for the analysis of Olmesartan medoxomil (OLMD) in tablets. The influences of pH, buffer concentration, applied voltage and capillary temperature on the migration time of OLMD were investigated. About 50 mM pH 6.5 phosphate buffer were used as background electrolyte. The optimum instrument parameters were found to be 30 °C temperature with 30 kV applied voltage and diode array detection was carried out at 210 nm. OLMD was hydrodynamically injected (P_{inj} = 50 mbar, t_{inj} = 3 s) and an internal standard, diflunisal (IS), was used to improve the precision and repeatability. Under these conditions, the migration time of OLMD was 2.32 min and the total analysis time was shorter than 5 min. Linearity range for the developed method was found to be 2.0–50.0 μg mL^{-1} and the limit of detection was 0.5 μg mL^{-1}. The developed method was applied for the analysis of OLMD in pharmaceutical tablet formulations.

Keywords

Capillary zone electrophoresis
Method optimization and validation
Pharmaceutical preparations
Olmesartan medoxomil

Introduction

Olmesartan medoxomil (OLMD) is a prodrug. It is hydrolyzed to olmesartan during absorption from the gastrointestinal tract [1–4]. Olmesartan is a selective AT_1 subtype angiotensin II receptor antagonist. OLMD is described chemically as 2,3-dihydroxy-2-butenyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]imidazole-5-carboxylate, cyclic 2,3-carbonate [5, 6].

Capillary electrophoresis (CE) is a simple analysis technique, which has been applied to the separation of a wide variety of compound types including pharmaceuticals [7–9]. CE has many advantageous features, such as extremely high efficiency, high resolution, rapid analysis and small consumption of sample and reagents in comparison to HPLC [10, 11]. The determination of some angiotensin II receptor antagonists has been carried out frequently by CE [12, 13]. However, only one UV spectrophotometric method is reported for the analysis of OLMD in pharmaceutical dosage forms [14]. Therefore, it is a necessity to develop a new and simple method. In this study, a CZE method was developed and it was validated according to the literature [15–18].

Experimental

Apparatus

All experiments were performed using an Agilent 3D CE (Waldbronn, Germany) system equipped with a diode array detector (DAD), a auto sampler, a temperature controller and 30 kV high voltage power supply. A CE Chemstation software was used for instrument control, data acquisition and data analysis. A fused silica capillary of 48 cm total length (40 cm effective length) and 50 μm i.d. was used. All statistical analyses were performed with SPSS software (version 10.7). The pH of solutions was measured by a pH meter (Metler Model MA 235, Switzerland).

Chemicals and Reagents

OLMD was kindly supplied by Daiichi Sankyo (Tokyo, Japan). Pharmaceutical preparations of OLMD (Olmetec® Tablets) were obtained from Pfizer (Istanbul, Turkey). Diflunisal (IS) was purchased from Sigma (St Louis, USA).

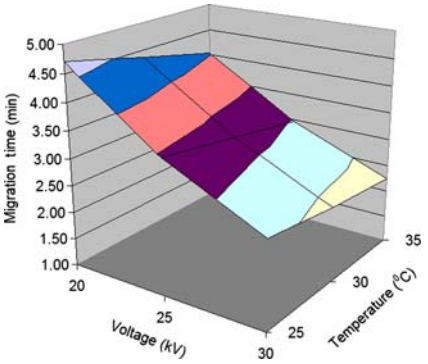

Fig. 1. The effect of applied voltage and temperature on migration time. Operating conditions: 50 mM pH 6.5 phosphate buffer, hydrodynamic injection (3 s at 50 mbar), λ = 210 nm (OLMD: 20.00 mg mL^{-1})

All other chemicals were of analytical reagent grade from Merck (Darmstadt, Germany). Milli-Q water system (Barnstead, USA) was used for the preparation of buffer and other aqueous solutions.

Standard Solutions

Standard solutions of OLMD (1,000 µg mL^{-1}) and diflunisal (IS) (1,000 µg mL^{-1}) were prepared in acetonitrile (ACN). 50.00 mg of OLMD were accurately weighed and transferred to a 50 mL volumetric flask and 30 mL of ACN were added. It was treated in ultrasonic bath for 15 min at 25 °C and then the volume completed with solvent. The same procedure was applied to prepare IS standard solution. These solutions were kept at +4 °C and prevented from daylight. After that, various aliquots of OLMD solutions were taken and a suitable amount of IS was added. These solutions containing identical amounts of IS (20 µg mL^{-1}) and a suitable amount of OLMD were diluted with 50 mM phosphate buffer (pH 6.5) to give the final desired OLMD concentration.

Running Buffers

About 50.0 mM phosphate buffer were prepared by dissolving 680 mg of potassium dihydrogenphosphate (KH_2PO_4) in about 80 mL water and adjusting the pH with 0.1 N sodium hydroxide to a pH of 6.5 and then making up the volume to 100 mL with water.

Sample Preparation

Ten tablets were weighed and finely powdered in a mortar. A quantity of the powder equivalent to one tablet was accurately weighed and transferred to a 50 mL volumetric flask. 30 mL of ACN was added. The flask was sonicated for 15 min and diluted to the mark with ACN. Then an aliquot was centrifuged at 5,000 rpm for 10 min. Clear supernatant was transferred to another flask.

Suitable amounts of tablet solution and IS standard solution were taken and diluted with 50.0 mM phosphate buffer (pH 6.5) to give the final concentrations (20 µg mL^{-1} OLMD and 20 µg mL^{-1} IS). All solutions were filtered through a 0.22 µm syringe filter and degassed with ultrasonic bath for 5 min before injection to the CE system.

Synthetic Tablet Preparation

For preparing the synthetic tablets, common inactive ingredients (microcrystalline cellulose, lactose monohydrate, talc, magnesium sterate, starch, titanium dioxide) and a standard OLMD (20 mg) equivalent amount to one tablet were weighed and transferred to a 50 mL volumetric flask. After that, the above-mentioned procedure was applied to prepare the synthetic tablet solutions including 20 µg mL^{-1} OLMD and 20 µg mL^{-1} IS.

Electrophoretic Procedure

Electrophoretic separations were carried out using a fused silica capillary having 50 µm i.d. and 48.5 cm total length (40.0 cm effective length), in a positive mode using constant voltage (30 kV). At the beginning of each working day, the capillary was rinsed with 0.1 M NaOH for 20 min. Between each injection, the capillary was rinsed with running buffer (4 min). Injection was performed hydrodynamically by the 50 mbar pressure for 3 s when the capillary temperature was 30 °C. OLMD and IS were detected using a diode array detector at 210 nm (bandwidth 10 nm).

Results and Discussion

Optimization of Electrolyte Parameters

Manipulation of buffer pH is a key strategy for optimizing the separation of ionizable analytes in CZE because of the fact that the buffer pH determines the extent of the ionization of each analyte and the magnitude of the electroosmotic flow (EOF). By the usage of different running buffers [phosphate (pH 5.5–8.5), acetate (pH 4.0; 5.0), citrate (pH 6.0; 6.5) and borate (pH 8.0–10.0)], the pH range from 4.0 to 10.0 was investigated in order to find the optimum conditions. Unfortunately, the ester structure of OLMD made it easy to hydrolyze in basic aqueous background electrolytes and it was hydrolyzed more and more rapidly by the increasing of pH. Therefore taking into consideration the different parameters (stability of OLMD, migration time, peak shape, height, baseline noise, etc.), 50 mM pH 6.5 phosphate buffer was selected as optimum electrolyte condition in order to preserve the OLMD's stability as long as possible and obtain short migration time.

Optimization of Instrument Parameters

The effect of applied voltage and capillary temperature on migration time and peak shape was studied. Therefore, three voltage values (20, 25 and 30 kV) and three temperature values (25, 30 and 35 °C) were evaluated and the effects of these parameters on experimental results were investigated simultaneously to find the shortest migration time with good efficiency and peak shape. The results for the migration time of OLMD are shown in Fig. 1 as a 3D diagram. Even though the migration time of OLMD and IS were shortened as expected by increasing the applied voltage and temperature, the best peak shape and baseline were observed for 30 kV applied voltage at a temperature of 30 °C. Therefore, these values were considered as optimum for the developed method. Injection time affects the peak width and peak height. Sample solutions were hydrodynamically injected at 50 mbar while the injection time was varied from 1.0 to 5.0 s. After 3 s, the peak shape of OLMD was deformed. Thus, 3 s of injection time with 50 mbar pressure was selected for the hydrodynamic injection. Since the OLMD has a sufficient absorption and the baseline was clear, 210 nm (bandwidth 10 nm) detection wavelength were selected in this study. Under these optimized conditions,

Table 1. Precision and accuracy of the developed method for the analysis of OLMD ($n = 6$)

Added (μg mL^{-1})	Intra-day			Inter-day		
	Found $\bar{x}$ (μg mL^{-1})	Precision RSD (%)	Accuracy Bias (%)	Found $\bar{x}$ (μg mL^{-1})	Precision RSD (%)	Accuracy Bias (%)
10	9.88 ± 0.08	2.0	−1.2	9.84 ± 0.09	2.2	−1.6
25	25.01 ± 0.12	1.2	0.0	25.23 ± 0.14	1.3	0.9
40	39.85 ± 0.17	1.0	−0.4	39.72 ± 0.20	1.3	−0.7

$\bar{x}$: mean ± SE, SE: standard error, RSD%: relative standard deviation, Bias %: [(Found − Added)/Added) × 100

the migration times of OLMD and IS were 2.32 and 3.41 min, respectively.

Method Validation

The use of an internal standard is recommended for quantitative analysis in order to improve injection precision [19, 20]. Diflunisal was found to be the best IS for the optimum conditions. To demonstrate the suitability of this proposed method for pharmaceutical analysis, it was validated using the following validation parameters: stability, linearity, sensitivity, repeatability, precision, accuracy, recovery, specificity and selectivity, robustness and ruggedness according to the literature [15–18].

Stability

The standard stock solution of OLMD was stored at +4 °C for 1 month and prevented from daylight. During this period, it was analyzed periodically and no unexpected peak appeared which might indicate the degradation of OLMD. Moreover, the obtained results between the freshly prepared solutions and those kept for one month were identical. A statistical comparison was done by the Wilcoxon Test and there was no statistically significant difference between these results ($n = 5$, $P = 0.705 > P = 0.050$). Therefore it could be concluded that the standard stock solution of OLMD was highly stable. However, OLMD could not preserve its stability in the using background electrolyte for more than 4 h. For this reason, the standard and the sample solutions of OLMD should be freshly solved in the background electrolyte before injection.

Linearity Range

In quantitative analysis, the calibration curves for OLMD were constructed under optimum conditions. The Peak Normalization (PN) ratio of OLMD to IS were plotted versus the nominal concentrations of the calibration standards and found to be linear over the range of 2.0–50.0 μg mL^{-1}. The regression equation of calibration curve for OLMD is $y = 0.0286x + 0.0218$; $r = 0.9998$ ($y = ax + b$ where x is the concentration of OLMD (μg mL^{-1}); y is the PN Ratio of OLMD to IS). The standard error of slope and intercept at regression equation are 0.0007 and 0.0035, respectively ($n = 6$).

Sensitivity [Limit of Detection (LOD), Limit of Quantitation (LOQ)]

LOD and LOQ were determined by the signal-to-noise ratio defined 3:1 for LOD and 10:1 for LOQ [17, 18]. For the developed method, LOD and LOQ values are 0.5 and 2.0 μg mL^{-1}, respectively.

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time [15]. In order to measure the repeatability of the method (while keeping the operating conditions identical), ten consecutive injections were performed with different standard solutions containing OLMD (20 μg mL^{-1}) and IS. The amount of OLMD was calculated by calibration equation. For the repeatability experiments, the mean of the results were 19.95 ± 0.05 ($\bar{x}$ ± SE where $\bar{x}$ is mean, SE: Standard error) and the relative standard deviation (RSD) of the results was 0.9%. According to the results, it can be said that the system is highly repeatable.

Precision

The precision of the analysis was determined by calculating the RSD [15]. Three different concentrations of standard OLMD solutions (in the linear range) were analyzed six consecutive days (inter-day precision) and six times in the same day (intra-day precision). The RSD values of intra- and inter-day studies varying from 1.0 to 2.2 percent show that the intermediate precision of the method is satisfactory [17, 18] (Table 1).

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found [15]. Bias values obtained from intra- and inter-day analysis were given in Table 1. The bias values of obtained results varying from −1.6 to 0.9 percent show that the developed method is highly accurate.

Recovery

In order to evaluate the effect of the presence of the excipients on the proposed method, six synthetic tablet solutions were prepared as mentioned in the experimental section and analyzed by the developed method. The mean of the recovery results was 99.6 ± 0.6 percent for the synthetic tablet solutions of OLMD. Another recovery study was performed by the standard addition technique. For this reason, four different solutions were prepared in 50.0 mM phosphate buffer (pH 6.5). These solutions contain the same amounts of OLMD tablet solution (20 μg mL^{-1}) and IS (20 μg mL^{-1}) but different amounts of OLMD standard solution (0, 5, 10, 15 μg mL^{-1}). The recoveries were calculated from percentage differences between the added (5.00; 10.00; 15.00 μg mL^{-1}) and found (5.06; 10.10; 14.89 μg mL^{-1}) concentrations. The obtained recovery results [101.1 ± 1.4; 101.2 ± 1.3; 99.1 ± 1.0 ($\bar{x}$ ± SE where $\bar{x}$ is mean, SE: standard error)] within the acceptable values show that there is not any interaction of

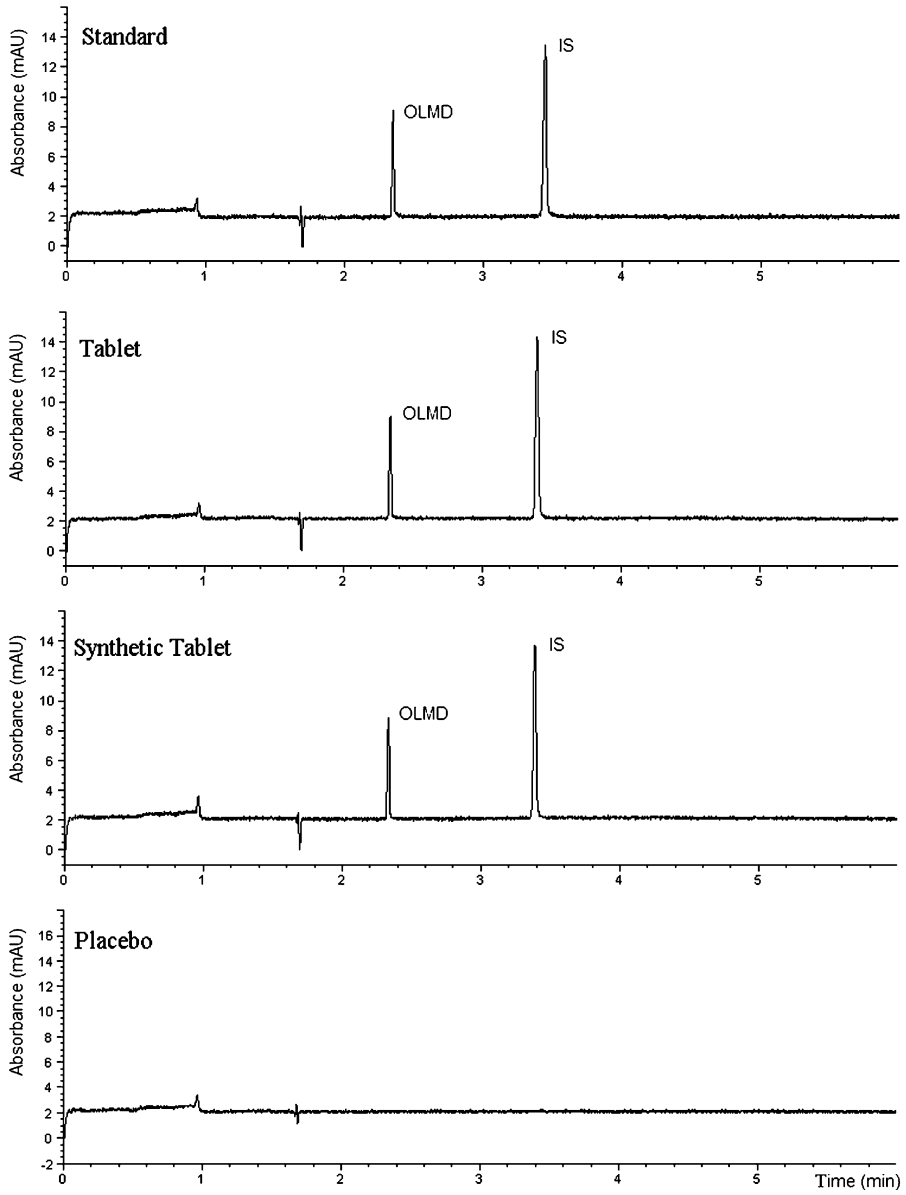

Fig. 2. The electropherograms of standard, tablet and synthetic tablet solutions containing 20.00 μg mL^{-1} OLMD and the electropherogram of excipients (placebo) solution. Operating conditions: 50 mM pH 6.5 phosphate buffer, hydrodynamic injection (3 s at 50 mbar), 30 kV, 30 °C, λ = 210 nm

exciptients in the analysis of OLMD in tablet dosage forms.

Specificity and Selectivity

The electropherograms, obtained from standard solution (Fig. 2) were identical to that obtained from tablet solution and synthetic tablet solution containing an equivalent concentration of OLMD (20 μg mL^{-1}). Moreover, there is not any interference from matrix components at the placebo electropherogram. These results show that the ability of the method to asses unequivocally the analyte in the presence of matrix components.

In addition to this, the standard addition technique was applied to the same preparations, which were analyzed by calibration curve technique. The regression equation of standard addition curve for OLMD was $y = 0.0291x + 0.6057$; $r = 0.9992$ ($y = ax + b$ where x is the concentration of OLMD (μg mL^{-1}); y is the PN ratio of OLMD to IS and $n = 6$). The slopes of calibration curve and standard addition techniques were identical. It was concluded that there were no interactions in the analysis of OLMD in pharmaceutical preparations by the developed method. Therefore, the tablet analyses were performed by calibration curve technique.

Robustness

In robustness testing of an analytical method, is aimed to explore the sensitivity of the responses to small changes in experimental conditions [15]. Ideally, a robustness test should show that the responses are not sensitive to small fluctuations in the experimental parameters. Four parameters (buffer pH, buffer concentration, temperature and wavelength) were varied around the optimum conditions in the method to reflect changes likely to arise in different test environments. Obtained results were close to those obtained under the optimum conditions. In addition to this, when a statistical comparison was done by the Friedman Analysis, there were no differences between the results ($n = 3$, $P = 0.097 > P = 0.050$). Therefore, it could be said that the developed method is robust under small changes in experimental conditions.

Ruggedness

The effect of two different analysts on the results for 20.00 μg mL^{-1} standard samples was evaluated. The mean values of the results obtained by two different analyst under optimum conditions were 20.22 ± 0.19 and 19.94 ± 0.11, respectively ($n = 5$). When a statistical comparison was done by the Wilcoxon Test, there was no statistically significant difference between these results ($P = 0.345 > P = 0.050$). Thus, the proposed method is considered rugged.

Analysis of Pharmaceutical Preparations

The developed method was successfully applied to the determination of OLMD in pharmaceutical tablet preparations [Olmetec® Tablet (10, 20 and 40 mg)].

The tablet solutions of OLMD were analyzed three times for three dosage forms by the calibration curve technique. The results are given in Table 2.

The obtained results were compared with those obtained by the reported UV

Table 2. Tablet analysis results (OLMD 20 μg mL^{-1}) ($n = 7$)

Olmetec® 10 mg	Olmetec® 20 mg	Olmetec® 40 mg
9.96	19.98	40.10
10.17	20.54	40.36
9.88	20.20	40.82
10.87	20.32	40.22
10.27	20.52	39.88
9.84	20.42	39.36
10.05	20.06	40.36
$\bar{x} = 10.01 \pm 0.06$	$\bar{x} = 20.29 \pm 0.08$	$\bar{x} = 40.16 \pm 0.17$
SD = 0.16	SD = 0.22	SD = 0.45
RSD = 1.6%	RSD = 1.1%	RSD = 1.1%

$\bar{x}$: mean ± SE, SE: standard error, SD: standard deviation, RSD%: relative standard deviation

spectrophotometric method [14] and when a Wilcoxon Test was done, no differences between the results ($n = 7$, $P = 0.237 > P = 0.050$) were found.

Conclusion

In this study, a new CZE method was developed and validated. The developed method, with its simplicity and ease of operation, is suitable for routine analysis of OLMD in pharmaceutical dosage forms (Olmetec® Tablet). Moreover, it is rapid, efficient and of low cost as a result of the advantages of CE in comparison to LC. Therefore, this method could be easily used in quality control laboratories.

Acknowledgments

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