Hacettepe University Journal of the Faculty of Pharmacy
Volume 27 / Number 2 / July 2007 / pp. 119-130

Development of a Capillary Zone Electrophoresis Method for the Indirect Determination of Olmesartan Medoxomil and Determination of Hydrochlorothiazide in Synthetic Tablets

Received : 06.11.2007
Revised : 21.11.2007
Accepted : 29.11.2007

Mustafa Çelebier*, Sacide Altınöz*o

Introduction

Olmesartan medoxomil (OLMD) is a selective AT1 subtype angiotensin II receptor antagonist[1,2]. Hydrochlorothiazide (HCT) is a thiazide diuretic that is commonly used in combination with other antihypertensive agents[3]. The structure of OLMD and HCT are given in Figure 1. It is reported that the combination of OLMD and HCT is a safe, well tolerated, and effective option for antihypertensive therapy, demonstrating greater blood pressure reduction than monotherapy[4].

Capillary electrophoresis (CE) is a simple analysis technique which is applied to the separation of a wide variety of compound types including pharmaceuticals[5-7]. The simultaneous determination of HCT with some other angiotensin II receptor antagonist was performed by CE recent years[8,9]. On the other hand, the determination of OLMD in tablets was carried out by CE[10], UV Spectrophotometry[11] and High Performance Liquid Chromatography (HPLC)[12]. There was reported a HPLC method for the simultaneous determination of OLMD and HCT in pharmaceutical dosage forms[12]. However, there wasn't any method described by CE for

* Hacettepe University, Faculty of Pharmacy, Department of Analytical Chemistry 06100 Sihhiye, Ankara, TURKEY

o Corresponding Author: E-mail: saltinoz@hacettepe.edu.tr. Tel: +90 312 305 40 15 Fax: +90 312 311 47 77

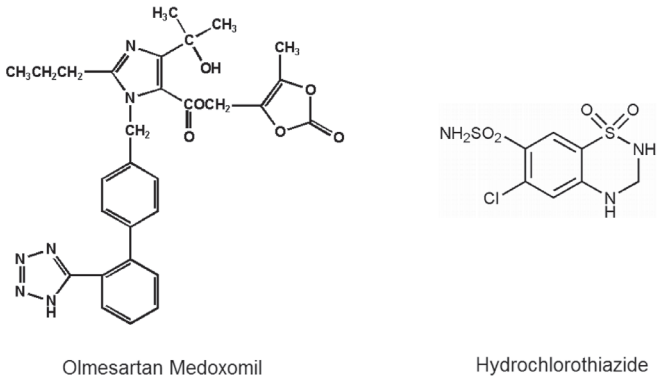

Figure 1
The chemical structures of OLMD and HCT

the simultaneous analysis. In the literature, it is indicated that the OLMD is not stable in methanol (MeOH) for more than a couple days[11] and in the aqueous solutions (especially in basic pHs) for more than 4 hours[10]. In order to eliminate the stability problem of OLMD in aqueous solutions, OLMD was completely degraded to its degradation product (OLH) in highly basic pHs through 0.01 N NaOH and then indirectly analyzed. The unstable behavior of OLMD in basic pHs and the complete degradation of OLMD to OLH are clearly shown in Figure 2. On the other hand, the HCT peak was not able to be seen in neutral pH. All these reasons caused working in basic pHs for the method development. The unstable behavior of the OLMD in aqueous solutions might be explained by either the ester structure of it[13] or the degradation of the -O-CO-O- group of the medoxomil part.

In this study, a new CE method was developed for the simultaneous determination of OLMD and HCT. The developed method was validated according to the literature[14-17] and applied to the synthetic tablet preparations containing OLMD (20.0 mg) and HCT (12.5 mg). The method was linear, sensitive, precise, accurate, selective and robust.

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 30kV high voltage power supply. A

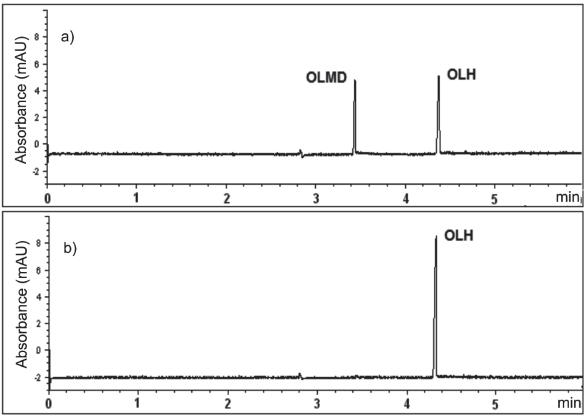

Figure 2
Electropherograms of OLMD (20 µg mL^{-1}) solved in different systems and waited for 20 min. a) OLMD solved in ACN and diluted with borate buffer (20 µM: pH 9.0) b) OLMD solved in 0.01 N NaOH and diluted with borate buffer (20 µM: pH 9.0)

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 for separation. 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 and HCT were kindly supplied by Daiichi Sankyo (Tokyo, Japan) and Pfizer Pharm. Ind. (Istanbul, Turkey) respectively. Diflunisal (IS) was purchased from Sigma (St Louis, USA). 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 Stock Solutions

Standard stock solutions (1000 µg mL^{-1}) of HCT and IS were prepared in acetonitrille (ACN). Standard stock solution of OLMD (1000 µg mL^{-1}) was prepared in 0.01 N NaOH in order to degrade it to OLH. 50.0 mg of the compound was accurately weighed and transferred to a 50 mL volumetric flask and 30 mL of solvent (ACN or 0.01 N NaOH) was added.

It was treated in ultrasonic bath for 15 minutes at 25 ^{0}C and then the volume completed with the solvent. These solutions were kept at +4 ^{0}C and prevented from daylight. In order to prepare the standard solutions of OLMD and HCT, various aliquots of standard stock solutions were taken and suitable amount of IS was added. These solutions, containing identical amount of IS (20 µg mL^{-1}) and suitable amount of OLMD and HCT, were diluted with 40 mM borate buffer (pH 9.5) to give the final concentrations of desired.

Synthetic Tablet Preparation

For preparing the synthetic tablets, OLMD (20.0 µg) and HCT (12.5 mg) with some inactive ingredients (microcrystalline cellulose, lactose monohydrate, talc, magnesium sterate, starch, titanium dioxide) were mixed and transferred to a 50 mL volumetric flask. 25 mL of ACN and 25 mL of 0.01 N NaOH were added respectively. It was treated in ultrasonic bath for 15 minutes at 25 ^{0}C and then an aliquot was centrifuged at 5000 rpm for 10 min. Clear supernatant was transferred to another flask. Suitable amounts of synthetic tablet solution and IS standard stock solution were taken and diluted with 40.0 mM borate buffer (pH 9.5) to give the final concentrations (24 µg mL^{-1} OLMD, 15 µg mL^{-1} HCT and 20 µg mL^{-1} IS). All solutions were filtered through a 0.22 µm syringe.

Electrophoretic Procedure

Electrophoretic separations were carried out using fused silica capillary having 50 µm i.d. and 48.5 cm total length (40.0 cm effective length). At the beginning of each working day, the capillary was rinsed with 0.1 M NaOH for 20 minutes. Between each injection, the capillary was rinsed with 40 mM borate buffer (pH 9.5) for 4 min. Injection was performed hydrodynamically by the 50 mbar pressure for 3 s when the capillary temperature was 30 ^{0}C and applying voltage was 30 kV. OLMD, HCT and IS were detected using a diode array detector at 210 nm (bandwidth 10 nm).

Results And Discussion

Method optimization

In order to find the optimum conditions for the developed method, different running buffers were tried as background electrolyte. These are

phosphate (pH 6.0, 7.0, and 8.0) and borate (pH 8.5, 9.0, 9.5, and 10.0) buffers. The optimum background electrolyte can be defined as a buffer solution to obtain the best peak shape with the shortest migration time (t_μ) on a good resolution (R), selectivity (α) and efficiency (N). There was no peak for HCT on acidic pHs. Therefore, basic borate buffer was used for the initial experiments. The effect of pH on migration time of OLMD and HCT are given in Figure 3. The efficiency was sufficient (> 90.000) for these pH values. Even though the shortest migration time was observed for pH 8.5, a better resolution between peaks (EOF (Electroosmotic Flow), HCT, OLMD) was observed for pH 9.5 (Figure 3). For pH 9.5, analysis time was shorter than 3 min on the different borate buffer concentrations (20, 30, 40, 50 µM). The best peak shape was observed for 40 µM buffer concentration. Thus, pH 9.5 40 µM borate buffer was selected as background electrolyte. The effect of capillary temperature and applied voltage on migration time was investigated simultaneously. Therefore, three voltage values (20, 25 and 30 kV) and three temperature values (25, 30 and 35 ^{0}C) were evaluated and the effects of these parameters on experimental results were investigated simultaneously. Figure 4 shows the effects of applied voltage and temperature on the migration time of OLMD and HCT. A short migration time with the best peak shape and baseline were observed for 30 kV applied voltage at 30 ^{0}C temperature. Therefore, these values were considered as optimum for the developed method. In

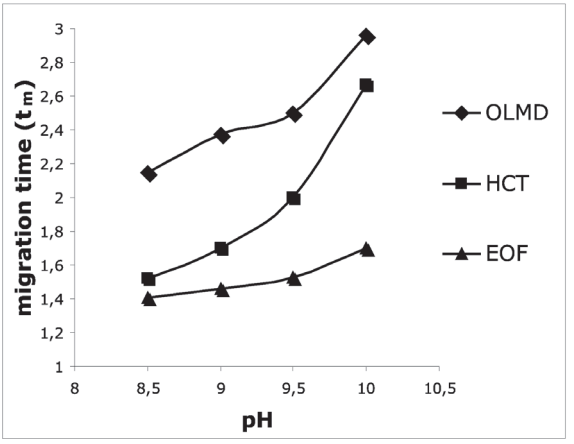

Figure 3
The effect of pH on migration time(tm), Operating conditions : Borate buffer (40 mM; pH 9.5), T = 25 ^{0}C, V = 30 kV, Detection at 210 nm, tinj=3 sec, Pinj=50 mbar (OLMD : 20 µg mL-1 , HCT : 20 µg mL^{-1}, and IS : 20 µg mL^{-1})

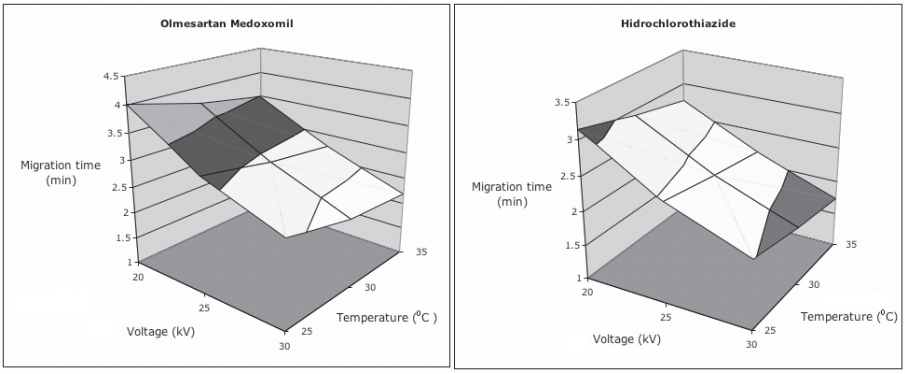

Figure 4
The effects of applied voltage and capillary temperature on migration times of OLMD and HCT Operating conditions: Borate buffer (40 µM; pH 9.5), Detection at 210 nµ, t_{inj}=3 sec, P_{inj}=50 mbar (OLMD : 20 µg mL^{-1} , HCT : 20 µg mL^{-1}, and IS : 20 µg mL^{-1})

CE studies, it is better to use IS and hydrodynamic injection to improve the precision and accuracy[18]. The peak shape of OLH was deformed after 3 s for the hydrodynamic injection. Thus, 3 s of injection time with 50 mbar pressure was selected as the optimum injection condition. Among the different compounds, migration time of diflunisal was between the OLH and HCT and the resolution was more than 1.5 for both. For this reason, diflunisal was preferred as IS. 210 nm (bandwidth 10 nm) detection wave lengths, in which OLH and HCT have sufficient absorption, was used for the developed method. Under the optimum conditions, the migration times of HCT, IS and OLH were 1.76, 2.06 and 2.25 minutes respectively.

Method validation

The developed method was validated for linearity, sensitivity, limit of detection (LOD) and limit of quantitation (LOQ), repeatability, precision, accuracy, recovery, specificity and selectivity, and robustness in accordance to the literature[14-17].

Linearity and Sensitivity (LOD and LOQ)

The calibration curves for OLMD and HCT were constructed under optimum conditions. The Peak Normalization (PN) Ratio of OLH and HCT to IS were plotted versus the nominal concentrations of the calibration standards. LOD and LOQ were estimated from the signal to noise ratio (S/N ratio 10:1 for LOQ and 3:1 for LOD). The results are given in Table I.

TABLE I

Data of the calibration curves for the developed method (n = 6)

	OLMD	HCT
Regression Equation*	y = 0.0286x+0.0218	y = 0.0256x+0.0344
Correliation coefficient (r)	0.9998	0.9996
Standard error of slope	0.0007	0.0010
Standard error of intercept	0.0035	0.0060
Linearity range (µg mL^{-1})	2.0 – 50.0	2.0 – 55.0
LOQ (µg mL^{-1})	2.0	2.0
LOD (µg mL^{-1})	0.5	1.0

*y = ax + b
y : Peak Normalization (PN) ratio
x : OLMD and HCT concentration (µg mL^{-1})

Repeatability

Repeatability was evaluated by assaying samples of the same concentration (OLMD : 20 µg mL^{-1} ; HCT : 20 µg mL^{-1}; IS : 20 µg mL^{-1}) on the same day by 10 consecutive injections. The amount of OLMD and HCT were calculated by regression equation. That, the relative standard derivation (RSD) of the results were less than 1 %, shows the repeatability of the system[19,20].

Precision and Accuracy

Three different concentrations of standard OLMD and HCT solutions on the linear range (10, 25 and 40 µg mL^{-1}) were analyzed six consecutive days (inter-day precision) and six times in the same day (intra-day precision). The RSD and Bias values within the acceptable values[15] for intra- and inter-day studies signify that the developed method is precise and accurate (Table II).

Specificity and Selectivity

The electropherograms, obtained from synthetic tablet solutions were identical with that obtained from standard solutions containing an equivalent concentration of OLMD, HCT and IS (Figure 5). In addition, the standard addition technique was applied to the sample preparations and there was no difference between the slopes of calibration curve and stan-

TABLE II

Precision and accuracy of the developed method (n=6)

		Intra-Day			Inter-day		
	Added (µg mL^{-1})	Found $\bar{x}$ (µg mL^{-1})	Precision RSD (%)	Accuracy Bias (%)	Found $\bar{x}$ (µg mL^{-1})	Precision RSD (%)	Accuracy Bias (%)
OLMD	10	10.17 ± 0.07	1.6	1.7	10.18 ± 0.09	2.2	1.8
	25	25.01 ± 0.12	1.1	0.0	24.81 ± 0.07	0.7	-0.8
	40	40.16 ± 0.36	2.2	0.4	39.58 ± 0.38	0.9	-1.0
HCT	10	9.97 ± 0.07	1.7	-0.3	10.11 ± 0.05	1.3	0.5
	25	25.03 ± 0.10	1.2	0.1	24.99 ± 0.08	0.8	-0.1
	40	40.16 ± 0.16	1.0	-0.4	40.21 ± 0.17	10	-0.5

$\bar{x}$: Mean ± SE
SE: standard error
RSD : Relative standard deviation
Bias % : ((Found – Added)/Added) x 100

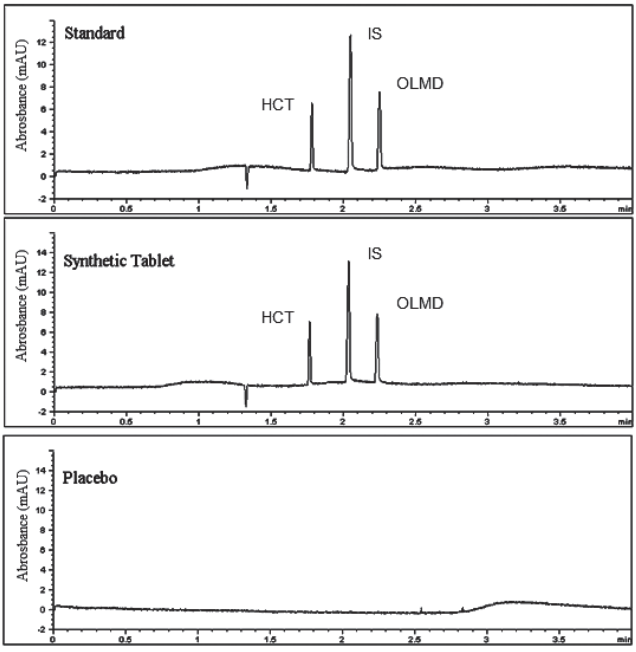

Figure 5
The electropherograms of standard, synthetic tablet and the excipients (placebo) solution. Operating conditions: 40 µM pH 9.5 borate buffer, hidrodynamic injection (3 s at 50 mbar), 30 kV, 30 ºC, 210 nm, (OLMD 24.00 µg mL^{-1} and HCT: 15.00 µg mL^{-1} and IS : 20 µg mL^{-1})

dard addition techniques. The regression equation for the standard addition technique was y= (0,0271 ± 0,0004)x + (0,5307 ± 0,0049), r=0,9994 (n=6) for OLMD and y= (0,0264 ± 0,0011)x + (0,3346 ± 0,0069), r=0,9994 for HCT where x: concentration of OLMD and HCT; y: PN ratio of OLMD and HCT to IS. These results show that there was no interference from matrix components.

Robustness

Robustness of the CE method was determined by analyses of samples under a variety of conditions such as small changes in buffer pH (9.4 and 9.6), buffer concentration (35 and 45 mM), capillary temperature (27 and 33 ^{0}C) and in the selected wave lengths (208 and 212 nm). When a statistical comparison (Friedman Analysis) was done between the obtained results and the results under optimum conditions, there was no difference found statistically (OLMD: 20 µg mL^{-1}, n=3, $p = 0.093 > p = 0.050$) and (HCT : 20 µg mL^{-1}, n=3, $p=0.066 > p = 0.050$).

Analysis of Synthetic Tablet Preparations

Since the pharmaceutical formulation containing OLMD (20.0 µg) and HCT (12.5 µg) (Benicar HCT®) cannot be found in the local markets, the developed method was applied to the synthetic tablet preparations. The synthetic tablet solutions of OLMD and HCT were analyzed seven times by calibration curve technique. The results are given in Table III.

TABLE III

Synthetic tablet analysis results obtained by the developed method (n=7)

Synthetic Tablet	
OLMD 20 µg	HCT 12.5 µg
20.06 20.08 19.54 20.45 20.22 20.04 19.86	12.60 12.34 12.28 12.54 12.50 12.40 12.52
$\bar{x}$ ± SE : 20.03 ± 0.11 RSD : 1.4 % Bias : 0.2 %	$\bar{x}$ ± SE : 12.45 ± 0.04 RSD : 1.0 % Bias : 3.6 %

$\bar{x}$ x ± SE : Mean ± Standard Error
RSD % : Relative Standard Deviation
Bias % = [(Found-Added)/(Added)] x 100

Conclusion

In this study, a simple and rapid CZE method was developed and validated for the simultaneous determination of OLMD and HCT in synthetic tablets. The validation assays were concluded that the developed method is linear, accurate, precise, sensitive and selective. Since the synthetic tablet excipients are identical with the commercial tablets, it is concluded that this proposed method might be applied to the real pharmaceutical formulations. On the other hand, this method should not be preferred if the degradation product of OLMD already occurred in the tablet preparations.

Summary

Development of A Capillary Zone Electrophoresis Method For The Indirect Determination of Olmesartan Medoxomil and Determination of Hydrochlorothiazide in Synthetic Tablets

A capillary zone electrophoresis method with ultraviolet detection for the simultaneous determination of Olmesartan medoxomil and Hydrochlorothiazide in synthetic tablets was developed. Since the Olmesartan medoxomil was not stable in aqueous solutions, first it was converted to its degradation product and then analyzed indirectly. A fused silica capillary (50 µm internal diameter, 48.5 cm total length, 40 cm effective length) was used and the separation was obtained by 40 µM pH 9.5 borate buffer followed by detection with an ultraviolet detector at 210 nm. The analysis were performed at a temperature of 30 °C with the application of 3 seconds hydrodynamic injection at 50 mbar pressure and a applied potential of 30 kV. Diflunisal (IS) was used as internal standard. The developed method was validated according to the literature.

Keywords: Olmesartan Medoxomil, Hydrochlorothiazide, Simultaneous determination, Capillary electrophoresis, Method development, Validation

Özet

Sentetik Tabletlerdeki Olmesartan Medoksomil'in Dolaylı Yoldan Analizi ve Hidroklorotiazid'in Analizi İçin Geliştirilen Bir Kapiler Elektroforez Yöntemi

Olmesartan Medoksomil ve Hidroklorotiazidin sentetik tabletlerden aynı anda analizi için UV dedetörün kullanıldığı bir capiler elektroforez yöntemi geliştirilmiştir. Olmesartan Medoksomil sulu çözeltilerde kararlı olamdığı için once bozunma ürününe çevrilmiş ve ardından dolaylı yoldan analiz edilmiştir. Eritilmiş silica kapiler (50 µm iç çap, 48.5 cm toplam uzunluk, 40 cm efektif uzunluk) kullanılmış ve ayrım 40 mM pH 9.5 borat tamponunda 210 nm dalga boyunda ultraviole dedektör kullanılarak gerçekleştirilmiştir. Analizler, 30 ^{0}C' de, 50 mbar basınç uygulanarak 3 saniye hidrodinamik enjeksiyonla ve 30 kV uygulama potansiyeli altında yapılmıştır. Diflunisal (IS), iç standart olarak kullanılmıştır. Yöntem, literature uygun olarak valide edilmiştir.

Anahtar Kelimeler: Olmesartan Medoksomil, Hidroklorotiazid, Aynı anda analiz, Kapiller elektroforez, Metot geliştirilmesi, Validasyon.

Acknowledgement

The authors thank Daiichi Sankyo and Pfizer for their kind supply of pure Olmesartan Medoxomil and pure HCT respectively.

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