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Short Communication

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Application of Capillary Electrophoresis to Investigate the Degradation Kinetic of Olmesartan Medoxomil

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SUMMARY. A simple capillary zone electrophoresis method was applied in order to investigate the degradation of olmesartan medoxomil in basic pHs. A kinetic approach was performed to enlighten its degradation process. Olmesartan medoxomil, solved in acetonitrile and diluted with 30 mM phosphate buffer, was injected six times within five hours by using running electrolytes of different pHs (30 mM phosphate buffer pH 7.5, 8.0 and 8.5). A fused silica capillary (i.d. 50.0 μm, total length 48.5 cm and effective length 40.0 cm) was employed for the analysis. The separation and best peak shape was achieved by applying 30 kV voltage at 30 °C capillary temperature. A diode array detector was used at 210 nm wavelength. Diflunisal was the internal standard. It was clarified that the degradation of olmesartan medoxomil progresses with a first order reaction kinetic. For pH 7.5, 8.0, and 8.5 the first order kinetic constants (k) were found to be 0.042, 0.092, and 0.171 respectively.

INTRODUCTION

Olmesartan medoxomil (OLMD) is a prodrug, which, after ingestion, liberates the only active metabolite, olmesartan. Olmesartan is a competitive and selective AII type 1 receptor antagonist. The hydrolysis of OLMD occurs readily by the action of esterases which are present abundantly in the gastrointestinal tract, liver and plasma [1-4]. In the literature there is reported UV Spectrophotometry [5], capillary electrophoresis (CE) [6] and a High Performance Liquid Chromatography (HPLC) methods [7,8] for the determination of OLMD in pharmaceutical dosage forms. In the previous studies, it is indicated that the OLMD is not stable in methanol (MeOH) [5] and in aqueous solutions especially in basic pHs for more than 4 hours [6]. However, there is not any stability indicating method reported up to date for the determination of OLMD. Therefore, the stability of OLMD is not known in different conditions clearly. In this study, the solution stability of

OLMD is screened on-line by CE [9] and the degradation kinetic of OLMD in basic pHs is enlightened. The degradation of OLMD might be explained by the ester hydrolyses of OLMD or the degradation of medoxomil group [10].

MATERIALS AND METHODS

Apparatus

All experiments were performed using an Agilent 3D CE (Waldbronn, Germany) system equipped with a diode array detector, an 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. The pH of solutions was measured by a pH meter (Metler Model MA 235, Switzerland)

Chemicals and reagents

OLMD (Fig. 1)was kindly supplied by Daiichi Sankyo (Tokyo-Japan). Diflunisal was purchased from Sigma (St Louis, USA). All other chemicals were of analytical reagent grade from Merck. Milli-Q water system (Barnstead, USA) was used

KEY WORDS: Capillary Electrophoresis, Degradation Kinetic, Method Development, Olmesartan Medoxomil, Stability Screening.

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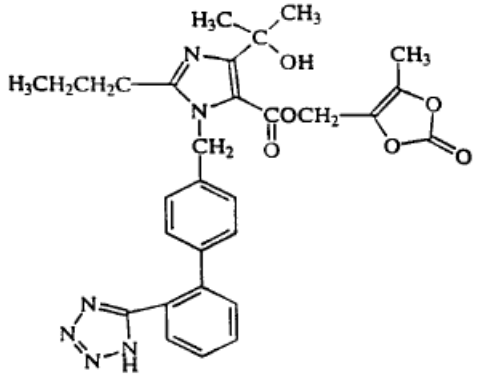

Figure 1. Olmesartan medoxomil (OLMD).

for the preparation of buffer and other aqueous solutions.

Standard and sample solutions

OLMD standard stock solution (1000 µg mL^{-1})

50.0 mg of OLMD was accurately weighed and transferred to a 50 mL volumetric flask and 30 mL of ACN, in which OLMD is stable, was added. It was treated in ultrasonic bath for 15 minutes at 25 °C and then the volume completed with ACN. This solution was kept at +4 °C and prevented from daylight.

Diflunisal standard stock solution

Diflunisal was used as internal standard. Standard stock solution of IS (1000 µg mL^{-1}) is prepared in ACN. 50.0 mg of diflunisal was solved with 50 mL of ACN in a volumetric flask.

Running buffers

In order to prepare the phosphate buffers, 2.04 g potassium dihydrogenphosphate (KH_2PO_4) was weighed and solved in 500 mL of water to give the final concentration. 0.1 N NaOH was used to adjust the desired pH values.

Experimental conditions

After the optimization studies the optimum conditions were found and used for the kinetic studies. Electrophoretic separations were carried out using fused silica capillary having 50 µm i.d. and 48 cm total length (40 cm effective length), in a positive mode using constant voltage (30 kV). At the beginning of each working day, the capillary was rinsed with 0.01 N NaOH for 20 minutes. Between each injection, the capillary was rinsed with the running buffer for 4 min. Injection was performed hydrodinamically by pressure (50 mbar) for 3 s. Capillary temperature was 30 °C and detection wavelength was 210 nm (bandwidth 10 nm).

RESULTS AND DISCUSSION

A kinetic approach was performed to investigate the degradation process of OLMD. OLMD (20 µg mL^{-1}), solved in acetonitrille (ACN) and diluted with phosphate buffer (30 mM), was injected six times within five hours by using different running pHs (30 mM phosphate buffer pH 7.5, 8.0 and 8.5). The OLMD, IS and OLH peaks were seen respectively. The degradation of OLMD in pH 8.5 is shown as an example of this application. As seen in Figure 2, the OLMD is degraded to its degradation product OLH while the time is passing. If the peak area ratio of OLMD to Diflunisal (IS) is accepted to be 100% at the beginning, the remaining amount of OLMD before degraded is able to be calculated proportional with the given formula in the next page.

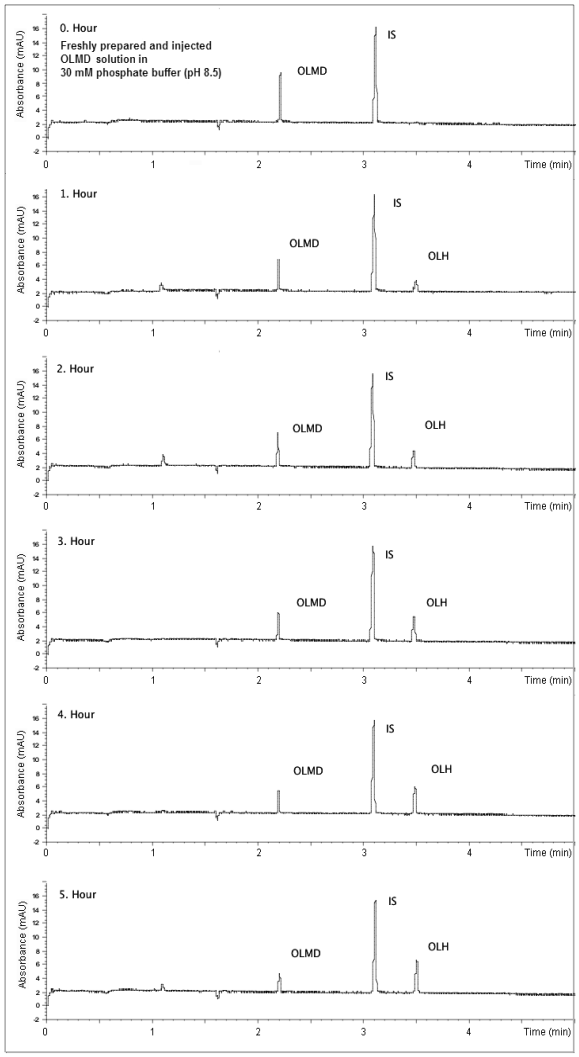

Figure 2. The degradation of OLMD in phosphate buffer (30 mM; pH 8.5). Operation conditions : T = 30 °C, V = 30 kV, Detection at 210 nm, t_{inj} = 3 sec, P_{inj}= 50 mbar (OLMD : 20 µg mL^{-1} and IS : 20 µg mL^{-1}).

$$\text{Remaining OLMD\%} = \frac{\left(\dfrac{\text{Peak area of OLMD (x hours later)}}{\text{Peak area of IS (x hours later)}}\right)}{\left(\dfrac{\text{Peak area of OLMD (Freshly prepared)}}{\text{Peak area of IS (Freshly prepared)}}\right)} \times 100$$

x hour : The passing time after the solution was prepared freshly (x: 1-5)

According to the experimental studies it was indicated that the degradation speed accelerated when the pH of the buffer was increased. The degradation of OLMD was independent from the kind of the buffer solutions. The remaining amount of OLMD in different hours for various pHs are given in Table 1.

If the logarithm (ln) of given values in Table 1 are calculated and plotted versus the time (hour), it can be easily seen that there is a linear relation. When the linear regression equations of this relation for different pHs are calculated, it is clarified that the degradation of OLMD progresses with the First Order Reaction Kinetic [11]. For pH 7.5, 8.0, and 8.5 the first order kinetic constants (k) are found 0.041, 0.092, and 0.171, respectively, by the intercepts of the linear regression equations (Table 1) [11]. The half-life times ($t_{1/2}$) of OLMD according to the pHs are also given in Table 1. It can be easily seen on the table that the $t_{1/2}$ values are changed dramatically by the increasing of pH values from 7.5 to 8.5. It was not possible to investigate the degradation kinetic for pHs more than 8.5, because of the fact that the OLMD was not stable even for the first injection time. Thus, the pH values from 7.5 to 8.5 were inside the range focused in this study. The stability of OLMD was also screened in acidic pHs, and it was seen that the OLMD was stable for acidic pHs for more than 4 hours. The chemical structure of OLH was not investigated in the scope of this study..

CONCLUSION

Degradation kinetic constants (k) are important on both drug development and production process in order to understand the stabilities of drugs in different pH values both inside and outside the body. In this study, it is clearly indicated that the OLMD is degraded to its degradation product in basic pHs but stable at acidic pHs for more than 4 hours. Thus, CE is proposed for the determination of degradation kinetic constants (k) and it is applied successfully

	30 mM Phosphate Buffer								
	pH 7.5			pH 8.0			pH 8.5		
	Peak area ratio*	Remaining OLMD %	Logaritmic values (ln) of the remaining OLMD %	Peak area ratio*	Remaining OLMD %	Logaritmic values (ln) of the remaining OLMD %	Peak area ratio*	Remaining OLMD %	Logaritmic values (ln) of the remaining OLMD %
0 Hour	0.39	100.00	4.60	0.38	100.00	4.60	0.35	100.00	4.60
1 Hour	0.37	94.43	4.55	0.35	92.67	4.52	0.29	83.49	4.42
2 Hour	0.35	90.63	4.51	0.32	83.97	4.43	0.24	69.85	4.25
3 Hour	0.33	86.34	4.46	0.28	73.82	4.30	0.21	59.52	4.09
4 Hour	0.32	83.80	4.43	0.26	69.39	4.24	0.17	50.06	3.91
5 Hour	0.31	80.90	4.39	0.24	63.83	4.15	0.14	36.03	
Regression equation**	y = -0.041x + 4.594			y = -0.092x + 4.603			y = -0.171x + 4.596		
Half-time of OLMD(t1/2)***	16.6 hours			7.5 hours			3.9 hours		

Table 1. The remaining amount of OLMD before degraded in basic pH values. * Peak ratio = Peak area of OLMD / Peak area of IS; ** y = ax + b where y: ln remaining amount of OLMD % ; x: time (hour) ; a: -1 x (kinetic constant (k)) ; b: slope; *** Half-life time of OLMD means when the remaining amount of OLMD is 50%.

for the determination of the "k" values of OLMD in basic pH values. It is also indicated that the degradation process of OLMD in basic pHs follows first order reaction kinetic.

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