Anal. Bioanal. Electrochem., Vol. 6, No. 2, 2014, 219 - 233

Analytical & Bioanalytical Electrochemistry

Full Paper

Development and Validation of a New Capillary Electrophoresis Assay for Monitoring of Atenolol Photodegradation Product in Non-aqueous Solution

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Received: 21 February 2014/ Accepted: 15 April 2014/ Published online: 30 April 2014

Abstract- The characterization of atenolol photodegradation product was performed using capillary electrophoresis (CE) with ultraviolet diode array detector (UV-DAD). The optimum separation for this assay was achieved in <7 min at 298 K with a fused-silica capillary column (57 cm length and 75 µm I.D.) and a running buffer containing 60 mM acetate buffer at pH 5.3 dissolved in methanol/ethanol mixture (20:80% v/v). The samples were injected with applied voltage of 16.0 kV. It's established that capillary electrophoresis method enables to detect atenolol photodegradation product. The suggested method has been used for the determination of the studied drug in human serum and the results were compared to the other reported methods.

Keywords- Capillary electrophoresis (CE), Atenolol, Ultraviolet diode array detector (UV-DAD), Photodegradation

1. INTRODUCTION

It is recognized that light can change the properties of different materials and products. This is often observed as bleaching of colored compounds like paint and textiles or as a discoloration of colorless products. For many years photostability has been a main concern within several fields of industry, e.g., the textile, food, cosmetic and agricultural industries. In the pharmaceutical industry, photostability plays an important role. Since a number of drugs are photochemically unstable is steadily increasing. The European pharmacopoeia prescribes light protection for more than 250 medical drugs and also for a number of adjuvant [1].

On the other hand, while a drug is shown to be photochemically inert in the sense that it does not decompose during exposure to light, it can still act as a source of free radicals or form phototoxic metabolites *in vivo* [2]. After administration the drug will be photoreactive, if the patient is exposed to light, causing light-induced adverse effects [3]. This emphasizes the importance of including studies of reaction mechanisms and sensitizing properties of the drugs and its degradation products in the evaluation of photostability of drugs.

Atenolol (AT) is a cardioselective ß-blocking agent prescribed for the treatment of cardiac arrhythmias and management of hypertension, angina pectoris, and myocardial infarction [4,5]. Although, several methods have been developed for the monitoring the photodegradation of AT [6-9]; however, the lack of studies on its photodegradation in non-aqueous focused our attention in this work which describes how AT responds to light exposure in non-aqueous solution. The objective of this work (as continuing our studies on beta-blockers and impurity analysis [10,11] is to develop a CE method for photodegradation study of AT.

2. EXPERIMENTAL

2.1. Reagents and chemicals

AT was supplied by DaruPakhsh Pharmaceutical Co. (Tehran, Iran). Methanol, ethanol, glacial acetic acid, sodium acetate, sodium hydroxide were purchased from Merck (Darmstadt, Germany). De-ionized water was used for preparing the acetate buffer. Stock solution was prepared by dissolving AT in 10 mL running buffer.

2.2. Apparatus

The electrophoresis analyses were performed using an Agilent 7100 capillary electrophoresis (Waldbronn, Germany) driven with Agilent Chem. Station software. A fused silica capillary (57 cm length and 75 µm I.D.) was used for electrophoresis experiments. The running buffer consisted of acetate buffer (60 mM) dissolved in methanol/ethanol mixture (20:80% v/v). Samples were injected into the capillary by pressure (50 mbar), for 6 s. The

applied voltage was 15-30 kV range. The capillary was washed between runs with a sequence of rinses: 0.1 M sodium hydroxide (1 min), water (0.5 min), 0.1 M hydrochloric acid (0.5 min), and water (0.5 min), followed by running buffer for 3 min to ensure reproducibility of the assay. Buffer ionic strength and pH were optimized. Detection was performed by a UV-DAD detector at 220-335 nm range. FT-IR spectra (4000-400 cm^{-1}) were recorded with a Shimadzu FT-IR Prestige 21 spectrophotometer as KBr disks.

3. RESULTS AND DISCUSSION

3.1. Photodegradation of AT

A standard stock solution of AT (1.0 mg mL^{-1}) was prepared by dissolving an appropriate amount in acetate buffer solution (60 mM). The standard solution was obtained by diluting the standard stock solution with acetate buffer solution to yield a solution containing 0.4 mg mL^{-1}.

A new CE method was developed for monitoring AT photodegradation in non-aqueous solution. Fig. 1 (A-C) respectively depict electropherograms of the sample solutions immediately after preparation and then after 16 days. A high peak corresponding to AT (Fig. 1A) observed after injection of AT which was prepared immediately after preparation. After ~8 days, 10% of AT (Fig. 1B) was degraded and height of peaks for AT was decreased; a low peak at migration time=5.12 min was also observed. Later we performed similar experiments after ~16 days with the same environmental conditions. It is observed that peak height of AT was decreased and the peak height of AT degradation product was increased. These results suggest that the proposed method is a useful tool to elucidate the degradation process of AT and is able to detect one of its degradation products.

AT can be exposed to daylight to obtain its photodegradation products. As the photo degradation product, 4-[2-hydroxy–3-[(1methylethyl)amino]propoxy]benzeneacetamide (Scheme 1) was found by electrophoresis method which confirm by other reports [12,13].

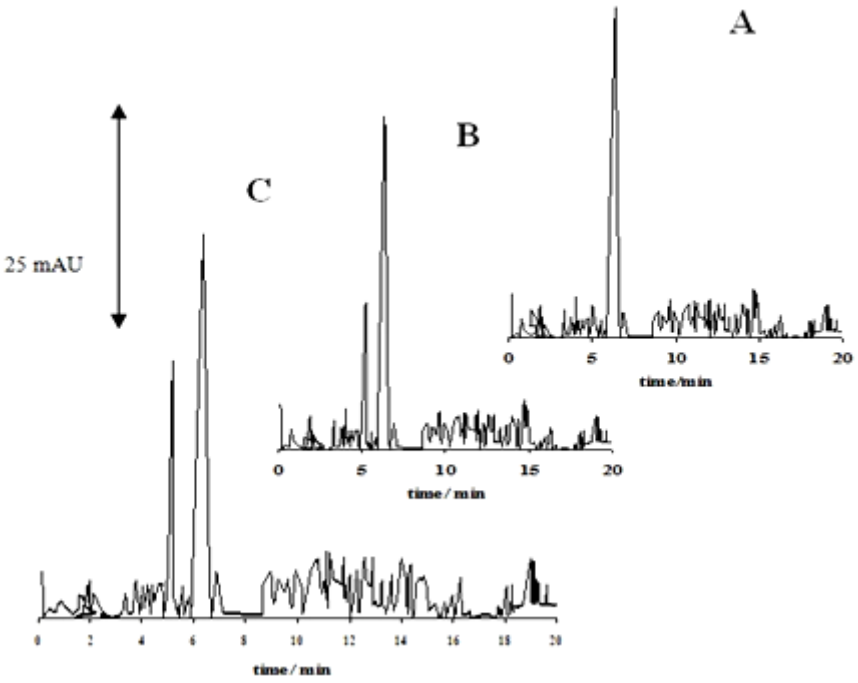
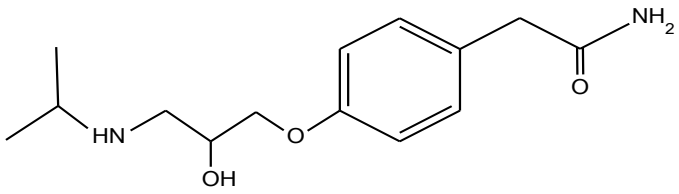
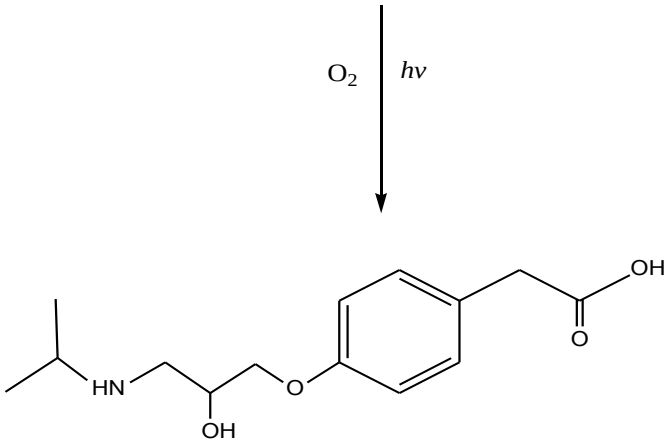


Fig. 1. Electrophorogram of 1.0 mg mL^{-1} AT for time=0 (immediately after preparation of AT) **(A)** and **(B-C)** after 8-16 days of exposure to daylight. Acetate buffer in methanol: ethanol (20:80% v/v) with the apparent pH of 5.3



4-[2-Hydroxy–3-[(1–methylethyl)amino]propoxy]benzeneacetamide



2-(4-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)acetic acid

Scheme 1. Degrading procedure of AT on photodegradation conditions

Fig. 1 (A-C) indicated that photodegradation product with the migration time of 5.12 min is the only detectable product of photodegradation pathway which observed in all electrophorograms in exposed time after ~8 days. In addition, the presence of 4-[2-hydroxy–3 [(1methylethyl)amino]propoxy]benzeneacetamide wasn't detectable on the electrophorogram (Fig. 1A) under described conditions.

The ability of a drug to degrade or undergo a gradual change in color upon light exposure is not an uncommon property. Drug substances can even exhibit different sensitivity to light [14]. In practice, the drug substance would mainly experience exposure to visible light during storage and production. AT is white; essentially, no visible light will be absorbed by AT (Fig. 2a). A change in color upon exposure is necessarily to correlate with the extent of chemical degradation of the material [15]. For these reasons, variation of AT color was record in different days (after and before degradation) (Fig. 2 (a-e)). It's observed AT color was vary after ~8 days. The cause of this phenomenon is the presence O and N in the structure which is usually associated with the ability of the molecule to absorb light. On the other hand, construction of carboxylic acid on the photodegradation product is cause of this observable fact. Similar results were obtained by other reports [16].

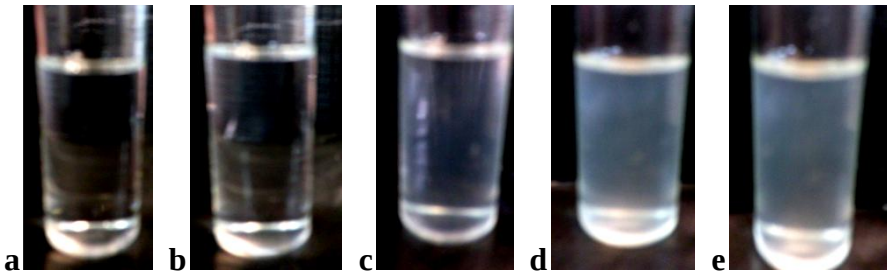


Fig. 2. AT discolor *vs.* time of exposition

For further study, the variations of AT and photodegradation products absorbance were obtained versus time of exposure. Fig. 3 shows that the absorbance of AT and photodegradation products is proportional to the time from 0-16 days, pointing to the degradation of the AT. As evident from the figure, the variations are more significant after ~8 days of exposure. Fig. 3 indicates that AT absorbance decreased with the time, signifying a degradation process of AT. This result was confirmed by increasing on the degradation product absorbance. Also, Fig. 3 shows that photodegradation product was detected after ~8 days. In summary, from a complementary study of the stability of AT under photodegradation conditions, we observed that the peak of AT was decreased, and a supplementary peak appeared. When a photodegradation was performed, the peak of degradation product was increased and the peak of AT decreased severely which this result was obtained after ~8 days. Photodegradation product was probably formed by photo-

oxidation of the acetamide functional group to a carboxylic acid [12,13]. Under normal storage conditions it is hardly likely that this product would be formed. The possibility of separating the two substances (AT and photodegradation product) proves once more the selectivity of the proposed method. On the bases of the CE results, the proposed method is provides accurate way for simultaneous detection of AT and its photodegradation product in non-aqueous conditions. Therefore, AT can be exposed to daylight to obtain its degradation product.

The FT-IR spectra of AT were recorded in different time of exposure of to daylight after and before degradation. The degradation procedure of AT was confirmed by IR spectra (Scheme 2). The oxidation of acetamide group of AT to the corresponding carboxylic acid can be explained from its IR spectrum. At time=0, two peaks at 3371 and 3356 cm^{-1} were observed due to amine groups, respectively. After photodegradation (time of exposure=8 days) FT-IR spectra indicating the conversion of CONH to COOH group. Also after ~8 days this variation was sever which can be observed in Scheme 2. In addition, IR spectra database for organic compounds (SDBS) [17] confirmed these results.

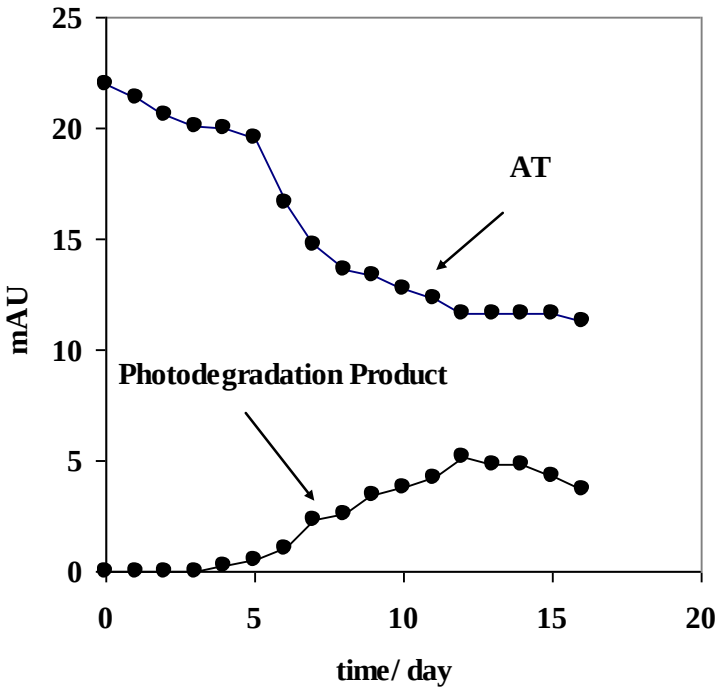
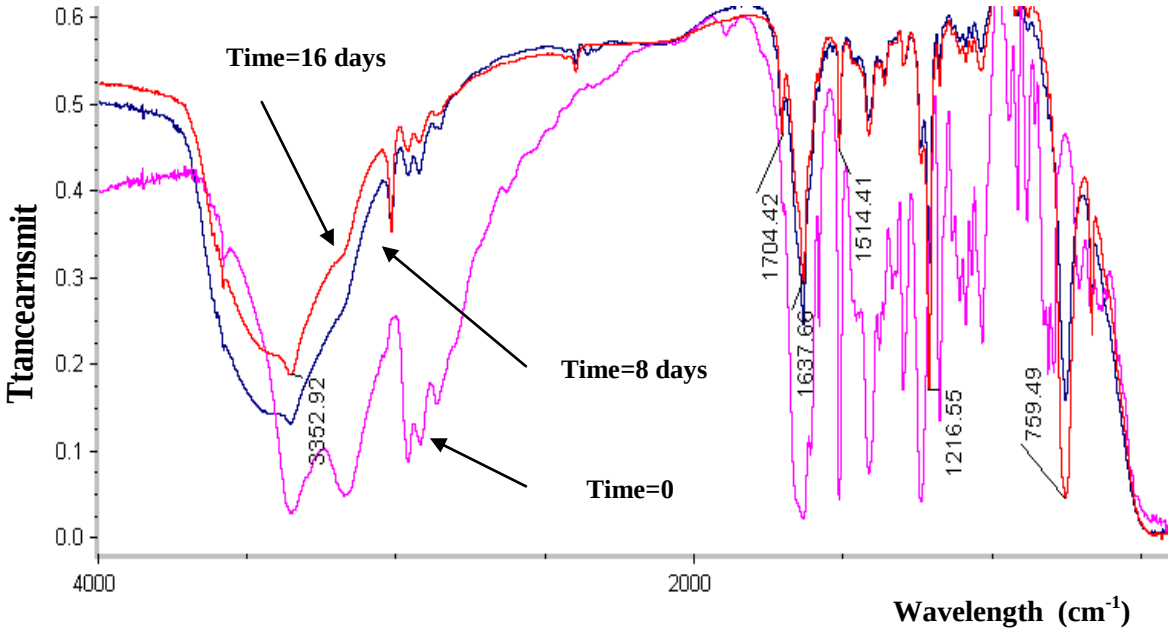


Fig. 3. Electrophoretic behaviors in photodegradation conditions; variation of mAU *vs.* time of daylight exposure for AT and detected degradation products



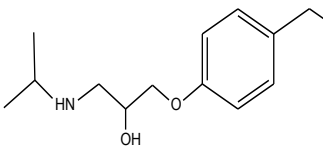
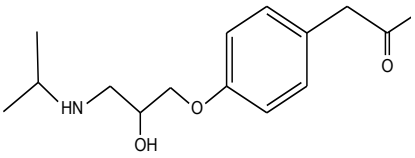
Scheme 2. FTIR spectrum of AT at t=0, t=8 and t=16 days of exposition to daylight

For further study some of physicochemical property of AT and its photodegradation product were extracted by CS ChemProp software which reported in Table 1. On the bases of Table 1, in photodegradation conditions, AT has Gibbs energy=-50 kJ mol^{-1} while 2-(4-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)acetic acid has Gibbs energy=-331.44 kJ mol^{-1}. On the other hand, heat of form 2-(4-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)acetic acid is more negative than AT which shows non-spontaneous of proposed photodegradation reaction. Based on Gibbs-dohem law, activity of photodegradation product is higher than AT. Insomuch, molecular weight (MW) of AT and photodegradation product are approximately equal, hence shorter migration time of 2-(4-(2-hydroxy-3(isopropylamino)propoxy)phenyl) acetic acid is expected because of existence of easy ionizable groups. The reason of this phenomenon is difference of two functional group CONH and COOH in AT and photodegradation product, respectively. Based on difference pK_a (Table 1), photodegradation product has inclinable ionization capability in pH=5.3 when compared with AT. Hence, photodegradation product has shorter migration time than AT. The direct correlation of photodegradation product structures and pK_a values with ionization groups and also relationship of these results with the migration time of the AT are presented in Table 1. It can be seen that photodegradation product has three accessible ionizable groups (N-H, O-H and COOH) and AT has two accessible ionizable groups (N-H, O-H). Also photodegradation product has higher K_a than AT. This may be the reason of shorter migration time of photodegradation product compared to AT. It is clear that the presence of three ionizable groups and higher K_a facilitates the interpretation of shorter migration time of AT photodegradation product than AT. Similar results were obtained by other reports [12, 13]

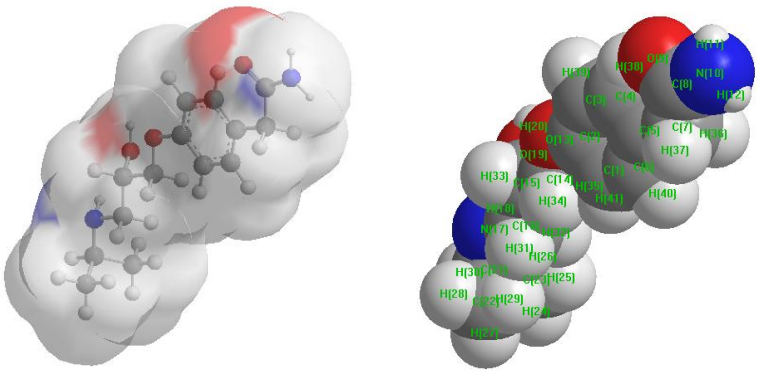
which confirm these results. To provide more comprehensive information, Table 2 was compared CE method with other available reports [6, 8, 12, 13, 18 and 19]. Based on Table 2, it is observed that proposed mechanism is mild in photodegradation conditions. Carefully inspection of Table 2 shows that similar degradation products were obtained by Abdelwahab [6] and Radjenovic et al. [12] and this work in various conditions and on different methods. It's find that, major degradation product of AT is 4-[2-hydroxy–3-[(1methylethyl) amino]propoxy]benzeneacetamide which was discovered by other researchers. Also, Table 2 shows that in stress degradation of AT, the product is different with mild degradation conditions.

All of above results was discussed by investigation of partial surface area (PSA) of AT and degradation products. In this study, PSA was used for evaluation of AT and photodegradation product ionization groups. Its established molecular surface properties have been used to describe solvation and partial ionization processes for a long time. One of the most useful surface properties has been shown to be PSA, characterizing the polar part of the molecular surface, defined simply as the part of the surface corresponding to oxygen and nitrogen, and including also the hydrogen's attached to these atoms [20]. Scheme 3 indicated an extremely rapid method to obtain PSA which describe simply from the sum of contributions of polar fragments in a molecule. This figure shows that in photodegradation conditions the PSA vary as degradation product >AT which verifies our results on CE developed method. These results indicated that the proposed method for AT photodegradation in non-aqueous solution is coherent.

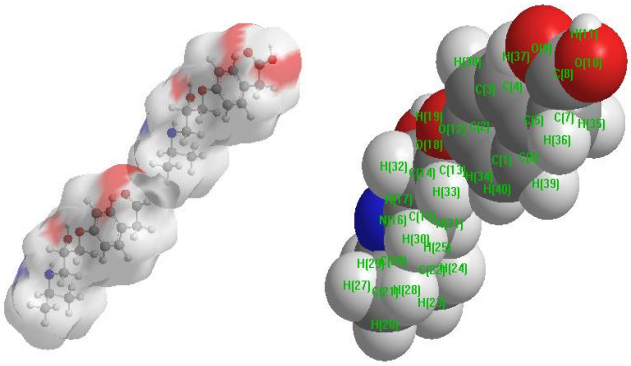
Table 1. Some of important physiochemical property report generated by CS ChemProp and ACD Lab software

Physiochemical Properties	**AT**	**Photodegradation Product**
Structure		
MW [a]	266	252
Gibbs Energy	-50 [kJ mol^{-1}]	-331.44 [kJ mol^{-1}]
Heat of Form	-427.56 [kJ mol^{-1}]	-699.04 [kJ mol^{-1}]
pK_a	9.6	4.5

[a] MW is Molecular of weight



AT



Photodegradation product

Scheme 3. Partial surface area of AT photodegradation products

An ab initio calculation, as well as PSA have been employed for the investigation of the energy (SCF, in Hartree) and atomic charges for AT and its photodegradation product in two forms occurring via Gaussian 03 program [21]. We performed an ab initio calculation with a PC computer. The geometries of the AT and its derivative were optimized by Moller-Plesset approach (MP2) with the 6-31G basis set. All of results were reported on Table 3 which confirmed our results on PSA calculations. On the basis of Table 3 results, it's find that total net charge of photodegradation product is lower than AT in two forms of base set. Therefore, it can include migration time of photodegradation product is shorter than AT. In addition ab initio calculation indicated that calculation results by PSA are according to CE results which electrophoresis has been defined as the differential movement of charged species.

3.2. Validation of the Method

CE method is validated with respect to linearity range, limits of detection (LOD) and quantitation (LOQ) as well as precision. The terms are used according to the definition of the ICH guideline Q_2B [22].

AT was calibrated in the concentration range of 15-130 μg mL^{-1} and the calibration curve is constructed from seven concentrations. Each concentration was primed in triplicate and

each individual solution was injected twice and the results are summarized in Table 4. A linear relationship with the regression coefficient (R) of 0.9920 is obtained. At the lowest concentrations assayed, the signal/noise ratio is at least 10:1, and this concentration was subsequently regarded as LOQ for the present assay. The LOD defined as a signal/noise ratio of 3:1 which is 4.2 µg mL^{-1}.

Table 2. Comparison of reported assays with proposed method for detection of AT degradation products

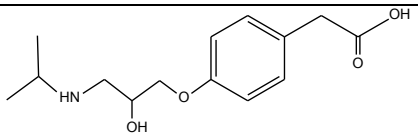
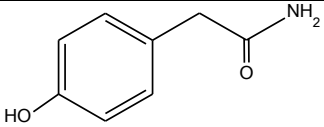
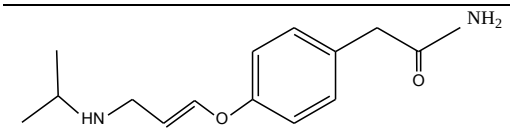
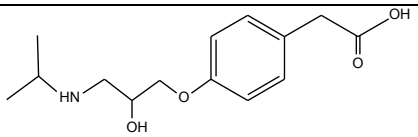
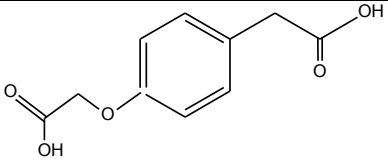
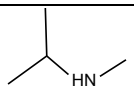
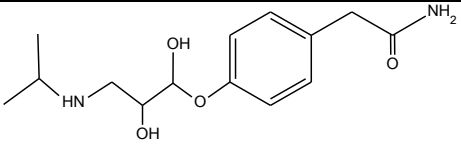
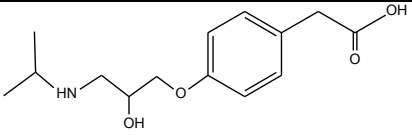
Method	**Condition**	**Degradation Products**	**MW**	**Ref.**
TLC-densitometric and Chemometric methods	Hydrolytic degradation		267	[6]
LC chromatograms	Photodegradation UVA-UVB radiations		151	[8]
			263	
UHPLC/ESI (+) -TOF-MS	Microbial degradation		267	[12]
LC-ESI-MS	Oxidative degradation		226	[13]
			73	
RP-HPLC	Force degradation	-	-	18
MS/Ms	Biological advanced oxidation		282	[19]
CE	Photodegradation		267	This Work

Table 3. Energy (SCF, in Hartree) and atomic charges for AT and its degradation product in two form calculated by MP2/6-31G

	Atenolol	**Atenolol Ion Form**	**Photodegradation Product**	**Photodegradation Product Ion Form**
SCF	-876.07290	-876.42958	-895.88459	-895.31876
R	-0.40	-0.33	-0.40	-0.44
Benzene	+0.435	+0.486	+0.467	+0.334
CH_2	+0.001	+0.135	+0.027	-0.096
Agent group	-0.03	+0.71	-0.08	-0.78
C	+0.76	+0.86	+0.77	+0.76
Carbonyl's O	-0.64	-0.68	-0.56	-0.78
N	-0.93	-0.87	-	-
H_1	+0.38	+0.47	-	-
H_2	+0.394	+0.443	-	-
O	-	-	-0.73	-0.76
H	-	-	+0.44	-

Repeatability and precision of the developed method is evaluated by analyzing AT concentrations in seven independent series on the same day (repeatability of intra-day precision) and on three consecutive days (intermediate precision of inter-day precision). Within these series, each sample was analyzed twice and the data are summarized in Table 4. Generally, precision was found for the CE assay with relative standard deviations (R.S.D.) varying between 3.1 and 4.0%. The results obtained for detection of AT by the proposed CE method was compared with those of previously reported techniques in Table 5 [6, 8, 23-41]. Based on Table 5 results, although limits of detections are lower with HPLC, Chemiluminescence, TLC-densitometry and electrochemistry, the CE assay offers the advantage of faster analysis times and low consumption of solvents. Resulting is benefit in lower overall costs as well as a greater environmental assay.

The accuracy of the proposed method is also investigated by recovery experiments performed by adding known amounts of AT to the human serum. Nine samples are used for each recovery level. Samples are treated as described in the above method for sample preparation. The results obtained are shown in Table 6, from which it is clear that both the recoveries and repeatabilities are according to degradation of AT.

Table 4. Some validation data of AT from new CE developed method in prepared solutions with pH=5.3 acetate buffer (60 mM) on methanol/ethanol mixture (20:80% v/v)

Rang (µg mL^{-1})	33-525	
R^2	0.9920 (n = 7)	
LOD [a] (µg mL^{-1})	4.2	
LOQ [b] (µg mL^{-1})	9.00	
Repeatability	Inter-day measured concentration	RSD [C] (%): 3.1
	Intra-day measured concentration	RSD (%): 4.0

[a] LOD is Limit of Detection

[b] LOQ is Limit of Quantitation

[c] RSD is Relative Standard Deviation

Table 5. Comparison of the results obtained by CE developed method with other reports

Method	Linear Range	LOD	Sample	Ref.
Chemometric	32-40 µg mL^{-1}	-	Pharmaceutical preparation	[6]
TLC-Densitometric	2-10 mg	0.5 mg	Pharmaceutical preparation	[6]
RP- HPLC	0.4-12.8 mg	-	Pharmaceutical preparation	[8]
Chemiluminescence	8-1000 ng mL^{-1}	3 ng mL^{-1}	Human urine	[23]
HPLC	4- 48 µg mL^{-1}	26.61 ng mL^{-1}	Pharmaceutical formulations	[24]
	1-100 pg mL^{-1}	0.4 µg mL^{-1}	Pharmaceutical formulations	[25]
	6.25-200 µg mL^{-1}	0.62 µg mL^{-1}	Pharmaceutical formulations	[26]
	-	5.07 µg mL^{-1}	Pharmaceutical formulations	[27]
	-	4.30 µg mL^{-1}	Pharmaceutical formulations	[28]
	-	0.7 µg mL^{-1}	Pharmaceutical formulations	[29]
	20-200 µg mL^{-1}	0.02 µg mL^{-1}	Pharmaceutical formulations	[30]
	4-10 µg mL^{-1}	0.046 µg mL^{-1}	Pharmaceutical formulations	[31]
Electrochemical	0.4-80 µg mL^{-1}	26.6 µg mL^{-1}	Commercial tablets	[32]
	12-96 µg mL^{-1}	1.12 µg mL^{-1}	Human Serum	[33]
RP- HPLC	2.5-125 µg mL^{-1}	0.5 µg mL^{-1}	Human urine	[34]
HPLC	30-585 ng mL^{-1}	-	Human plasma	[35]
Spectrophotometry	0.5-30 µg mL^{-1}	0.0107 µg mL^{-1}	Commercial tablets	[36]
CE	1-10 µg mL^{-1}	0.1165 µg mL^{-1}	Human urine	[37]
	-	5.0 µg mL^{-1}	Human urine	[38]
	0.6-10 µg mL^{-1}	0.6 µg mL^{-1}	Human urine	[39]
	1-250 µg mL^{-1}	0.78 µg mL^{-1}	pharmaceutical formulations	[40]
	-	1.0 µg mL^{-1}	Tablet dosage	[41]
	15-130 µg mL^{-1}	4.2 µg mL^{-1}	Human Serum	This Work

Table 6. Results obtained from recovery analysis of AT in photodegradation conditions in human serum

Degradation Condition	Time	Added AT to human Serum (μg mL^{-1})	Measured AT on human Serum (μg mL^{-1})	Recovery (%)
Sunlight	0 day	10.00	9.91	99.5
	2 days		9.90	99.1
	4 days		9.90	99.1
	6 days		9.47	90.3
	8 days		8.00	88.3
	10 days		8.00	84.0
	12 days		7.25	82.7
	14 days		7.20	80.7
	16 days		6.40	76.5

4. CONCLUSION

In summary, sensitive and rapid electrophoretic method was used and validated for the analysis of AT photodegradation in non-aqueous solution. The separation of AT and its photodegradation product takes place in less than 10 min. The results indicated that CE method is appropriate for simultaneous detection of AT and its photodegradation product in serum samples. The proposed method is a promising means to elucidate the photodegradation process of AT.

Acknowledgments

This is a report of a database from thesis entitled «Development of sensitive, routine and reliable methods for determination of cardiovascular drugs in biological samples" registered in Drug Applied Research Center. We gratefully acknowledge the financial support of this work by the Drug Applied Research Center, Tabriz University of Medical Sciences.

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