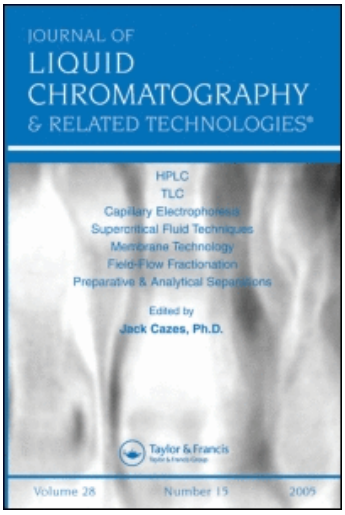

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M. Itxaso Maguregui[a]; Rosa M. Alonso[a]; Rosa M. Jiménez[a]
[a] Departamento de Química Analítica, Facultad de Ciencias Universidad del País Vasco/EHU Apdo, Bilbao, Spain

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CAPILLARY ZONE ELECTROPHORETIC METHOD FOR THE QUANTITATIVE DETERMINATION OF THE β-BLOCKER ATENOLOL IN HUMAN URINE

M. Itxaso Maguregui, Rosa M. Alonso, Rosa M. Jiménez

Departamento de Química Analítica
Facultad de Ciencias
Universidad del País Vasco/EHU
Apdo. 644
48080 Bilbao, Spain

ABSTRACT

A simple capillary zone electrophoresis method was developed for the quantitation of the β-blocker 4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy] benzeneacetamide, atenolol.

The electrophoretic separation was performed using a 78 cm × 75 μm I. D. fused silica capillary. The electrolyte consisted of a buffer Na_2HPO_4 (25mM)-$Na_2B_4O_7$ (25 mM) (50-50; V/V), pH 9.7. The introduction of the sample was made hydrostatically for 25 s and the running voltage was 20 KV at the injector end of the capillary. Photometric detection was used and a wavelength of 214 nm. The method was applied to the determination of atenolol in urine samples obtained from a hypertensive patient under medical treatment with the pharmaceutical formulation "Tenormin 100 mg" (atenolol 100 mg), another patient under treatment with the pharmaceutical combination "Tenoretic"

(atenolol 100 mg + chlorthalidone 25 mg), and also in urine samples obtained from a healthy volunteer after the ingestion of a 100 mg atenolol tablet, "Tenormin 100 mg". Using a simple solid phase extraction, a recovery of 70.69±3.27% and a very good separation from the urine matrix are achieved. A good reproducibility, linearity, and accuracy are obtained, and a quantitation limit of 0.1 μg/mL in urine, allows the method to be applied to pharmacokinetic studies of the compound.

INTRODUCTION

Atenolol, 4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy] benzene acetamide, is a cardioselective β-adrenoreceptor antagonist (β-blocker), widely used in the treatment of hypertension, angina pectoris, cardiac dysrhythmias, and heart attacks. The β-blockers are also subjected to certain restrictions in sports, by the Medical Commision of the International Olympic Committee (IOC) in order to prevent their abuse in sports.[1]

Following oral administration, about 50 to 60% of an atenolol dose is absorbed with maximum plasma concentrations reached within 2 to 4 hours, less than 5% of the dose is bound to plasma proteins, and most of the absorbed atenolol is excreted unchanged in the urine.

In adult patients with normal renal function, the elimination half-life is about 5 to 7 hours and total clearance is about 6 L/h per 1.73 m^2. However, there are wide intra and interindividual differences in the pharmacokinetic properties of atenolol.[2]

Procedures for the determination of atenolol in plasma and urine included phosphorence techniques,[3] HPLC with photometric detection,[4-12] HPLC with fluorimetric detection,[13-18] and Liquid Micellar Chromatography with laser induced fluorescence.[9] Ion Pair Chromatography,[20-21] and Gas Chromatography-Mass Spectrometry[22-23] were also used.

There are also several papers dealing with the use of Micellar Electrokinetic Capillary Chromatography,[24-25] and Capillary Zone Electrophoresis (CZE).[26-28] But none of these papers show a quantitative application of these techniques for the analysis of β-blockers in biological fluids. The aim of this paper is the application of a simple CZE system with photometric detection to the quantitation of atenolol in urine, preceded by a simple and fast solid-phase (SP) extraction procedure.

MATERIALS AND METHODS

Chemicals and Reagents

Methanol (HPLC grade) was purchased from Lab-Scan (Dublin, Ireland). Chloroform, glacial acetic acid, ammonia solution, disodium tetraborate, boric acid, and sodium hydroxide were Merck pro analysis (Bilbao, Spain). Isopropyl alcohol and disodium hydrogenphosphate were purchased from Fluka (Bilbao, Spain).

The water used was obtained by the Milli-RO and Milli-Q systems.

Reference standard of atenolol was provided by Sigma Chemical (Bilbao,Spain). A stock standard solution of the drug (1000 μg/mL) was prepared in water and stored at 4°C. Working solutions were prepared by appropiate dilution, just before use.

Bond-Elut Certify columns were supplied by Varian (Barcelona, Spain).

Apparatus and Electrophoretic Conditions

This work was performed in a 78 cm × 75 μm I.D. fused-silica capillary tube (Composite Metal Services LTD., United Kingdom). The effective separation distance was 70 cm.

The Capillary Electrophoresis system was a Waters Quanta 4000, and data were collected with the help of a PC and treated with the software Millenium 2010 (Waters Chromatography Division, Barcelona, Spain). The wavelength used for photometric detection was 214 nm. Injections were carried out hydrostatically for 25 s and the running voltage was 20 KV at the injector end of the capillary. The electrolyte consisted of a buffer Na_2HPO_4 (25 mM) - $Na_2B_4O_7$ (25 mM) (50+50 V/V), pH 9.7.

The capillary was conditioned every day with an initial wash cycle consisting of NaOH 1 M, 20 min, and deionized water, 20 min. Wash cycles before each injection: NaOH 0.1 M, 2 min, and running buffer, 3 min, in order to reduce fouling. Daily wash cycles after finishing experiments: NaOH 1M, 5 min, and deionized water, 5 min.

The system required some equilibration to ensure consistent migration behaviour (due to urine matrix effects). To this purpose, the analysis of blank urine samples was included at the beginning of an analytical sequence.

Procedure for Urine Samples

The pH of the urine samples (2.5 mL) was adjusted to 9 with borate buffer (500μl), vortex mixed for 5 s, and filtered through a 45 μm membrane filter.

Bond Elut Certify SP extraction columns were inserted into a vacuum manifold and conditioned by washing once with 2 mL methanol and 2 mL deionized water. The columns were prevented from drying. Filtered samples were poured into each column reservoir and drawn slowly through the column. The columns were washed with 2 mL deionized water, 1 mL 0.1 M acetate buffer (pH 4), and 2 mL methanol. Elution of the analyte was performed with 2 mL of a mixture of chloroform-isopropyl alcohol (80:20 v/v) containing 2% ammonia. The eluate was evaporated to dryness at 30°C under a stream of nitrogen, using a Zymark Turbo Vap LV evaporator (Barcelona, Spain). The residue was dissolved in 100 μl of electrolyte (sometimes a smaller volume of electrolyte was used) and measured under calibration conditions.

The reproducibility and efficiency of the extraction procedure was determined by extracting replicate spiked urine samples doped with 2 ppm of atenolol. The urine samples were obtained from 4 different volunteers, and as no significant diferences were observed in between these samples (in relation to matrix interferences and recoveries after the Solid-Phase extraction), 10 replicates were arbitrarily analysed for the determination of the percentage of recovery. A quantitative recovery of (mean value ± R.S.D. %) 70.69±3.27 was achieved (n=10).

RESULTS AND DISCUSSION

The primary objective of this work was the development of a validated Capillary Electrophoresis method for the quantitation of the β-blocker atenolol in human urine samples. Several parameters were optimized to ensure a reproducible and accurate method.

The samples were injected hydrostatically for 25s and the running voltage was 20kV inducing a 48μA current across the capillary. Electrokinetic injection was also assayed, and although improved, the sensitivity proved to be quite irreproducible, because a sampling bias is introduced based on the ionic

charge of the molecule. The introduction of another β-blocker as internal standard did not improve the area reproducibility, with an R.S.D around 10%, The R.S.D is calculated by the formula R.S.D = (standard deviation/mean of the peak areas)·100%.

A linear increase in peak area and height were observed with increasing hydrostatical injection times, since the amount of sample introduced into the capillary also increases linearly. For sampling times longer than 25 s no further peak height increase was observed, due to band broadening effects, therefore an optimum 25 s injection time was chosen.

The effect of temperature on the migration time of atenolol was studied. A decrease in migration times with increasing temperature was observed, as well as higher currents inside the capillary which caused baseline disturbances. The temperature was kept constant at 25°C to ensure reproducible results.

The length and diameter of the capillary were also optimized. Shorter capillaries didn't provide enough resolution between atenolol and the endogenous substances present in the urine, therefore an optimum effective length of 70 cm was chosen. Internal diameters smaller than 75 μm didn't provide enough sensitivity.

The study of the influence of pH and composition of the electrolyte gave an optimum value of pH 9.7 and a Na_2PO_4(25mM):$Na_2B_4O_7$(25mM) (50:50; V:V) ratio.

Once the optimum electrophoretic conditions had been established, a quantitative method for the determination of atenolol in urine samples was developed, (Table 1).

The relative standard deviation of the retention times is less than 1% for both intra and interday assays (intraday reproducibility determined by injecting replicate samples, n=10; and n=5 for the interday reproducibility), thus indicating high stability for the system.

The accuracy of the method was determined by the analysis of 5 control urine samples (obtained from 4 different volunteers) and spiked with 2 ppm of atenolol. Aceptable accuracy, defined as mean (found concentration/actual concentration) × 100, was achieved: 98.29 ± 2.45 %. The experimental quantitation limit, defined as the minimum atenolol concentration in urine which gives rise to a signal able to be quantified by the computer program used (after extraction of 2.5mL urine and final preconcentration to 25μl), was 0.1μg/mL.

Table 1

Determination of Atenolol*

Migration Time ± S.D. (min.)	6.54 ± 0.06
Linear Range	10-140μg/mL
Slope of Calibration Graph (area/conc.)	373.34
r^2	0.998
Area Repeatability Intraday R.S.D. (%)	0.26[a]
Area Repeatability Interday RS.D. (%)	2.24[b]
Experimental Detection Limit (μg/mL)	10

* The optimization of the electrophoretic system and estimation of the given parameters was done with spiked urine samples. (For electrophoretic conditions see the experimental section.)

[a] Five determinations at the 30ppm level.

[b] Three determinations at the 30ppm level.

Analytical Applications

The method developed was applied to the determination of atenolol in urine samples obtained from patients suffering from hypertension and under medical treatment with atenolol (Tenormin 100mg) and with a pharmaceutical combination of atenolol and chlorthalidone (Tenoretic), and urine samples obtained from a healthy volunteer after the ingestion of atenolol (Tenormin 100mg), Figure 1.

Urine was collected at different time intervals for the quantitative determination of the β-blocker: 0-2 hours, 2-4 hours, 4-8 hours and 8-24 hours. Urine samples were treated following the clean-up procedure described in the experimental section.

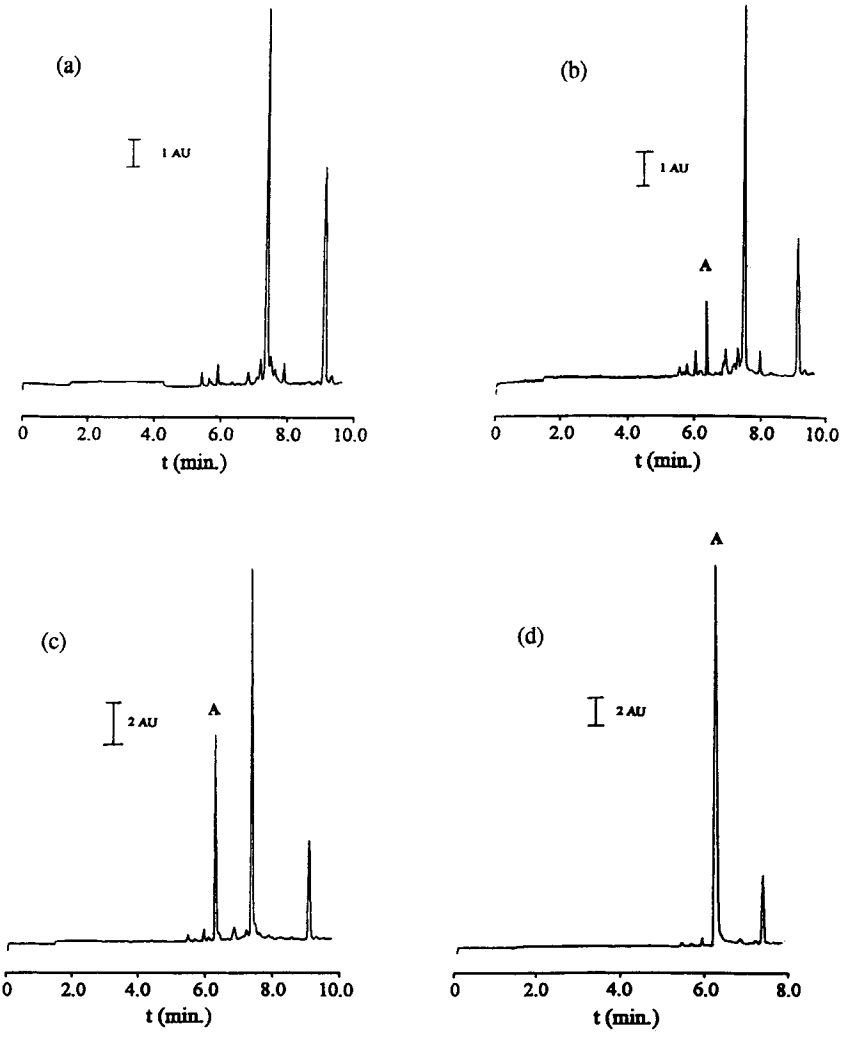

Figure 1. Electropherograms obtained from an extract of: a) Blank urine sample, b) urine sample spiked with 0.4ppm of atenolol c) Urine sample 4-8hours after the oral administration of 1 tablet of "Tenoretic" (atenolol 100mg + Chorthalidone 25mg) d) Urine sample 4-8hours after the oral administration of 1 tablet of "Tenormin 100mg" (atenolol 100mg).

Table 2

Quantitative Determination of Atenolol in Urine

Urine Samples	Time Inverval (Hours)	Atenolol (mg)	Total Atenolol Amount (mg)
Patient under	0-2	1.43	
treatment with	2-4	12.76	35.25
"Tenormin 100mg"	4-24	21.06	
Patient under	0-2	1.07	
treatment with	2-4	0.19	
"Tenoretic"*	4-8	4.55	
	0-2	1.35	
Healthy	2-4	6.07	
Volunteer	4-8	6.74	25.06
"Tenormin 100mg"	8-24	10.9	

* It was not possible to collect samples between 8 and 24 hours.

The compound was easily detected at all time intervals and the found concentrations, collected in Table 2, were in agreement with the pharmacokinetic data[2]. A considerably minor excretion was observed for the patient taking the pharmaceutical combination "Tenoretic". This decrease in the excretion was probably due to the effect of the diuretic Chlorthalidone.

DISCUSSION

The described CZE method was succesful for the determination and quantitation of atenolol in real human urine samples. The clean-up procedure is very simple and effective, and the electrophoretic separation is made in less than 8 minutes.

The assay validation was adequate in terms of reproducibility, linearity, and accuracy, showing that the CZE can also be used with quantitative purposes for the analysis of β-blockers.

The limit of quantitation of 0.1 μg/mL (with a preconcentration of a 100 factor) is sensitive enough for the determination of free atenolol in urine samples at all time intervals studied. A further preconcentration could also be possible, improving the forementioned limit of quantitation.

The poor sensitivity of the CZE-Photometric Detection method can be overcome by the preconcentration of the samples, as only a very low volume is needed for the injection.

REFERENCES

1. Medical Commission, International Olympic Committee, "List of Doping Classes and Methods," in International Olympic Charter against Doping in Sport, IOC, Lausane, Switzerland, 1990, (update May 1992).

2. A. N. Wadworth, D. Murdoch, R. N. Brogden, Drugs, **42(3)**, 468-510 (1991).

3. L. M. Cabalín, J. J. Laserna, A. Rupérez, Anal. Chim. Acta, **270**, 239-245 (1992).

4. C. Verghese, A. McLeod, D. Shand, J. Chromatogr. B, **275**, 367-375 (1983).

5. A.Tracqui, P. Kintz, J. Himber, A. A. J. Lugnier, P. Mangin, Forensic Sci. Int., **38**, 37-41 (1988).

6. M. Johansson, H. Forsmo-Bruce, J. Chromatogr., **432**, 265-272 (1988).

7. M. T. Kelly, M. R. Smyth, D. Dadgar, J. Chromatogr., **473**, 53-62 (1989).

8. M. T. Kelly, M. R. Smyth, J. Pharm. & Biomed. Anal., **7(12)**, 1757-1764 (1989).

9. M. Bogusz, M. Wu, J. Anal. Toxicol., **15**, 188-197 (1991).

10. M. S. Leloux, F. Dost, Chromatographia, **32(9/10)**, 429-435 (1991).

11. W. E. Lambert, E. Meyer, A. P. Leenheer, J. Anal. Toxicol., **19(2)**, 73-78 (1995).

12. M. T. Saarinen, H. Sirén, M. L. Riekkola, J. Chromatogr. B, **664**, 341-346 (1995).

13. K. U. Bühring, A. Garbe, J. Chromatogr. B, **382**, 215-224 (1986).

14. R. Mehvar, J. Pharm. Sci., **78(12)**, 1034-1039 (1989).

15. S. K. Chin, A. C. Hui, K. M. Giacomini, J. Chromatogr. B, **489**, 438-445 (1989).

16. T. A. Kolbah, F. Plavsi´c, A. W. Coporda, J. of Pharm. & Biomed. Anal., **7(12)**, 1777-1781 (1989).

17. A. D. Dale, S. E. Turner, J. Pharm. & Biomed. Anal., **8 (8-12)**, 1055-1058 (1990).

18. R. G. Morris, N. C. Saccoia, B. C. Sallustio, R. Zacest, Ther. Drug Monit., **13**, 345-349 (1991).

19. I. Carretero, M. Maldonado, J. J. Laserna, Química Analítica, **11(2)**, 107-119 (1992).

20. H. Siren, M. Sarinen, S. Hainari, P. Lukkari, M. L. Riekkola, J. Chromatogr., **632**, 215-227 (1993).

21. P. Lukkari, H. Sirén, J. Chromatogr. A., **717**, 211-217 (1995).

22. F. T. Delbeke, M. Debackere, N. Desmet, F. Maertens, J. Chromatogr., **426**, 194-201 (1988).

23. A. Solans, M. Carnicero, R. de la Torre, J. Segura, J. Anal. Toxicol., **19**, 104-114 (1995).

24. P. Lukkari, H. Sirén, M. Pantsar, M. L. Riekkola, J. Chromatogr., **632**, 143-148 (1993).

25. P. Lukkari, T. Nyman, M. L. Riekkola, J. Chromatogr. A, **674**, 241-246. (1994).

26. E. Gonzalez, J. J. Laserna, Electrophoresis, **15**, 240-243 (1994).

27. J. M. Vadillo, M. E. Gonzalez, I. Carretero, J. J. Laserna, Mikrochim. Acta, **118**, 273-282 (1995).

28. J. C. Hudson, M. Golin, M. Malcolm, Can. Soc. Forens. Sci., **28(2)**, 137-152 (1995).

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