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Research Article

Analysis of Atenolol in Rabbit Plasma by HPLC Method

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Abstract

Atenolol is one of the most used drugs today. Therefore, it is important to develop new and simple methods for the determination of atenolol. The goal of this research is to develop a high-performance liquid chromatography method for analyzing atenolol levels in rabbit plasma and apply this method to the pharmacokinetic study. The liquid-liquid extraction technique was used to prepare blood samples from rabbits. Separation of atenolol was achieved on an Ace C_{18} column. The method's calibration curve was plotted between 5 and 250 ng/mL. The accuracy results were better than 2.97% and the precision results were less than 6.30% in rabbit plasma for atenolol. The method had recovery values >93.1% for all samples in rabbit plasma. In addition, the validated method was used to study atenolol pharmacokinetics in rabbits. The maximum atenolol plasma concentration is 240.1 ± 33.41 ng/mL. The duration to attain the greatest atenolol concentration and the area under the curve from ($AUC_{0\text{-}12\ h}$) were 3.0 ± 0.64 h and 1184.1 ± 235.13 ng/mL h, respectively.

Keywords: Atenolol, HPLC, extraction, pharmacokinetic, rabbit

Tavşan Plazmasında Atenololün HPLC Yöntemi ile Analizi

Öz

Atenolol günümüzde en çok kullanılan ilaçlardan biridir. Bu nedenle, atenolol tayini için yeni ve basit yöntemlerin geliştirilmesi önemlidir. Bu araştırmanın amacı tavşan plazmasında atenololün analizi için bir yüksek performanslı sıvı kromatografi yöntemi geliştirmek ve farmakokinetik çalışmaya uygulamaktır. Tavşan kan örnekleri sıvı-sıvı ekstraksiyon yöntemi ile hazırlanmıştır. Atenololün ayırımı Ace C_{18} kolon ile yapılmıştır. Yöntemin kalibrasyon eğrisi 5 ve 250 ng/ng/ arasında çizildi. Tavşan plazmasında atenolün kesinlik sonuçları %6.30'dan küçüktü ve doğruluk sonuçları %2.97'den daha iyiydi. Yöntemin tavşan plazmasındaki tüm örnekler için geri kazanım değerleri >%93.1'dir. Ayrıca, tavşanlarda atenololün farmakokinetiğini incelemek için valide edilen yöntem uygulanmıştır. Maksimum atenolol plazma konsantrasyonu 240.1 ± 33.41 ng/mL idi. Atenololün maksimum konsantrasyona ulaşma süresi ve eğrinin altındaki alan ($AUC_{0\text{-}12\ h}$) sırasıyla 3.0 ± 0.64 h ve 1184.1 ± 235.13 ng/mL h idi.

Anahtar Kelimeler: Atenolol, HPLC, ekstraksiyon, farmakokinetik, tavşan

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1. Introduction

The chemical formula structure of atenolol is 4-(2-hydroxy-3-isopropyl-aminopropoxy) phenylacetamide (Figure 1). It is a type of β-blockers constitute. It has been widely used to angina pectoris, hypertension, myocardial infarction and cardiac arrhythmias (Al-Ghannam, 2006). Therefore, atenolol may be used alone or concomitantly with other antihypertensive agents (Pires de Abreu et al., 2003).

In literature research, UV-Visible spectrophotometry (Saleem, 2019; Antakli et al., 2020; Mohammad et al., 2019; Vaikosen et al., 2020), spectrofluorometry (Tabrizi and Yousefzadeh, 2019), high-performance liquid chromatography (HPLC) (Pires de Abreu et al., 2003; Iha et al,. 2002; Chiu et al., 1997; Madhusudhan et al., 2018; El-Alfy et al., 2019; Elkady et al., 2020), gas chromatography-mass spectrometry (GC-MS) (Yilmaz et al., 2009) and capillary electrophoresis (Arias et al., 2001) methods for determining atenolol in invitro conditions and biological materials have been reached.

However, the HPLC method was not available for detecting atenolol in rabbit plasma. Therefore, a new and easy HPLC approach for the analysis of atenolol in rabbit plasma was devised in this study. For this, liquid-liquid extraction technique and fluorescence detection were used to increase sensitivity. The developed method was then validated with respect to the Center for Drug Evaluation and Research guidelines (CDER, 2001).

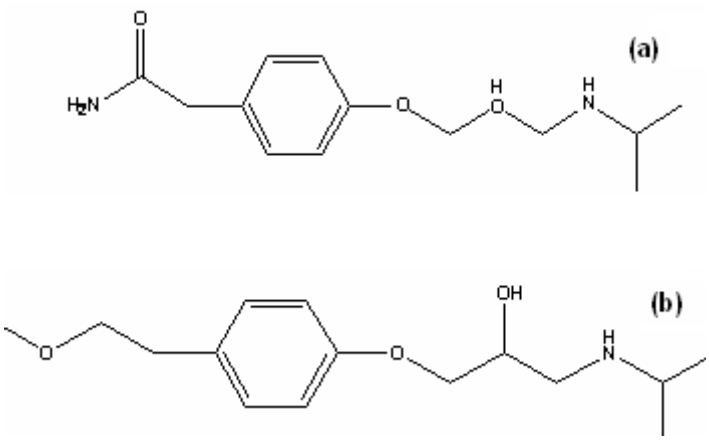

Figure 1. Atenolol (a) and metoprolol (b)

The presented method is based on a simple and single extraction step in a short time using inexpensive chemicals. At the same time, the approach was also used to examine atenolol levels in rabbit plasma. For this, Tensinor tablet containing atenolol was given to six rabbits. Bloods was drawn from the rabbits at different times. The analysis was performed after extraction. From the results obtained, the plasma pharmacokinetic parameters of atenolol were calculated.

2. Materials and Methods

2.1 Chemicals and reagents

Atenolol (99.6% purity) was obtained from Abdi Ibrahim Pharmaceutical Industry. Ethylacetate, chloroform, butanol, methanol, acetonitrile and metoprolol (IS, ≥98% purity) were acquired from Sigma-Aldrich. Tensinor tablet that included 50 mg atenolol was purchased.

2.2 HPLC system and chromatographic conditions

HPLC analyses were performed with the Agilent HPLC system. In this work, an Ace C_{18} column (4.6×250 mm, 5 μm) was used. 20 μLs injection volume was selected for the analysis. As the mobile phase, methanol-water (50:50, *v/v*) containing 0.1% TFA was employed with a flow rate of 1 mL/min. Fluorescence detector was used employed for HPLC system. Fluorescence detection of atenolol and IS were selected as 276 nm and 296 nm (excitation and emission), respectively.

2.3 Preparation of standard and quality control samples

Methanol was used to make a 1.0 mg/mL atenolol solution. Atenolol standard solutions were diluted with methanol. Standard calibration samples were prepared 05-250 ng/mL (5, 15, 45, 100, 150, 200 and 250 ng/mL). The solutions were all kept at 4 ^{0}C. Atenolol quality control standard solutions were produced 10, 125 and 225 ng/mL.

2.4 Extraction procedure

The extraction procedure in rabbit plasma samples was carried out using the liquid-liquid extraction method. Butanol, acetonitrile, hexane, dichloromethane, chloroform and ethylacetate were tried to find the most suitable extraction solvent. In the end, 5 mL chloroform and butanol mixture (4:1, *v/v*) was chosen.
0.5 mL rabbit plasma was used in the study. 0.1 mL atenolol standard solutions (5, 10, 25, 50, 100, 150 and 250 ng/mL) containing 20 μL IS solution were added and then 1 M 0.5 mL sodium hydroxide solution was added. The vortex procedure was performed for 30 seconds. 5 mL chloroform and butanol mixture (4:1, *v/v*) was added in rabbit plasma. The rabbit plasma sample was vortexed for 30 seconds, and the plasma was then centrifuged for 5 minutes at 3000 × g. The supernatant was transferred into another plastic tube. The supernatant was vaporized under nitrogen gas. The dry residue sample was dissolved in 1 mL methanol and then 20 μL plasma sample was analyzed in HPLC system.

2.5 Rabbits

Before starting the research, the ethics committee report was obtained from the Ethical Committee for Medical Experimental Research and Application Centre of Ataturk University.

12.5 mg kg^{-1} of atenolol were six rabbits by oral administration. 2.0 mL blood was taken from the ear vein of six rabbits at 0, 0.5, 1, 2, 3, 4, 6, 8 and 12 h. EDTA tubes were used to collect the bloods. The blood samples were centrifuged. The plasma samples were taken, and samples were extracted. After that, they are analyzed by the HPLC system.

2.6 Statistical analysis

The statistical analyses were done with SPSS V.15.0 version at computer program. Regression analyses were used in the preparation of the atenolol standard line and calculations. The results were given with the mean ± standard deviation.

3. Results and Discussion

3.1 Method development and optimization

In this work, the reversed-phase column (C_{18}) was used because atenolol is a polar molecule. For the sensitivity of the HPLC method, fluorescence detection was used to atenolol and IS. Several mobile phase components were performed to achieve a good peak shape and resolution in chromatogram. In the end, methanol-water (50:50, *v/v*) containing 0.1% TFA was employed as the mobile phase. Atenolol retention time was quite short than that studied by Giachetti et al., 1997; Iha et al., 2002). In the proposed HPLC method, the buffer mobile phase system was not used as in the previously published articles (Chatterjee et al., 1995; Giachetti et al., 1997). In this research, there is no column washing after the analysis. This is an advantage of the method. In HPLC chromatogram, atenolol and IS retention times were 2.8 and 4.7 min, respectively. A representative chromatogram of atenolol and IS was given in Figure 2.

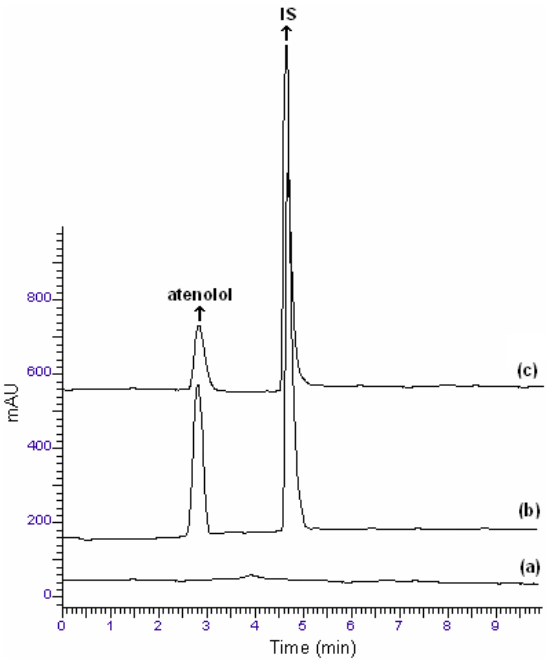

Figure 2. (a) Pre-drug rabbit plasma chromatogram, (b) rabbit plasma chromatogram spiked with 100 ng/mL atenolol, (c) rabbit plasma chromatogram obtained 6h after administration 12.5 mg/kg of atenolol

3.2 Validation of the method

HPLC method was validated with validation parameters according to CDER. These parameters were selectivity, linearity, precision, accuracy, recovery, limits of detection (LOD) and quantification (LOQ), stability and dilution integrity.

i. Selectivity

The selectivity of the developed technique was checked by comparing the chromatograms of spiked rabbit plasma with the blank rabbit plasma. Atenolol and IS retention times were approximately 2.8 and 4.7 min (Figure 2). The symmetry of the peaks and the short retention times are the advantages of the method. In addition, the blank plasma sample was analyzed. Endogenous interferences were not observed.

ii. Linearity

Standards calibration curves were drawn according to peak area ratio (*y*) of atenolol and IS versus atenolol concentration. It was found to be linear over the 5-250 ng/mL concentration range for atenolol. The mean calibration equation of method is y=0.1824x + 0.0192. The correlation coefficient value was higher than 0.99 for the mean calibration curve (n=3).

iii. Precision and accuracy

Intra-day and inter-day precision were used to assess the proposed method's precision. Six replicates for each of three different concentrations (7.5, 75, 175 ng/mL) were analyzed to determine intra-day precision. The same samples of plasma were analyzed in three successive days to measure the intermediate precision. The percent relative standard deviation (RSD %) was used to assess precision.

In addition, the percentage relative error was used to assess the method's accuracy. The precision and accuracy were gratifying for atenolol from plasma samples. RSD is obtained as lower than 6.27%. In addition to this, accuracy is detected to be within ± 2.97% with relative error. It is understood that both the precision and the accuracy of this method are good in statistical comparison with previously reported methods (Miller et al., 1991; Iha et al., 2002).

iv. Recovery

In order to prepare the samples at rabbit plasma, the extraction technique was used for this work. Therefore, dichloromethane, ethylacetate, butanol and chloroform were tried. In the end, chloroform and butanol mixture (4:1, *v/v*) was decided to be used as the most suitable solvent for extraction. Atenolol recovery values of rabbit plasma are between 93.1 and 98.6% (Table 1). The mean recovery of atenolol was 96.1% in rabbit plasma.

Table 1. Recovery of atenolol in human plasma

Added (ng/mL)	Found (Mean ± SD[a])	% Recovery	% RSD[b]
5	4.9 ± 0.23	98.0	4.69
10	9.4 ± 0.47	94.0	5.00
25	24.1 ± 0.63	96.4	2.61
50	48.1 ± 2.32	96.2	4.82
100	98.6 ± 5.59	98.6	5.67
150	145.1 ± 6.49	96.7	4.47
250	232.8 ± 8.62	93.1	3.70

SD[a]: Standard deviation of six replicate determinations, RSD: Relative standard deviation

[b]Average of six replicate determinations

In the literature, atenolol was extracted with a solid-phase extraction method from human plasma in previous studies (Chatterjee et al., 1995; Arias et al., 2001; Miller et al. 2002). The solid-phase extraction technique is an expensive method. In our technique, the liquid-liquid method in plasma from rabbits is used as the extraction method. In addition, the extraction technique was very simple, cheap and could be done in one step.

v. Limits of detection (LOD) and quantification (LOQ)

The LOD value was evaluated as the minimum concentration in this work. The reason behind this is the fact that the signal-to-noise ratio of it is found to be 3 for atenolol at HPLC chromatogram. In addition, the LOQ value was evaluated as the minimum concentration of the plasma spiked with atenolol. The LOD and LOQ values were 1.5 and 5.0 ng/mL in the work, respectively. Both precision and accuracy of the LOD and LOQ values were within the criteria (CDER, 2001). In addition, this method is better than reported previosly methods (Giachetti et al., 1997; Miller et al., 1991).

vi. Stability

Atenolol stock solution stability was evaluated for at least 72 hours at room temperature. In addition, the stabilities of atenolol in rabbits were investigated under various storage conditions. By analyzing the low and high concentrated samples (15 ng/mL and 225 ng/mL), the method's short-term stability was determined. Therefore, the samples were thawed at room temperature. They were stored in room condition for 24 h. The samples were analyzed at -20 °C for three

days to determine long-term stability. No significant degradation product of atenolol in the storage conditions.

vii. Dilution integrity

The dilution integrity was performed on higher atenolol concentrations above the upper LOQ. The accuracy and precision of atenolol were between 97.9 to 102.1 and 2.46 to 3.27 % for 1/5th and 1/10th dilution.

3.3 Pharmacokinetic analysis

Pharmacokinetic results of atenolol were calculated in rabbit plasma by the linear trapezoidal rule (Yilmaz et al., 2009). In addition, Figure 3 illustrates the average atenolol concentration-time curve for six rabbits.

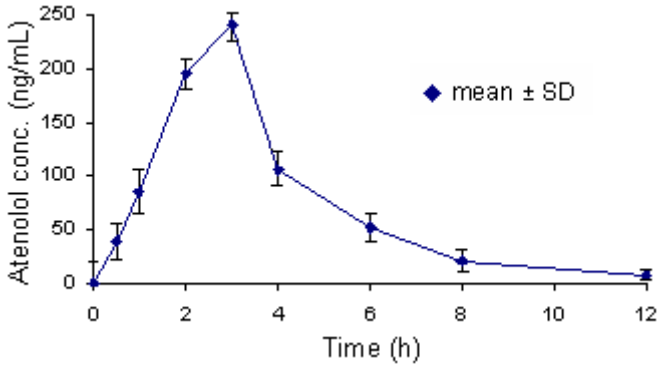

Figure 3. Atenolol concentration-time profile in rabbit plasma (n=6).

The mean $AUC_{0\text{-}12h}$, C_{max}, T_{max} and $t_{1/2}$ of atenolol obtained from six rabbits were 1184.1 ± 235.13 µg/mL h, 240.1 ± 33.41 ng/mL, 3.0 ± 0.64 h and 3.18 ± 0.41 h, respectively. Using this method, the obtained pharmacokinetics results are in agreement with the previously researh papers (Giachetti et al., 1997; Iha et al., 2002).

However, in the previously papers especially it has been used with 1.0 mL plasma (Giachetti et al., 1997; Iha et al., 2002). In the proposed method, low plasma volume (0.5 mL) is used This can be advantageous in pharmacokinetic studies. Because, atenolol is one of the most used β-blockers drugs today. Therefore, it is important to develop and validate new and simple methods for the determination of atenolol. In the literature, there are different chromatographic studies on the determination of atenolol in biological materials. Among these, the most used methods are usually HPLC, capillary electrophoresis and GC-MS.

The GC-MS method is a widely used chromatographic method for the analysis of volatile or volatile substances. In this study, atenolol, a polar compound, was volatilized by derivatization. In this way, the sensitivity of the study was increased. With this, it was possible to determine the amount of atenolol in rabbit plasma even at low concentrations. However, the GC-MS

method is not always accessible because it is a very expensive method. Therefore, the HPLC method developed in this study is cheaper than the GC-MS method.

4 Conclusion

In this research, a new and fast HPLC technique has been completely developed in order to analyze atenolol in rabbit plasma. Furthermore, validation parameters were used to validate the procedure. The advantages of the method are that 0.5 mL of plasma is sufficient for the application of the method and that it has an easy extraction method. In addition, the proposed method was applied to six rabbits. Pharmacokinetic parameters were calculated in rabbit plasma. Therefore, the proposed method can be easily used in the plasma of people using atenolol in the clinic.

Ethics in Publishing

Before starting the study, the ethics committee report was obtained from the Ethical Committee for Medical Experimental Research and Application Centre of Ataturk University (2009/4).

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References

Al-Ghannam, S.M. 2006. ‘‘A simple spectrophotometric method for the determination of β-blockers in dosage forms’’, *Journal of Pharmaceutical and Biomedical Analysis,* 40, 151-156.

Antakli, S., Nejem, L., Joumaa, M.A. 2020. ‘’Determination of atenolol in tablet formulation by analytical spectrophotometry’’. *Research Journal of Pharmacy and Technology,* 13(2), 609-614.

Arias, R., Jimenez, R.M., Alonso, R.M., Telez, M., Arrieta, I., Flores, P., Ortiz-Lastra, E. 2001. ‘‘Determination of the β-blocker atenolol in plasma by capillary zone electrophoresis’’, *Journal of Chromatography A,* 916, 297-304.

Chatterjee, D.J., Li, W.Y., Hurst, A.K., Koda, R.T. 1995. ‘‘High-performance liquid chromatographic method for determination of atenolol from human plasma and urine: simultaneous fluorescence and ultraviolet detection’’, *Journal of Liquid Chromatogr,* 18, 791-806.

Chiu, F.C., Zhang, J.N., Li, R.C., Raymond, K. 1997. ‘‘Efficient assay for the determination of atenolol in human plasma and urine by high-performance liquid chromatography with fluorescence detection’’, *Journal of Chromatography B,* 691, 473-477.

El-Alfy, W., Ismaiel, O.A., El-Mammli, M.Y., Shalaby, A. 2019. ‘’Determination of atenolol and trimetazidine in pharmaceutical tablets and human urine using a high-performance liquid chromatography-photo diode array detection method’’, *International Journal of Analytical Chemistry.* Article ID 9625849.

Elkady, E.F., Fouad, M.A., Faquih, A.A. 2020. ‘’A versatile stability-indicating liquid chromatographic method for the simultaneous determination of atenolol, hydrochlorothiazide and chlorthalidone’’, *Current Pharmaceutical Analysis,* 16(8):1037-1051.

Giachetti, C., Tencoti, A., Canali, S., Zanolo, G. 1997. ‘’Simultaneous determination of atenolol and chlorthalidone in plasma by high-performance liquid chromatography Application to pharmacokinetic studies in man’’, *Journal of Chromatography B,* 698, 187-194.

Guidance for Industry, Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Rockville, MD, 2001.

Iha, M.H., Martinez, A.S., Bonato, P.S. 2002. ‘’Enantioselective analysis of atenolol in biologic fluids: comparison of liquid-liquid and solid-phase extraction methods’’, *Journal of Chromatography B,* 767, 1-9.

Li, W., Li, Y., Francisco, D.T., Naidong, W. 2005. ‘’Hydrophilic interaction liquid chromatographic tandem mass spectrometric determination of atenolol in human plasma’’, *Biomedical Chromatography,* 19, 385-393.

Madhusudhan, P., Reddy, K.V., Singh, S. 2018. ‘’A novel isocratic RP-HPLC method development and validation for simultaneous determination of atenolol and nifedipine in tablet dosage form’’, *Universal Review* 7(9): 412-421.

Miller, R.B. 1991. ‘’A validated high-performance liquid chromatographic method for the determination of atenolol in whole blood’’, *Journal of Pharmaceutical and Biomedical Analysis,* 9, 849-853.

Mohammad, M.Y., Abdullah, M.S., Sabir, S.S. 2019. ‘’Simultaneous determination of atenolol and amlodipine using second derivative spectroscopy’’, *Polytechnic Journal,* 9(2), 25-29.

Morris, R.G., Saccoia, N.C., Sallustio, B.C., Zacest, R. 1991. ‘’Improved high-performance liquid chromatography assay for atenolol in plasma and urine using fluorescence detection’’, *Therapeutic Drug Monitoring,* 13, 345-349.

Pires de Abreu, L.R., Calafatti de Castro, S.A., Pedrazzoli Jr, J. 2003. ‘’Atenolol quantification in human plasma by high-performance liquid chromatography: application to bioequivalance study’’, *AAPS PharmSci,* 5, 1-7.

Saleem, B.A. 2019. ‘’Spectrophotometric determination of atenolol using ındigo carmine dye’’, *Kirkuk University Journal for Scientific Studies,* 14(2), 15-39.

Sethi, P.D. 2001. ‘‘High Performance Liqid Chromatography-Quantitative Analysis of Pharmaceutical Formulations’’, *CBS Publishers & Distributors, Mumbai,* 3-212.

Tabrizi, A.B., Yousefzadeh, F. 2019. ‘’Spectrofluorimetric determination of atenolol and carvedilol in pharmaceutical preparations after optimization of parameters using response surface methodology’’, *Pharmaceutical Sciences*, 25(3), 262-267.

Vaikosen, E.N., Bioghele, J., Worlu, R.C., Ebeshi, B.U. 2020. ‘’Spectroscopic determination of two beta-blockers–atenolol and propanolol by oxidative derivatization using potassium permanganate in alkaline medium’’, *Reviews in Analytical Chemistry,* 39(1): 56-64.

Yilmaz, B., Arslan, S. 2009. ‘‘GC-MS determination of atenolol plasma concentration after derivatization with N-Methyl-N-(trimethylsilyl) trifluoroacetamide’’, *Chromatographia,* 70(9-10), 1399-1404.