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Comparative study of atenolol in human plasma by high performance liquid chromatography and capillary zone electrophoresis

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Abstract

Atenolol is a beta-blocker that is cardioselective, meaning it only affects beta receptors. It is used to treat angina pectoris and high blood pressure. The HPLC and Capillary Zone Electrophoresis analytical technique was developed for the purpose of detecting and quantifying Atenolol in human plasma, according to the study paper you're reading right now. The internal standard and atenolol were recovered from the solution after being extracted from plasma using the Liquid-Liquid Extraction method. A mobile phase of 10mM sodium hydrogen phosphate, 7.3mM Sodium Lauryl sulphate (pH=3), methanol, and acetonitrile (40:57:3, v:v:v) is used, with a flow rate of 1.0ml/min. A fluorescence detector was used to detect the isolated materials, which had an excitation wavelength of 229 nm and an emission wavelength of 298 nm. With this in view, Atenolol's and the internal norm's survival times are observed to be 5.4 and 8.3 minutes, respectively. The linear correlation coefficient (R20.9992) was found in the Atenolol calibration curve. The recovery rate for atenolol and an internal norm was estimated to be between 76 and 87 percent. Solid-phase extraction was performed on an uncoated silica capillary with a diameter of 58.5 cm 75 m, and detection was performed at 194 nm in the Capillary Zone Electrophoresis procedure. For an electrolyte solution containing 50mM H3BO3 and 50mM Na2B4O7 (50:50 V/V), atenolol was determined to be present in the solution in less than 3 minutes. Energized with a voltage of 25kV and injected with a hydrodynamic configuration for 4S. Under various conditions, this method was used to assess the stability and capability of measuring Atenolol in human plasma.

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INTRODUCTION

Atenolol is a beta-blocker medication that is often used to control high blood pressure and heart disease (specifically chest pain due to heart disease) (Atenolol Monograph for Professionals, 2018). 4-(2-hydroxy-3-isopropylaminopropoxy) phenylacetamide, also known as atenolol, is a beta-adrenergic receptor blocking agent used in the Indian Pharmacopoeia [Indian pharmacopoeia;1996]. While atenolol is quickly absorbed, just about half of it reaches the gastrointestinal tract. During oral administration, the average amount of atenolol absorbed is around half of the

total atenolol concentration. The rest is excreted in its natural state in the urine. The kidneys are the main organs from which atenolol is removed (Wander *et al.*, 2009; Heel *et al.*, 1979). Atenolol has antihypertensive effects, which are due to its blood pressure-lowering effect, which is achieved with a single daily dosage (Miller, 1991). A peak plasma concentration of 200-300 ng/ml is obtained as a result of this procedure (Braza *et al.*, 2000).

HPLC (Chiu *et al.*, 1997; Chatterjee *et al.*, 1995; Morris *et al.*, 1991), Liquid chromatography-tandem mass spectrometry (Li *et al.*, 2007; Gonzalez *et al.*, 2010), and GC-MS (Yilmaz and Arslan, 2009; Amendola *et al.*, 2000) are some of the methods used to determine atenolol.

The measurement of atenolol in human plasma is generally done using fluorometric techniques (Dale and Turner, 1990; Miller, 1991). Many of these methods (De Abreu *et al.*, 2003; Hebert *et al.*, 2005) depend on time-consuming multi-step liquid or solid-phase extraction procedures. As a result, minimum sample volume requirements have been established (Spanakis and Niopas, 2013; Gwak and Chun, 2007). Figure 1 shows the structural equations for atenolol.

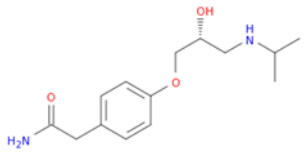

Figure 1: Structure of Atenolol

The overall goal of this research is to develop and validate a straightforward, selective, and repeatable HPLC mechanism. Capillary electrophoresis is being used to demonstrate the procedure's suitability for this function. HPLC and CZE are used to determine the amount of atenolol in human plasma using Ses techniques.

MATERIALS AND METHODS

HPLC and Capillary Zone Electrophoresis

Apparatus

A Waters Alliance HPLC 2695, which includes a pump, autosampler, and fluorescence detector, was used for chromatography. Using the empower chromatography manager method, data was obtained on a computer.

A Hewlett Packard HP 3D CE capillary electrophoresis device with a diode array detector was used to carry out CZE.

Chemicals and Reagents

Both reagents were of the highest analytical reagent grade. Atenolol and nadolol are acquired from a pharmaceutical producer. These products were all of HPLC quality: Acetonitrile, methanol, 85% phosphoric acid, sodium hydroxide, sodium lauryl sulphate, and sulfuric acid were all HPLC grade. Hollow-polymer ion-exchange (HP-HILIC) grade water was prepared using reverse osmosis and was further filtered using a Milli-Q method.

In the CZE, Merck provides analytically pure Methanol and n-hexane; the majority of the chemicals were supplied by Merck.

Chromatographic Conditions

A Symmetry shield RP_{18} 4.5×150mm column, guarded by Pak pre-column module with Nova-Pak C_{18}, was used. Mobile phase containing 10mM Sodium hydrogen phosphate (ph=3) mixed with 7.3mM sodium lauryl sulphate, methanol and acetonitrile (40:57:3, v:v:v). And it was filtered through a 0.45μm size and delivered at a flow rate of 1.0 ml/min. A fluorescence detector is used and its wavelength of 229 and 289nm.

Electrophorectic Conditions

The fused silica capillaries had an I.D. of 58.5mm and an O.D. of 75μm with a detection window of 50mm. This allowed for the detection wavelength to be 194nm. The capillary temperature was set at 25.0°C ± 0.1°C. Buffer was formed by mixing 50mM sodium tetraborate and 50mM boric acid at a ratio of 50:50 (v/v), which produced a pH of 9. Although the concentration of the salt and acid is 0.5M, the buffer was used to carry out the clean-up process, which allows the buffer to be polluted.

Capillary Conditioning

Capillary was primed on a daily basis with an initial wash cycle consisting of 15 minutes of 1M NaOH treatment, 10 minutes of deionized water, and 5 minutes of electrolyte run. The capillary was rinsed at the end of each injection. The capillary was rinsed with 1M NaOH for 10 minutes before being rinsed with deionized water for 10 minutes in the regular experiment. It was then flushed for three minutes of fresh air.

Experiments

High-Performance Liquid Chromatography

Preparation of Standard and Quality Control Samples

A stock solution of Atenolol and IS were prepared in Methanol and diluted with blank human plasma to produce a working solution of 10μg/ml

and 2.0μg/ml. Nine calibration standards in the range of 0.01-15μg/ml and 4 quality control samples(0.01,0.03,0.8 & 1.4μg/ml) were prepared in human plasma. Samples are vortexed for 1min & transferred to glass culture tubes and stored at -20^o C until used.

Sample Preparation

In each tube, 100μl of IS solution & 100μl of 0.1M NaOH was added and vortexed for 10sec. After that, with the addition of 3.5ml of solvent, again the samples are vortexed for 1min and centrifuged at 4000rpm for 1min. The organic layer was transferred into a clean tube containing 0.05M Sulfuric acid. 150μl of the aqueous layer was transferred into an autosampler & 50μl were injected into an HPLC system with a run time of 10mins (Yusuf *et al.*, 2016).

Method Validation

The method was validated according to USFDA guidelines: The parameters include accuracy, precision, linearity, recovery and stability (FDA, 2018).

Capillary Zone Electrophoresis

Stock Solution

Because of its tolerance to photodegradation, atenolol was made in water and stored in an amber glass volumetric flask. To allow for further dilution, a solvent is prepared in advance.

Sample Collection and Sample Clean Up

Blood was extracted by syringe and was transferred into tubes containing EDTA. Performed at 5000 RPM, combined, and centrifuged. The next move was to allow the plasma to thaw at room temperature and then add 1ml of human plasma. In this experiment, vortex mixing was done and then 20μl of perchloric acid was applied to precipitate proteins. After vortexing the mixture once, it was centrifuged for 5 minutes at 5000 rpm to isolate the solids and change the pH to 9.

In order to neutralize and activate the Bond-Elut Certify LRC cartridge, 2ml of plasma solution was used. This solution was previously combined with 4ml of methanol and deionized water. When the samples have been placed in the column, they are pulled into the column by gravity. Then, the cartridge was put in a dish, which held a 2ml mixture of water, 1ml of 1M Acetate buffer, and 1ml of n-hexane. The atenolol was derived with 2ml of chloroform-isopropanol (80:20, v/v). The removed components were evaporated to dryness at 50^{o}C under nitrogen and then the aqueous solution was reconstituted with 50μl of deionized water (Arias *et al.*, 2001).

Quantitative Parameters

The spike human plasma samples were infused with known quantities of atenolol, as well as samples containing unknown concentrations of atenolol. With the peak area of atenolol vs the concentration of atenolol, the calibration equation was discovered using least-squares regression. The detection and quantification limits were calculated to be detectable and quantifiable (Meissner *et al.*, 1998; Minamide *et al.*, 2011).

The precision was estimated at two concentration levels, with spiked plasma being matched to concentration and then producing a calibration map.

The recovery was noticed by calculating the peak area of atenolol.

A measured concentration of three was chosen for the reproducibility test: 100, 200, and 400ng/ml. In order to make assumptions about intraday and interday activity, Figure 2

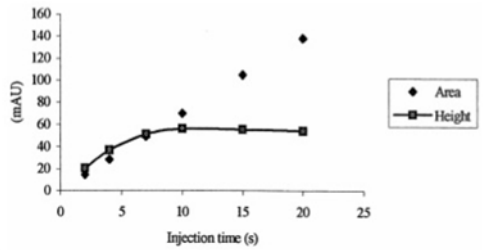

Figure 2: Time of injection and height of the peak of atenelol

RESULTS AND DISCUSSION

Optimization of Chromatography Conditions

The mobile process is used in the tests and is distributed at a 1ml/min flow rate. Within 10 minutes of running time, the atenolol, IS, and plasma components separated under these conditions. As a result, the retention times of atenolol and IS were discovered to be 5.4 and 8.3 minutes, respectively, as seen in Figure 3.

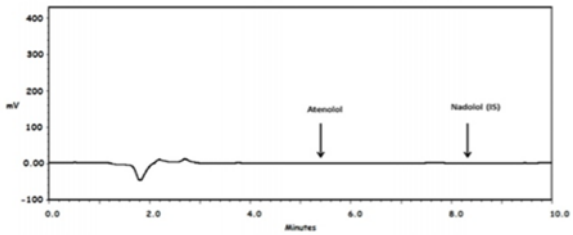

Figure 3: Retention time for Atenolol and IS

Linearity was evaluated by analyzing 8 curves, each with nine standard concentration is shown

Table 1: Back-calculated Atenolol concentration from eight calibration curves

Nominal level (μg/ml)	Measured level (μg/ml) Mean	SD	CV (%)	Bias (%)
0.01	0.01	0.00	13.9	15.9
0.02	0.02	0.00	8.6	0.2
0.05	0.05	0.00	2.4	-1.3
0.10	0.10	0.00	2.6	0.3
0.25	0.25	0.00	1.6	0.9
0.50	0.50	0.01	1.1	0.0
0.75	0.75	0.01	1.5	-0.4
1.00	1.00	0.02	2.1	-0.3

The limit of quantification, defined as the lowest measured concentration with precision and bias of $\leq$ 20%, was 10μg/ml.

Table 2: Intraday and interday level of precision and accuracy

	Intra day				Inter day			
Nominal level (μg/ml)	Mean measured level (μg/ml)	SD	CV (%)	Bias (%)	Mean Measured level (μg/ml)	SD	CV (%)	Bias (%)
0.01	0.01	0.00	5.3	17.0	0.01	0.00	4.6	16.2
0.03	0.03	0.00	3.4	0.0	0.03	0.00	3.1	-0.2
0.80	0.83	0.02	2.1	4.0	0.80	0.04	5.2	-0.6
1.40	1.45	0.05	3.3	3.6	1.40	0.07	4.8	-0.2

Table 3: Recovery of Atenolol & IS from 0.23ml human plasma

Nominal levels μg/ml	Human plasma	Mobile phase	Recovery (%)
Atenolol			
0.01	4760 (203)	5837 (366)	82
0.03	14214 (492)	16615 (255)	86
0.80	402492 (3124)	522114 (2998)	77
1.40	707508 (5632)	935930 (6335)	76
Internal standard			
2.0	376632 (4722)	434740 (3398)	87

Table 4: Variations in the parameters of analysis of atenelol

Parameters	Values Obtained
Regression coefficient	0.9995
Intra-day (RSD %)	5.05,2.42,2.27
Inter-day (RSD %)	2.50,6.91,8.22
Accuracy	76.9; 6.1
Detection limit	27
Quantitation limit	90
Recovery (%)	82.3

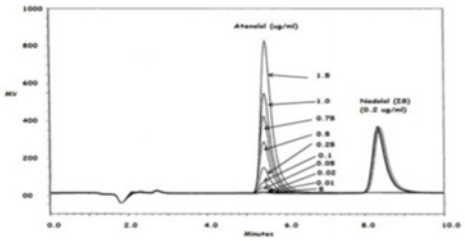

Figure 4: overall chromatograms

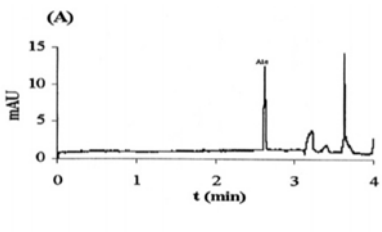

Figure 5: The Retention time for Atenolol

in Table 1. The calibration curve were linear $R^2 \geq 0.9992$. Mean concentrations back-calculated from peak height ratios using individual regression equations and overall chromatograms are given Figure 4.

Precision and BIAS

To reliably forecast intra-day and inter-day accuracy by using the amounts of atenolol at various intervals. Over the timeframe, from 2.1% to 5.3% and from 0.0% to 17.0%, it varied from 2.1% to 5.3% and from 0.0% to 17.0%. The inter-day varied from 3.1% to 5.2%. Table 2 outlines the conclusions.

Recovery

For direct comparison of peak height from plasma and mobile samples, using 5 replicates for five QC samples, 5 cm replicates were employed. In comparison, the recovery of the Islamic State remains unchanged. The values are listed in Table 3.

Robustness and Ruggedness

Robustness is characterized as a slight shift in chromatographic conditions. to assess the robustness of the current assay, we attempted to alter the power of the buffer. Theoretically, no significance effects were determined. Ruggedness was put to the test by doing split sample testing. Two different kinds of samples were sampled by two different types of analytical equipment.

Quantitative Determination of Parameters in CZE

The calibration curve for atenolol in plasma is created by generating a calibration curve using spiked plasma blanks and then extracting the samples with the SPE process. The electrophoretic separation takes place in less than three minutes. And, the graph can be seen in Figure 5. The Parameters values are shown in Table 4.

CONCLUSION

The HPLC and CZE assay method is precise, simple and effective. In HPLC, the Liquid-Liquid Extraction method is used, wherein the CZE Solid Phase Extraction method is used. The assay was applied to monitor atenolol under various conditions. CZE, the electrophoretic separation takes place in less than 3mins, wherein HPLC the retention time is within 10mins. So, CZE has a better separation than HPLC.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

Funding Support

The authors declare that they have no funding support for this study.

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