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A validated high-performance liquid chromatographic method for the determination of atenolol in whole blood*

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Abstract: A validated reversed-phase high-performance liquid chromatographic (HPLC) procedure employing fluorescence detection for the analysis of atenolol (Tenormin®) in human whole blood is reported. The method is rapid and coupled with standard HPLC procedures leads to a sensitive, accurate and reproducible assay. The retention times of atenolol and internal standard, bamethan, are 4.4 and 6.3 min, respectively. The peak height ratio versus plasma concentration is linear over the range of 20.0–800 ng ml^{-1}, with a detection limit below 10 ng ml^{-1}. The mean absolute recovery of atenolol using the described assay is 90%. The inter- and intra-day accuracy and precision are within 8% of the actual values for all concentrations investigated. Furthermore, this procedure was applied to the analysis of whole blood samples from healthy subjects receiving a single 100 mg oral dose of Tenormin®.

Keywords: *Atenolol; whole blood; reversed-phase HPLC; fluorescence detection.*

Introduction

The use of β-blocking drugs in the management of cardiovascular disorders is well established. Atenolol [4-(2-hydroxy-3-isopropylaminopropoxy) phenyl acetamide] is a cardioselective β_1-adrenergic receptor blocking agent prescribed for the treatment of hypertension [1]. The antihypertensive effects of atenolol are such that it need only be administered in a single daily dose. As a consequence of the widespread use of β-blockers, there are many published methods for their determination in plasma incorporating a variety of techniques [2–7]. However, the analysis of atenolol in whole blood could be required if a limited blood draw is necessary and/or it is given in combination with a drug that could only be analysed in whole blood. Consequently, this prompted the development of a method in whole blood which is specific, sensitive and robust.

The method reported herein for the detection of atenolol is linear over the range of 20.0–800 ng ml^{-1} in human whole blood. This range has been selected on the basis of its C_{max} of 640 ng ml^{-1} in plasma [8], and extrapolating five half-lives yields a lower limit of 20.0 ng ml^{-1}. Furthermore, this procedure can be applied to ascertain the pharmacokinetics of a single 100 mg dose of Tenormin® in humans.

Experimental

Materials

Atenolol and the internal standard, bamethan sulfate, were purchased from Sigma (St Louis, MO, USA). HPLC grade phosphoric acid 85%, isopropanol, and triethylamine, along with sodium carbonate were purchased from Fisher Scientific (Montréal, PQ, Canada). HPLC grade methanol, acetonitrile, and glass distilled ethyl acetate were purchased from Caledon (Georgetown, ON, Canada). The water was deionized Type 1, reagent grade (Millipore, Ville St Laurent, PQ, Canada). All reagents were used without further purification.

Instrumentation and chromatographic conditions

The chromatographic system consisted of a Waters Model 590 pump, a WISP 710B autosampler (Waters Associates, Milford, MA, USA) and a Shimadzu RF-535 fluorescence detector (RP Instruments, Montréal, PQ, Canada). A stainless-steel column (15 cm × 4.6 mm i.d.) was packed with Nucleosil C18,

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particle size 5 μm (prepared in-house). The column was maintained at ambient temperature. Atenolol and bamethan were determined by fluorescence at an excitation wavelength of 230 nm and an emission wavelength of 300 nm. The mobile phase consisted of 2% acetonitrile, 8% methanol in 0.05 M phosphate-triethylamine buffer, pH 3.3, and was delivered at a flow rate of 1.2 ml min^{-1}. Under these conditions, the retention times for atenolol and internal standard were 4.4 and 6.3 min, respectively.

Biological samples

Blood samples were collected from healthy male volunteers after receiving a single 100 mg oral dose of Tenormin®. Blood was drawn into evacuated EDTA collection tubes (Becton Dickinson Vacutainer Systems, Rutherford, NJ, USA) and stored at −15°C.

Preparation of standards

A stock solution of atenolol was prepared at 1.0 mg ml^{-1} in methanol. Appropriate dilutions of the stock were made with deionized water and the spiking solutions were used to prepare spiked whole blood standards at concentrations of 20.0, 40.0, 120, 200, 400, 600 and 800 ng ml^{-1}. Spiked whole blood quality control samples (QCs) were prepared in pools of 20.0 ml at final concentrations of 80.0, 360 and 720 ng ml^{-1}. Individual aliquots of 500 μl were stored in polypropylene tubes. A stock internal standard solution of bamethan was prepared at 100 μg ml^{-1} and diluted to 2.5 μg ml^{-1} with 1 M sodium carbonate. All solutions were stable for at least 3 months at −15°C.

Sample preparation

Aliquots of whole blood (500 μl) were added to polypropylene tubes. Samples were spiked with 500 μl of the internal standard spiking solution and vortexed. To each tube 5 ml of ethyl acetate was added. Samples were extracted at low speed (230 ± 30 oscillations min^{-1}) on a reciprocating shaker for 15 min. After centrifugation for 10 min at *ca* 1500**g**, the organic layer was transferred to a 13 × 100 mm disposable borosilicate tube and evaporated to dryness at 37°C under a gentle stream of nitrogen. The residue was reconstituted in 350 μl of mobile phase, and 100 μl was injected onto the liquid chromatograph under the previously stated conditions. The reconstituted samples were stable at room temperature for at least 24 h.

Data acquisition

Peak heights of atenolol and internal standard were measured with a Spectra-Physics model 4270 integrator and down loaded to Chrom-Station (Spectra-Physics Inc., Mountain View, CA, USA). The chromatographic data were automatically processed for peak height ratios of atenolol and internal standard, followed by weighted ($1/C$) linear regression analysis.

Results and Discussion

Chromatography

Typical chromatograms obtained from an extracted whole blood bank and a calibration sample at the limit of quantification (LOQ) are illustrated in Fig. 1(a) and (b). Chromatograms of a subject at predose and 2 h after receiving a 100 mg tablet of Tenormin® are provided in Fig. 2(a) and (b). The retention times of atenolol and internal standard are 4.4 and 6.3 min, respectively. The overall chromatographic run time was 9.0 min.

Linearity and quantitation limit

A linear response in peak height ratios of atenolol to internal standard over the concentration range of 20.0–800 ng ml^{-1} was observed. The correlation coefficients for atenolol were 0.9970 or better ($n = 5$).

A minimum signal-to-noise ratio of 5:1 was obtained with the lowest standard, allowing a quantitation limit of less than 10 ng ml^{-1}. Thus, the limit of quantitation used (20.0 ng ml^{-1}) is higher than the absolute limit of the assay.

Recovery

The absolute recovery of atenolol was based on the selection of two concentrations, one at four times the LOQ and the other at 90% of the upper limit of the assay. The recoveries were determined using a calibration curve representing 100% atenolol recovery and bamethan as an external standard.

The absolute recovery of the internal standard was also performed at two concentrations, one at half and the second at the recommended concentration for the assay. The results are given in Table 1.

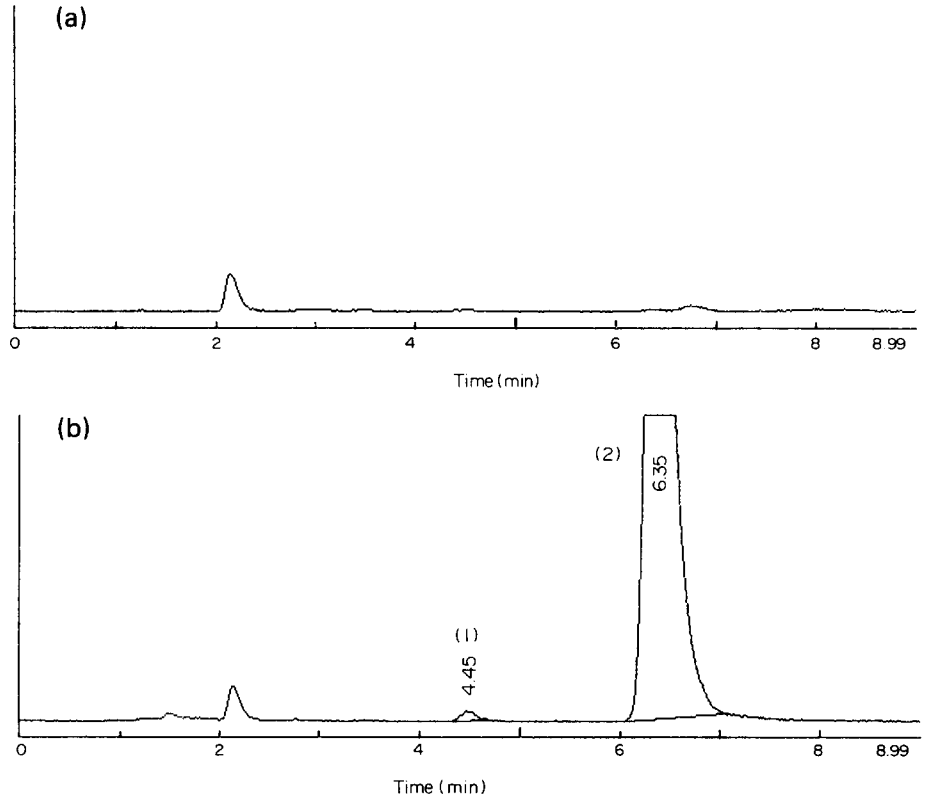

Figure 1
Samples prepared according to the described procedure. (a) Whole blood blank, attenuation = 2, (b) whole blood spiked at 20.0 ng ml^{-1} atenolol (1) and 50.0 μg ml^{-1} bamethan (2), attenuation = 2.

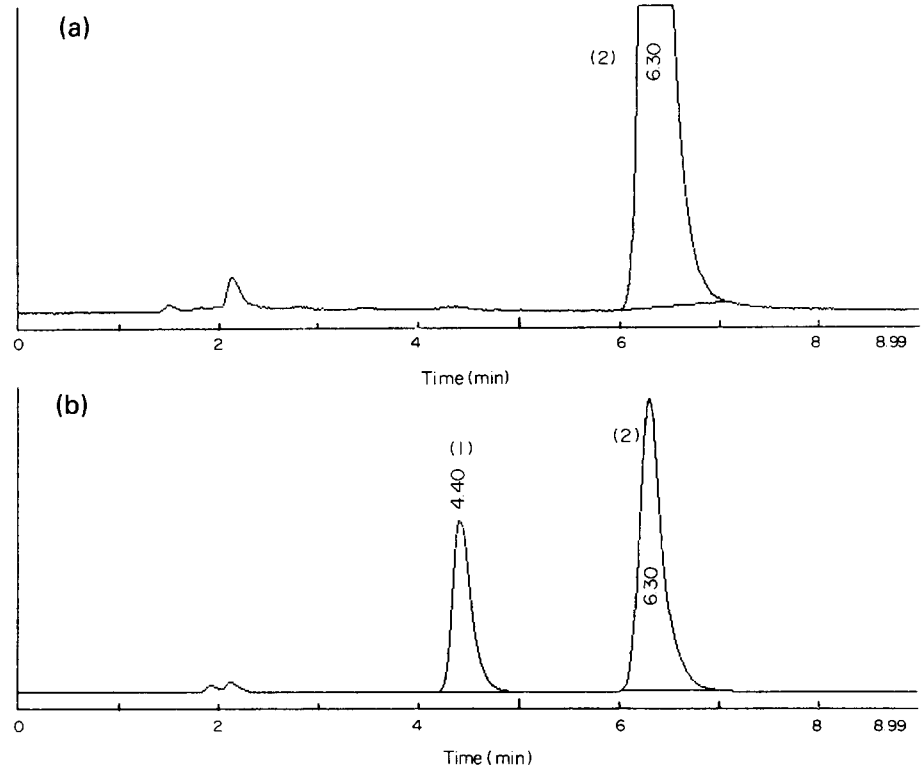

Figure 2
Samples prepared according to the described procedure. (a) Whole blood of subject at predose, showing bamethan peak at 6.3 min, attenuation = 2, (b) whole blood of subject 2 h after a 100 mg oral dose of Tenormin® [atenolol (1)] and bamethan (2), attenuation = 13.

Table 1
Recovery of atenolol and internal standard from human whole blood*

Drug	Concentration (ng ml^{-1})	Recovery (%)	RSD (%)
Atenolol	80.0	93.5	5.1
	720	87.3	2.4
Bamethan	1250	86.8	3.3
	2500	87.6	3.0

*n = 8 in all cases.

Specificity

Human whole blood was collected from healthy donors and screened for interference at the retention times of atenolol and internal standard. No significant interference had been observed in drug-free blood samples.

Precision and accuracy

The inter-day precision and accuracy was assessed by the repeated analysis of whole blood specimens containing different concentrations of atenolol (Table 2). Two samples at each QC concentration, low, medium and high, together with a calibration curve were run as a single batch. To be regarded as a separate batch, the entire sample processing must take place in a time domain completely separate from one another. At spiked blood concentrations of 80.0, 360 and 720 ng ml^{-1} atenolol, the method yields RSDs of 5.4, 3.7 and 7.7%, respectively. The mean concentrations obtained from the calibration curve range from 96.9 to 103.5% of the nominal concentrations for atenolol.

The intra-day precision and accuracy was determined by the evaluation of a typical production run consisting of atenolol concentration of 20.0, 80.0, 360 and 720 ng ml^{-1}. The RSDs were 6.0, 5.6, 5.4 and 4.7%, respectively, while the mean concentrations ranged from 98.7 to 105.7% of the nominal concentrations for atenolol. These results are presented in Table 3.

Applications

Whole blood was collected in Vacutainers

Table 2
Inter-day precision and accuracy of atenolol in human whole blood

Nominal concentration (ng ml^{-1})	n	Concentration found (ng ml^{-1}) Mean ± SD	RSD (%)	% Found
Std 20.0	5	20.7 ± 1.42	6.9	103.5
Std 40.0	5	38.7 ± 1.25	3.2	96.9
Std 120	5	122 ± 3.6	2.9	101.5
Std 200	5	198 ± 6.8	3.4	98.8
Std 400	5	398 ± 16.0	4.0	99.5
Std 600	5	587 ± 34.2	5.8	97.9
Std 800	5	816 ± 35.3	4.3	102.0
				Mean 100.0
				RSD (%) 2.4
QC 80.0	10	84.6 ± 4.58	5.4	106.0
QC 360	10	373 ± 13.9	3.7	103.7
QC 720	10	737 ± 56.6	7.7	102.3
				Mean 104.0
				RSD (%) 1.8

Table 3
Intra-day precision and accuracy of atenolol in human whole blood

Nominal concentration (ng ml^{-1})	n	Concentration found (ng ml^{-1}) Mean ± SD	RSD (%)	% Found
Std 20.0	10	19.7 ± 1.19	6.0	98.7
QC 80.0	10	82.0 ± 4.59	5.6	102.5
QC 360	10	358 ± 19.4	5.4	99.3
QC 720	10	761 ± 35.9	4.7	105.7

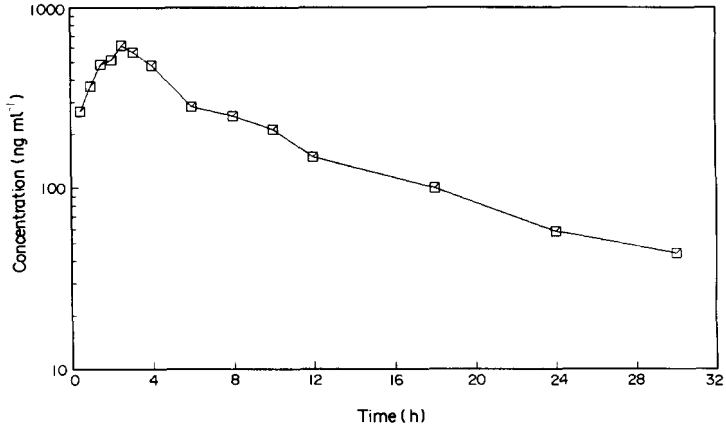

Figure 3
Representative concentration–time profile of a subject after a single 100 mg oral dose of Tenormin®.

containing EDTA prior to dosing and at 14 subsequent time points. Following collection, the samples were stored at −15°C until analysed. All samples were analysed by the method presented here. A typical concentration–time profile after a 100 mg oral dose of Tenormin® is depicted in Fig. 3.

Conclusion

The described method for the analysis of atenolol in human whole blood is specific, sensitive and robust. The intra- and inter-assay precision of the method was below 8%, while the accuracy of the method was within 7% even at the lowest concentration. The inter-day means of the per cent nominal found for the standards and QC samples were 100 and 104%, respectively, exemplifying the accuracy of the assay. Furthermore, the method is fast and requires a relatively simple sample preparation. A large number of samples can be processed daily (*ca* 90). This method has been used to monitor whole blood levels in clinical trials generating over 700 samples. More than 800 injections of spiked whole blood have been made on a single analytical column with minimal loss of chromatographic integrity.

This method allows the quantitation of whole blood levels of atenolol for at least 30 h following a single 100 mg oral dose of Tenormin®, and permits the complete characterization of the resulting whole blood profile.

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