DRUG FORMULATIONS AND CLINICAL METHODS

Determination of Enantiomers of Atenolol and Propranolol in Pharmaceutical Formulations by HPLC

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A validated HPLC-UV method was developed for the determination of R(–), S(+)-atenolol and R(–), S(+)-propranolol in pharmaceutical formulations. The proposed method required no elaborate sample preparation and was found to be selective, linear, and repeatable within the established ranges. Atenolol and propranolol isomers were separated using a Chirex 3022 (S) column with the mobile phases hexane–dichloromethane–methanol–trifluoroacetic acid (35 + 35 + 5 + 0.25, v/v/v/v) and hexane–dichloromethane–ethanol–trifluoroacetic acid (55 + 40 + 5 + 0.25, v/v/v/v), respectively. The LOD values of R(–) and S(+)-atenolol were 12.3 and 9.86 µg/mL, respectively, and 0.61 and 0.89 µg/mL, respectively, for R(–) and S(+)-propranolol. Retention times of R(–)-propranolol and S(+)-propranolol were 12.4 and 14.3 min, respectively, and 29.06 and 32.71 min, respectively, for (R)-atenolol and (S)-atenolol. The proposed method was applied to the determination of enantiomers in pharmaceutical formulations, and no interference from any excipients was found.

Atenolol and propranolol have beta-blocking activity in common, but their pharmacokinetic properties are largely different (1, 2). Chemically, atenolol and propranolol share an important feature, namely, an asymmetric carbon atom, as shown in Figure 1. Thus, both substances are racemic mixtures consisting of the (R)- and (S)-enantiomers in a fixed 1:1 ratio. Optically pure (R)- and (S)- propranolol not only differ pharmacodynamically but pharmacokinetically, as well; stereoselectively controlled processes, such as drug absorption, distribution, metabolism, and elimination have to be considered and studied carefully (3–6).

Enantiomer separation of these beta-blocker drugs was performed on commercial columns, such as Chiral AGP, C18, Chiralcel OD, and Chirex 3022. Hermansson and Grahn (7) separated atenolol and proranolol enantiomers by HPLC with a Chiral AGP column. The resolution factors (Rs) of atenolol and propranolol were found to be 1.46 and 2.62, respectively. Tran and Dotlich (8) used a C18 column for separation of enantiomers, and the Rs values of atenolol and propranolol were 1.11 and 0.63, respectively. Williams et al. (9) used HPLC with a Chirex 3022 column and reported that Rs values of atenolol and propranolol were 1.40 and 1.00, respectively. Phinney and Sander (10) used HPLC with a Chirex 3022 column for separation of propranolol enantiomers and found that the Rs value was 0.85.

The chiral selector for Chirex 3022 consists of (S)-indoline-2-carboxylic acid and (R)-1-(α-naphthyl) ethylamine urea linkage, an electron donor designed for the separation of amines, amino alcohols, and alcohols (11). This chiral stationary phase resolves underivatized β-blockers and β-antagonists through a combination of π-π, hydrogen bonding, dipole-dipole, and steric interactions (12). We present here a validated HPLC-UV method for the determination of (R)-, (S)-atenolol and (R)-, (S)-propranolol in pharmaceutical formulations. The optimal column was determined to be Chirex 3022. Our results indicated that enantioresolution factors (R_s) determined by using Chirex 3022 were better than in the other studies. The proposed method requires no elaborate sample preparation and was found to be selective, linear, and repeatable within the established ranges. The applicability of the developed method was successfully validated and applied for the analysis of pharmaceutical formulations.

Experimental

Standards and Reagents

Atenolol and propranolol were obtained from Sigma-Aldrich (St. Louis, MO) and (S)-timolol (used as an internal standard) was a gift from Bayer (Istanbul, Turkey). HPLC-grade reagents, such as methanol, ethanol, hexane, and dichloromethane, used in mobile phases were bought from Sigma-Aldrich.

Instrumentation

The HPLC system used was an Agilent Technologies (Santa Clara, CA) 1200 series with a G1311A pump, G1322A degasser, G1316A column heater, G1328B

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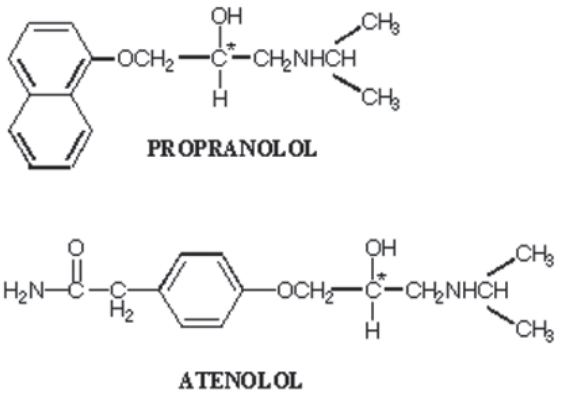


Figure 1. Structural formula of propranolol and atenolol.

manual injector, and G1314B variable wavelength detector. A Chirex (S)-ICA/(R)-NEA column (Part No. 00G-3022-E0; 250×4.6 mm) was used as the stationary phase. A brush-type phase having π-donor characteristics was obtained from Phenomenex (Torrance, CA; 9).

Chromatographic Conditions

The separations were performed under isocratic conditions. The isomers of atenolol and propranolol were separated using the mobile phases hexane–dichloromethane–methanol–trifluoroacetic acid (60+35+5+0.25, v/v/v/v) and hexane–dichloromethane–ethanol–trifluoroacetic acid (55+40+5+0.25, v/v/v/v), both at a flow rate of 2 mL/min. The mobile phase was filtered through a 0.45 µm nylon-membrane filter (Millipore, Barcelona, Spain) and degassed with suction prior to use. The monitoring wavelength of atenolol was 280 nm, and the injection volume was 10 µL. The monitoring wavelength of propranolol was 254 nm, and the injection volume was 20 µL. Peak areas were recorded, and the column temperature was set to 35°C for atenolol and 20°C for propranolol.

Preparation of Stock and Standard Solutions

Individual stock solutions of (R)-, (S)-atenolol (1000 µg/mL) and (S)-timolol (1000 µg/mL) were prepared in methanol. Individual stock solutions of (R)- and (S)-propranolol (1000 µg/mL) and (S)-timolol (1000 µg/mL) were prepared in ethanol. These solutions were stored in the dark under refrigeration at 4°C. The stability of the standard solutions was checked over this period by HPLC analysis. Standard solutions of atenolol and propranolol were prepared by diluting stock solutions with methanol. These standard solutions were directly injected into the HPLC system. Concentrations of standard solutions were prepared at 50, 75, 100, 125, and 150 µg/mL for atenolol, and 5, 15, 25, 35, 45, and 55 µg/mL for propranolol.

Preparation of Pharmaceutical Atenolol Tablets

Nortan® tablets contain the active ingredient atenolol and titanium dioxide, sunset yellow, and erythrosine as excipients. Ten Nortan tablets (50 mg atenolol/tablet) were powdered, then 428.6 mg powder was weighed and dissolved in 100 mL methanol to prepare a 1000 µg/mL stock solution. The solution was shaken mechanically for 5 min. Sample solutions of 80, 100, and 120 µg/mL were prepared by adding 2 µg/mL (S)-timolol internal standard and diluting with methanol.

Preparation of Pharmaceutical Propranolol Tablets

Dideral® tablets contain the active ingredient propranolol and the coloring agent Ponceau 4R as an excipient. Ten Dideral tablets (40 mg propranolol/tablet) were powdered, then 502.6 mg powder was weighed and dissolved in 100 mL ethanol to prepare a 1000 µg/mL stock solution. Sample solutions of 20, 30, and 40 µg/mL were prepared by adding 10 µg/mL (S)-timolol internal standard and diluting with ethanol.

Method Validation

Evaluation of the NP-HPLC methods was based on proportionality (linearity assay), LOD, LOQ, precision (repeatability and reproducibility assays), and accuracy (13–16). Method validation was carried out according to the guidelines of the main regulatory agencies, such as those issued by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH; 17).

(**a**) *Linearity.*—The linear range of detectability is dependent on the compound analyzed and detector used.

Table 1. System suitability parameters

	Selectivity factor (α)	Capacity factor (k')	Resolution (R_S)	Theorical plate number (N)	Tailing factor (TF)
(R)-Atenolol	1.14	16.8	2.13	4791.9	1.14
(S)-Atenolol	1.14	19.1	2.13	4998.9	1.08
(R)-Propranolol	1.18	5.9	2.10	3565.8	1.17
(S)-Propranolol	1.18	6.9	2.10	3545.8	1.15

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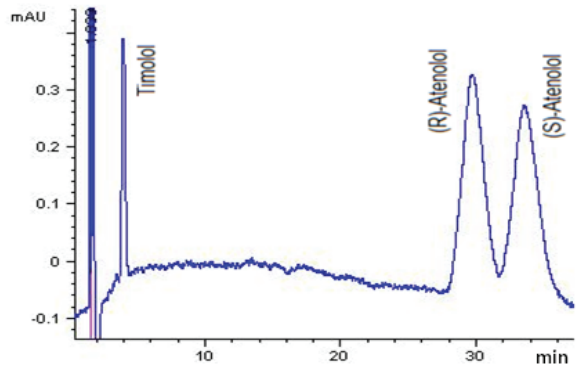


Figure 2. Chromatogram of (R)- and (S)-atenolol enantiomers.

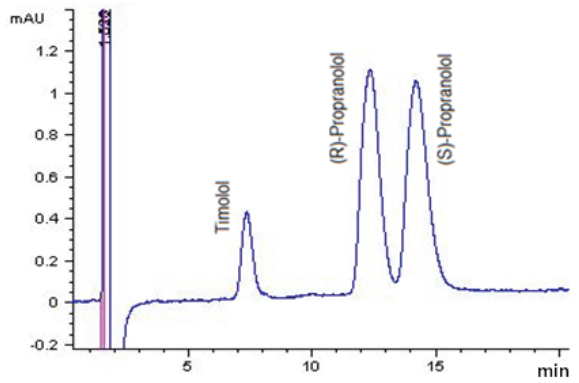


Figure 3. Chromatogram of (R)- and (S)-propranolol enantiomers.

The working sample concentration and samples tested for accuracy should be in the linear range. The linearity range for examination depends on the purpose of the test method. For example, the recommended range for an assay method for content would be ±20%, and the range for an assay/impurities combination method based on area (for impurities) would be ±20% of the target concentration down to the LOQ of the drug substance or impurity. Under most circumstances, the regression coefficient (r) is ≥0.999 and the intercept and slope should be indicated (18).

In this study for the system linearity, standard solutions of atenolol and propranolol at five concentration levels were prepared: 50, 75, 100, 125, and 150 µg/mL for atenolol; and 5, 15, 25, 35, 45, and 55 µg/mL for propranolol. Each concentration level was injected in triplicate. The experimental results were plotted to obtain a calibration curve, and the corresponding statistical study was carried out.

(**b**) *LOD and LOQ.*—The LOD and LOQ values were calculated from the calibration curves that defined linearity. LOD, which is expressed in units of concentration, describes the lowest concentration level that can be distinguished from the blank. The slope of the calibration curves (b) and the standard error of the independent term of the regression (Sb) were used to calculate the LOD and LOQ with reference to International Union of Pure and Applied Chemistry definitions (19).

(**c**) *Precision.*—Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. The ICH has defined precision to contain three components: repeatability, intermediate precision, and reproducibility (18).

Two different standard solutions of propranolol (15 and 35 µg/mL) and atenolol (50 and 100 µg/mL) were analyzed six times on the same day to obtain method repeatability (intraday precision). Three different solutions of propranolol (15, 35, and 55 µg/mL) and atenolol (50, 100, and 150 µg/mL) were prepared on different days to obtain intermediate precision (interday precision) and analyzed in triplicate. All of the data were expressed as RSD values.

(**d**) *Recovery.*—The method of sample fortification with standards is used in instrumental analysis to determine the concentration of a substance in an unknown sample by comparison to a set of samples of known concentration, similar to using a calibration curve. Sample fortification with standards can be applied to most analytical techniques, and is used instead of a calibration curve to solve the matrix effect problem (20). Five different solutions containing samples in the same volume (100 mL) were prepared and analyzed in triplicate. Five atenolol samples were prepared from the stock sample solution at a concentration of 50 µg/mL. Standard solutions were added to four of these solutions at levels of 10, 20, 30, and 40 µg/mL. Then a calibration curve was prepared with these solutions. In the same way, five propranolol samples were prepared from the stock sample solution at a concentration of 20 µg/mL.

Table 2. Quantitative parameters and statistical data for HPLC determination of (R)- and (S)-atenolol and (R)- and (S)-propranolol

	Linearity range, µg/mL	LOD, µg/mL	LOQ, µg/mL	Slope (m)	RSD of slope, %	Intercept (n)	RSD of intercept, %	Correlation coefficient (r)
(R)-Atenolol	50–150	12.30	36.90	0.0634	4.73	0.310	31.00	0.9949
(S)-Atenolol	50–150	9.86	29.59	0.0578	3.46	0.518	57.92	0.9944
(R)-Propranolol	5–100	0.61	1.83	0.0991	3.03	–0.1651	43.00	0.9967
(S)-Propranolol	5–100	0.89	2.67	0.1012	3.95	–0.1327	82.89	0.9942

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Table 3. Recoveries of atenolol and propranolol enantiomer standard solution added to commercially available samples

Sample No.	Added concentrations of standard solution, µg/mL	(R)-Atenolol, %	Parameters
1	—	96.37	$\bar{x}$ = 98.95[a]
2	10	100.02	SD = 2.24
3	20	96.77	RSD, % = 2.26
4	30	101.41	CI = 96.17 – 101.73[b]
5	40	100.00	(*P* = 0.05)
		(S)-Atenolol, %	
1	—	92.86	$\bar{x}$ = 98.45
2	10	98.60	SD = 3.41
3	20	99.75	RSD, % = 3.46
4	30	102.09	CI = 94.22 – 102.68
5	40	98.96	(*P* = 0.05)
		(R)-Propranolol, %	
1	—	93.84	$\bar{x}$ = 98.06
2	5	96.20	SD = 3.54
3	10	101.90	RSD, % = 3.61
4	15	96.77	CI = 93.67 – 102.45
5	20	101.58	(*P* = 0.05)
		(S)-Propranolol, %	
1	—	93.57	$\bar{x}$ = 98.12
2	5	95.97	SD = 4.04
3	10	103.19	RSD, % = 4.12
4	15	96.39	CI = 93.11 – 103.13
5	20	101.49	(*P* = 0.05)

[a] $\bar{x}$ = Mean.

[b] CI = Confidence interval.

Standard solutions were added to four of these solutions at levels of 5, 10, 15, and 20 µg/mL.

Results and Discussion

Chromatographic Conditions and Selectivity

In order to obtain the separation of the (R)-, and (S)-atenolol, and (S)-timolol (internal standard) peaks under isocratic conditions, the mobile phase composition was optimized as shown in Table 1. Different mobile phases were used for separation of atenolol enantiomers using a Chirex 3022 column; column temperature was changed between 10 and 40°C. The best separation was achieved using hexane–dichloromethane–methanol–trifluoroacetic acid (60 + 35 + 5 + 0.25, v/v/v/v) at a flow rate of 2 mL/min and 35°C. The elution order was (R)-

Table 4. Assay variability of (R)-, (S)-atenolol and (R)-, (S)-propranolol (intraday)

	Area of (R)-atenolol/ area of internal standard (timolol)		Area of (S)-atenolol/ area of internal standard (timolol)		Area of (R)-propranolol/ area of internal standard (timolol)		Area of (S)-propranolol/ area of internal standard (timolol)	
ppm/ppm	50/2	100/2	50/2	100/2	15/10	35/10	15/10	35/10
$\bar{x}$	3.44	6.86	3.14	6.65	1.08	3.31	1.07	3.29
SD	0.03	0.05	0.07	0.05	0.03	0.04	0.02	0.04
RSD, %	0.90	0.65	2.21	0.80	2.87	1.28	1.93	1.18

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Table 5. Assay variability (R)-, (S)-atenolol and (R)-, (S)-propranolol (interday)

	Area of (R)-atenolol/area of internal standard (timolol)			Area of (S)-atenolol/area of internal standard (timolol)			Area of (R)-propranolol/area of internal standard (timolol)			Area of (S)-propranolol/area of internal standard (timolol)		
ppm/ppm	50/2	100/2	150/2	50/2	100/2	150/2	15/ 10	35/ 10	55/10	15/10	35/10	55/10
$\bar{x}$	3.39	6.90	9.70	3.19	6.54	9.01	1.07	3.35	4.99	1.07	3.32	4.93
SD	0.17	0.03	0.12	0.12	0.12	0.14	0.01	0.07	0.17	0.01	0.06	0.16
RSD, %	4.94	0.44	1.19	3.81	1.90	1.51	1.09	2.13	3.34	1.14	1.84	3.20

atenolol [retention time (t_r) 29.06 min], (S)-atenolol (t_r, 32.71 min), and (S)-timolol (t_r, 3.96 min), as shown in Figure 2.

In order to obtain the separation of the (R- and (S)-propranolol, and (S)-timolol (internal standard) peaks under isocratic conditions, the mobile phase composition was optimized as shown in Table 1. Different mobile phases were used for separation of propranolol enantiomers using a Chirex 3022 column; column temperature was varied between 5 and 30°C. The best separation was achieved using hexane–dichloromethane–ethanol–trifluoroacetic acid (55+40+5+0.25, v/v/v/v) at a flow rate of 2 mL/min and 20°C. (S)-Timolol was chosen as an internal standard. The elution order was (R)-propranolol (t_r, 12.4 min), (S)-propranolol (t_r, 14.3 min), and (S)-timolol (t_r, 7.3 min), as shown in Figure 3.

Response Function and Linearity

A linear response was obtained for (S)- and (R)-atenolol, and (S)- and (R)-propranolol. Complete response function parameters can be found in Table 2 for atenolol and propranolol. The maximum wavelength for detection was 280 nm for atenolol and 254 nm for propranolol, at which good detector response was obtained. The calibration curves for (R)- and (S)-atenolol were obtained in the range of 50–150 µg/mL, and showed excellent linearity, with r values of 0.9949 and 0.9944, respectively. The calibration equations exhibited average slopes of 0.0634 and 0.0578, respectively. The LOD values were found to be 12.3 µg/mL for (R)-atenolol and 9.86 µg/mL for (S)-atenolol. The calibration curves for (R)- and (S)-propranolol were obtained in the range of 5–100 µg/mL and showed excellent linearity, with r values of 0.9967 and 0.9942, respectively. The calibration equations exhibited average slopes of 0.0991 and 0.1012, respectively. The method run times for (R)-propranolol and (S)-propranolol were 12.4 and 14.3 min, respectively. The LOD values were found to be 0.61 and 0.89 µg/mL for (R)- and (S)-propranolol, respectively.

Precision and Accuracy

The amounts of atenolol and propranolol determined in relation to the added amounts were calculated. The results were reported as recovery, and are shown in Table 3. Standard solutions were prepared for precision studies as described above. The results of intraday and interday precision of (R)-, (S)-atenolol and (R)-, (S)-propranolol are shown in Tables 4 and 5, respectively.

We tried several chiral stationary phases for enantiomer separation. The selectivity factors and Rs values in various columns used are summarized in Table 6. The best separation was provided using a Chirex 3022 column for separation of anelolol and propranolol enantiomers. When system suitability parameters of atenolol and

Table 6. System suitability parameters of the proposed method and the studies in the literature

	Atenolol		Propranolol		
Applied method (column)	α	R_s	α	R_s	Reference
HPLC-UV (Chiral AGP Kolon)	1.23	1.46	1.52	2.62	7
HPLC-UV (C18 Kolon)	1.29	1.11	1.06	0.63	8
SFC-UV (Chiralcel OD)[a]	2.20	3.40	1.74	8.6	9
HPLC-UV (Chiralcel OD)	1.54	3.40	1.87	5.40	9
SFC-UV (Chirex 3022)	1.07	0.80	1.07	1.10	9
HPLC-UV (Chirex 3022)	1.17	1.40	1.11	1.00	9
SFC-UV (Chirex 3022)	—	—	1.00	0.00	10
HPLC-UV (Chirex 3022)	—	—	1.09	0.85	20
HPLC-UV (Chirex 3022)	1.14	2.14	1.18	2.10	Proposed method

[a] SFC = Supercritical fluid chromatography.

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propranolol were surveyed, enantiomer separation obtained using a Chirex 3022 column was preferable to columns in many reported studies. The Rs of atenolol and propranolol enantiomers were found to be 2.14 and 2.10, respectively. The selectivity factors (α) were found to be 1.14 and 1.18, respectively.

Conclusions

We developed an NP-HPLC method for the determination of (R)- and (S)-atenolol and (R)- and (S)-propranolol in tablet formulations, and compared this method to previous methods shown in Table 6, in which other commercial columns were used. The following facts were revealed: the proposed method is simple, selective, and easily adaptable for quantitative determination of atenolol and propranolol; there was no interference of any tablet excipients found for both methods; and no elaborate sample preparation or analyte derivatizion was required. The methods can be used for the routine analysis of atenolol and propranolol enantiomers in pharmaceutical formulations.

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