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Research Article

Liquid Chromatography Study on Atenolol-β-Cyclodextrin Inclusion Complex

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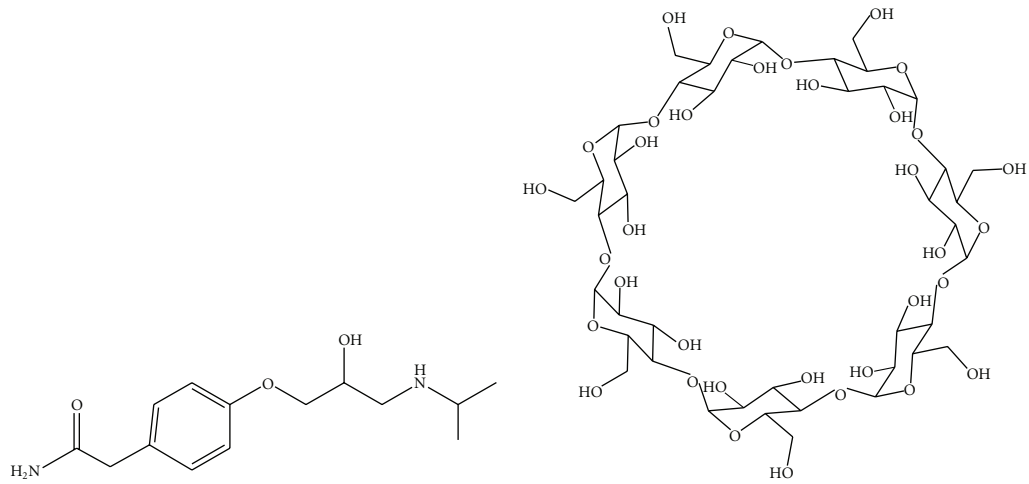
Inclusion complex formation of atenolol with β-cyclodextrin (β-CD) has been investigated by HPLC on different stationary phases, by varying pH and concentration of β-CD added as an additive in the mobile phase over a wide range of column temperature. Stationary phases of different polarity and hydrophobicity were evaluated to find the best conditions for complex formation. The optimum conditions for inclusion complexation were achieved on YMC ODS-AQ C18 (150×4.6 mm, $5\,\mu$) analytical column. The apparent formation constant (K) of the complex as evaluated by liquid chromatography using retention factors (k) was $179.47 \pm 2.5\,M^{-1}$ at 25°C. The stoichiometry of the complex was 1 : 1 as is evident from the straight line plot of $1/k$ versus β-CD concentration. The formation of inclusion complex was essentially enthalpy (-42.12 kJ/mol) driven and the binding forces included hydrophobic, van der Waals-London dispersion interactions. The enthalpy-entropy compensation criterion was used to prove the inclusion phenomena.

1. Introduction

Beta-blockers are a class of antihypertensive drugs that are used in the management of cardiac arrhythmias, cardioprotection after myocardial infarction (heart attack), and hypertension. They have revolutionized the medical management of angina pectoris and are recommended as first-line agents by national and international guidelines. Atenolol, [4-(2-hydroxy-3-isopropylamino propoxy) phenyl acetamide] (see Figure 1(a)) is a long established β-blocker widely used in the treatment of high blood pressure, arrhythmias, and angina pectoris [1]. Like other antihypertensive drugs, atenolol lowers the systolic and diastolic blood pressure by 15–20% in a single-drug treatment and reduces cardiovascular mortality [2]. The β-blockers are characterised by poor solubility in aqueous and gastric fluid, and therefore, the dissolution rate and their bioavailability directly affect their efficacy [3]. Consequently, in order to increase the solubility and dissolution rate of β-blockers, inclusion complex formation with cyclodextrins becomes imperative.

Cyclodextrins (CDs) are toroidal-shaped cyclic oligomers of α-(l,4)-D-glucopyranose units which contribute to several guest-associated phenomena in solution [4]. CDs are oligoglycosides of six (α-CD), seven (β-CD), or eight (γ-CD) glucose units (see Figure 1(b)) forming a relatively hydrophobic cavity in the middle and a relatively hydrophilic exterior part [5]. CDs as host molecules can thus form inclusion complexes with various drug (guest) molecules and are utilized for the improvement of drug properties such as solubility, stability, and bioavailability [6, 7]. Though the exact mechanism of interaction is difficult to ascertain between CDs and the guest molecules, nevertheless it can be understood as a combination of geometrical compatibility, release of CD-ring strain upon complexation, van der Waals forces, hydrogen bonding, electrostatic, and hydrophobic interactions [5]. They are also optically active and offer potential discrimination of enantiomeric substances. These characteristics of CDs permit them to be employed as stationary phase in gas and liquid chromatography [8, 9], as chiral additives in HPLC [10–12] and in capillary electrophoresis (CE) [13, 14] for the separation of drug enantiomers. Generally in liquid chromatography, modest attention has been focused on the importance of temperature, especially using the chiral mobile phase additives for β-blockers. The major advantage



(a) (b)

Figure 1: Chemical structures of (a) atenolol and (b) β-cyclodextrin.

in studying the effect of temperature is to determine the thermodynamic parameters for inclusion complexes and for understanding the mechanism and driving force of a wide variety of supramolecular interactions [15].

Few studies have reported CD-based inclusion complex formation, or chiral separation of β-blockers [16–20]. Wei et al. [16] have studied chiral separation of atenolol, metoprolol, isoproterenol, salbutamol, and clenbuterol using electrophoresis with dual cyclodextrin systems. The chiral separation was strongly influenced by the concentration of the reaction mixture, pH, and organic modifier. Similarly, stereoselective recognition of several β-blockers by capillary zone electrophoresis with various CD derivatives like hydroxypropyl-β-CD, methylated-β-CD, sulphated-β-CD, and sulphated-α-CD has been studied by Gagyi et al. [17]. An HPLC and solubility study of the interaction between CDs and pindolol has been investigated by Gazpio et al. [18]. Ficarra and coworkers [19] have determined the stability constant of inclusion complex of atenolol with β-CD by phase solubility to be 28.33 M^{-1} at 25°C, employing the Higuchi-Connors equation. To the best of our knowledge, there is no report on the systematic study of retention mechanism of atenolol with β-CD by reversed phase liquid chromatography. The primary reason behind selection of atenolol from the β-blocker category is its well-established therapeutic importance in the field of medical science and the interest in healthcare industries towards this drug.

The paper describes a reversed phase-HPLC approach to study the retention behaviour of atenolol-β-CD inclusion complex. The effect of temperature and pH of the mobile phase on the stability constant is evaluated and the impact of concentration of β-CD is discussed. Further, the modynamic parameters were calculated to elucidate the mechanism of inclusion complexation and retention.

2. Experimental

2.1. Chemicals and Solutions. β-Cyclodextrin (99.2%) and atenolol (99.5%) were obtained as a gift sample from Merck KGaA (Darmstadt, Germany) and Zydus Cadila Healthcare Ltd. (Ahmedabad, India), respectively. Standard stock solution of atenolol (1.0 mg/mL) was prepared in methanol and stored at 5°C. HPLC grade methanol, acetonitrile, and tetrahydrofuran were procured from Merck India Ltd. (Mumbai, India). Ultrapure water was obtained using Barnstead water purification system (Barnstead, IA, USA). AR grade sodium nitrate, sodium hydroxide, phosphoric acid, and sodium dihydrogen phosphate of purity ≥99% were purchased from S.D. Fine Chemicals Ltd. (Mumbai, India). All solutions analyzed were filtered through 0.45 μm mdi Nylon syringe filters from Advanced Microdevices Pvt. Ltd. (Ambala Cantt, India).

2.2. Equipment. The centrifuge tubes (1.5 mL capacity) were purchased from Tarson (Kolkata, India). Amber-coloured wide-opening HPLC vials, glass conical inserts with polymer feet and screw caps were purchased from Agilent (Waldbronn, Germany), while syringes were purchased from Becton Dickinson India Pvt. Ltd. (Haryana, India). Autopipettes were procured from Eppendorf (Hauppauge, NY, USA). Sonication was performed on Sonicator 8891 (Cole-Parmer, USA), while the vortexer-3020 was from Tarson (Kolkata, India). Weighing was done on Analytical balance Model AG245 from Mettler Toledo (Switzerland). pH measurements were carried out on Metrohm 780 pH meter (Herisau, Switzerland). Three YMC stainless steel HPLC columns, namely, ODS-AQ C18 and CN pack, were purchased from YMC India (Mumbai, India), while Kromasil C4 and NH_2 columns were procured from Eka Chemicals (Akzo Nobel, Bohus, Sweden). High-performance liquid chromatography measurement was performed on Agilent 1200 series HPLC-DAD (Waldbronn, Germany), equipped with degasser G1320A, binary pump G1312B, HiP Auto-sampler SL G13676 with cooler and thermostat ASL unit G1330B and thermo column compartment TCC SL G1316B. The data were collected and processed on PC equipped with ChemStation software.

2.3. Chromatographic Condition. The chromatographic experiments were conducted on preequilibrated stainless steel YMC-ODS-AQ (150 × 4.6 mm, 5 μ) column at different temperatures (15, 20, 25, 30, 35, and 40°C). The detection of atenolol was performed using ultraviolet detector at a wavelength maxima (λ_{max}) of 226 nm. The mobile phase consisted of 50 mM sodium dihydrogen phosphate (NaH_2PO_4 and methanol (92 : 08, v/v), pH adjusted to 6.8 by addition of 5% NaOH with various concentration of β-CD (0, 2, 4, 6, 8, and 10 mM) in water. The mobile phase flow rate was maintained at 1.0 mL/min. The injection volume was kept at 2 μL and all the experiments at different temperature and β-CD concentration were done in triplicate. The dead time was obtained experimentally using sodium nitrate.

2.4. Determination of Apparent Formation Constants. The apparent formation constant (K) of atenolol can be determined by the equation [20]

$$\frac{1}{k} = \frac{1}{k_0} + \frac{K[\beta\text{-CD}]^x}{k_0}, \quad (1)$$

where k is the atenolol retention factor in presence of β-CD, k_0 is the retention factor in the absence of β-CD in the mobile phase, [β-CD] is the concentration of cyclodextrin, and x is the stoichiometry of the complex. The retention factors were monitored in presence of increasing concentration of β-CD. For an inclusion complex with 1 : 1 stoichiometry, the value of x is 1 and a plot of $1/k$ versus [β-CD] must be a straight line. The value of K is then computed from the slope of the linear plot.

2.5. Temperature Studies and Evaluation of Thermodynamic Parameters. The chromatographic system was equilibrated for at least 1 h prior to each experiment and the retention factors were determined at 15, 20, 25, 30, 35, and 40°C. The standard enthalpy (ΔH^0) and entropy (ΔS^0) values for the transfer of atenolol from the mobile phase to β-CD were calculated using the following thermodynamic relationship [21]:

$$\ln K = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{T}, \quad (2)$$

where T is the temperature and R the gas constant. The values of enthalpy (ΔH^0) and entropy (ΔS^0) were computed from the slope, $\Delta H^0/T$ and intercept, $\Delta S^0/R$, respectively, form the van't Hoff plot of $\ln K$ versus $1/T$. The standard free energy (ΔG^0) was calculated from the well-known equation

$$\Delta G^0 = -RT \ln K. \quad (3)$$

3. Results and Discussion

There can be two approaches for applying CDs in reversed phase HPLC (i) CDs can be chemically bonded to the stationary phase or (ii) can be used as an additive in the mobile phase. Based on the interaction (host-guest type) of analyte (guest) with CD, the retention time of the analyte will change; it will be shorter when complexation occurs in the mobile phase and longer when it takes place in the stationary phase [18]. These changes in the retention behaviour are closely related to the apparent formation constants of the complexes formed.

3.1. Optimization of Chromatographic Conditions. Initially, different columns, namely, Kromasil C4 (150 × 4.6 mm, 5 μ), Kromasil NH_2 (250 × 4.6 mm, 5 μ), YMC CN pack (250 × 4.6 mm, 5 μ), and YMC ODS-AQ C18 (150 × 4.6 mm, 5 μ), were screened to get adequate retention of atenolol in the absence of β-CD. The amino and cyano are polar bonded silica columns having lower hydrophobicity but higher polarity. Both these columns have been used in reversed phase separations for polar compounds. Cyano columns are less sterically restricted and have lower hydrogen-bond acidity. On the other hand Kromasil C4 and YMC ODS-AQ C18 are less polar and have higher hydrophobicity of 0.965 and 0.733, respectively, [20, 21]. The general properties and physical characteristics of the columns are presented in Table 1 [22, 23]. Several solvent systems were tested, which included buffer solutions like acetate and phosphate (pH 3.0–7.0) and commonly used organic solvents like acetonitrile, methanol, along with modifier like tetrahydrofuran (THF) in different compositions. Use of ethanol and 1-propanol was deliberately avoided as they have higher association constants of 0.93 M^{-1} and 3.71 M^{-1} with β-CD [24]. Further, the effect of injection volume and flow rate was also studied to have an efficient chromatography. In purely aqueous system, the retention time was very high (>30 min) and thus small volume fractions of organic solvents (6–20%) were tried in the mobile phase during method development. Conversely, in higher organic solvent fractions the retention time of atenolol was considerably reduced. This is due to the higher solvation effect which results in less interaction with the stationary phase [25]. A similar observation was also seen in presence of β-CD (10 mM) when the volume fraction of methanol was greater than 8% for all the columns. Figure 2 shows a significant decrease in retention factor of atenolol with increase in methanol component in the mobile phase. Higher content of methanol makes the mobile phase less polar and thus the affinity of atenolol towards the hydrophobic cavity of β-CD is diminished. Another phenomena which can also effect the inclusion process is the competition of methanol for binding β-CD, though it weakly binds to β-CD with an association constant of 0.32 M^{-1} [26].

As it was expected to have further reduction in retention time in presence of β-CD in the mobile phase, the chromatographic conditions were suitably optimized to have adequate retention on these columns. The effect of mobile phase pH on different columns was also studied and the results are summarized in Table 2. Best chromatographic conditions were achieved in terms of peak shape, adequate response, and sufficient retention on YMC ODS-AQ column, using 0.05 mM NaH_2PO_4 in water and methanol (92 : 8, v/v) having pH 6.8 as the mobile phase. The effect of THF modifier in the mobile phase had little impact on the peak shape or retention time and hence was not taken in the optimized mobile phase. A flow rate of 1 mL/min gave acceptable peak

Table 1: Physical characteristics and general properties of HPLC columns studied.

Parameter	YMC ODS-AQ C18	Kromasil, C4	YMC CN Pack	Kromasil NH_2
Column length, mm	150	150	250	250
Internal diameter, mm	4.6	4.6	4.6	4.6
Particle size, μ	5	5	5	5
Pore size, Å	120	120	120	120
Surface area, m^2/g	330	340	330	340
Carbon content	14	8	7	1.9% N_2
pH range	2.0–7.5	2.0–7.5	2.0–7.5	1.5–9.5

Table 2: Effect of pH on different columns and apparent formation constants of atenolol. Mobile phase: 50 mM NaH_2PO_4, pH—6.8: methanol (92 : 8, v/v). Wavelength: 226 nm. Flow rate: 1.0 mL/min. Temperature: 25°C.

Analytical column	Retention time, min		Retention factor, k		Apparent formation constant, K (M^{-1})
	0 mM β-CD	10 mM β-CD	0 mM β-CD	10 mM β-CD	
			pH 3.0		
[a]Kromasil, C4	8.4	6.3	3.33	2.23	49.33
[b]Kromasil, NH_2	3.2	3.1	0.14	0.09	55.56
[c]YMC-CN	5.0	4.8	0.56	0.50	12.00
[d]YMC ODS-AQ C18	12.8	6.5	6.07	2.59	134.36
			pH 4.0		
[a]Kromasil, C4	9.2	6.7	3.72	2.44	52.46
[b]Kromasil, NH_2	3.7	3.3	0.32	0.18	77.78
[c]YMC-CN	6.1	5.6	0.91	0.75	21.33
[d]YMC ODS-AQ C18	14.6	7.1	7.06	2.92	141.78
			pH 5.0		
[a]Kromasil, C4	11.6	7.5	5.18	2.85	73.68
[b]Kromasil, NH_2	4.9	3.9	0.75	0.39	92.31
[c]YMC-CN	8.4	6.9	1.63	1.16	40.52
[d]YMC ODS-AQ C18	17.8	7.8	8.83	3.31	166.77
			pH 6.8		
[a]Kromasil, C4	13.5	8.4	5.92	3.32	78.31
[b]Kromasil, NH_2	5.8	4.3	1.07	0.52	98.15
[c]YMC-CN	10.6	8.0	2.31	1.51	52.98
[d]YMC ODS-AQ C18	22.8	9.3	11.62	4.16	179.47

Dead time of the columns: [a]1.94 min, [b]2.80 min, [c]3.20 min, [d]1.81 min.
All measurements were done in triplicate.

shape and run time. The % CV in the measurement of retention time was ≤1.5 for 50 injections on the same column, indicating high reproducibility and stability of the chromatographic system. The retention time observed for atenolol on Kromasil C4, Kromasil NH_2 and YMC CN pack, and YMC ODS-AQ columns was 13.5, 5.8, 10.6, and 22.8, respectively, using β-CD free mobile phase. By addition of β-CD (0–10 mM) in the mobile phase the retention time for atenolol decreased from 22.8 to 9.3, with a corresponding decrease in retention factor (k) from 11.62 to 4.16 on YMC ODS-AQ C18 column (Figure 3). The retention factors were calculated based on the dead time of 1.81 min on this column. This reduction was due to the formation of atenolol-β-CD inclusion complex, which enhances the solubility of atenolol in the mobile phase and thus reduces the residence time in the column. To confirm this, glucose which is a constituent of β-CD was added in the mobile phase (70 mM, equivalent to 10 mM β-CD); however, there was no change in the retention factor. This result is in agreement with the work reported by Clarot et al. [27]. Further, the number of theoretical plates (N) was reduced with increase in the concentration of β-CD in the mobile phase (Table 3). This signifies that the efficiency of atenolol to interact with the stationary phase decreases with increasing concentration of β-CD and this favours interaction of atenolol with the hydrophobic interior of β-CD cavity. At concentrations higher than 10 mM of β-CD, a considerable change in peak shapes was observed, probably due to aggregation of β-CD molecules in the mobile phase [28]. The retention factor (k) of atenolol on Kromasil C4 (5.92), Kromasil NH_2 (1.07) and YMC CN pack (2.31)

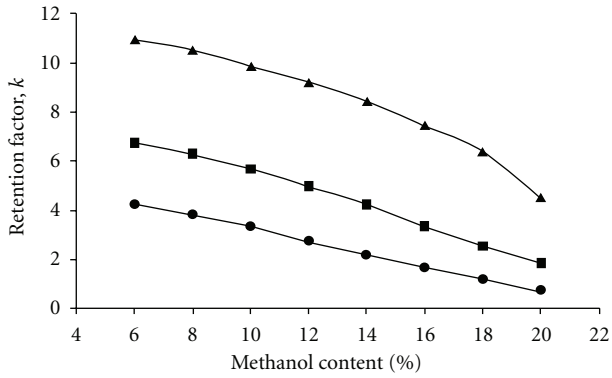


Figure 2: Correlation between the retention factor (k) of atenolol and the volumetric fraction of methanol in the water-methanol mobile phase at a flow rate of 1.0 mL/min at 25°C on YMC ODS-AQ C18 column. (▲) 0 mM β-CD, (■) 5 mM β-CD, (●) 10 mM β-CD.

Table 3: Variation in the retention factor (k) and number of theoretical plates with β-cyclodextrin concentration for atenolol. Mobile phase: 50 mM NaH_2PO_4, pH—6.8: methanol (92 : 8, v/v). Wavelength: 226 nm. Flow rate: 1.0 mL/min. Temperature: 25°C.

β-Cyclodextrin concentration ($M \times 10^{-3}$)	Retention factor (k)	Number of theoretical plates
0.0	11.62	3510
2.0	7.81	3305
4.0	6.49	2702
6.0	5.13	2298
8.0	4.68	1803
10.0	4.16	1597

All measurements were done in triplicate.

was too low under the optimum conditions of mobile phase composition and pH (Table 2). Due to further reduction in the retention time in presence of β-CD, they were not considered further in the present work.

3.2. Apparent Formation Constant: Role of pH and Temperature. The retention of atenolol on YMC ODS-AQ C18 column is based on its partition between the mobile and the stationary phase; however, in presence of β-CD in the mobile phase the retention of atenolol is divided into two physicochemical processes: (i) complexation of atenolol by β-CD and (ii) the transfer of free atenolol from the mobile phase to the stationary phase. The plot of $1/k$ versus [β-CD] at 25°C was linear with regression coefficient "r" = 0.998. From the value of "r" it is evident that the inclusion complex has 1 : 1 stoichiometry. With an increase in β-CD concentration there was a corresponding decrease in retention factors for atenolol (Table 3). This is in agreement with two previous reports which have studied inclusion complexation between atenolol and β-CD [19, 29]. The K value of $179.47 \pm 2.1\ M^{-1}$ obtained by the chromatographic experiment is considerably higher compared to that obtained by phase solubility study ($28.66\ M^{-1}$) for atenolol at 25°C [19].

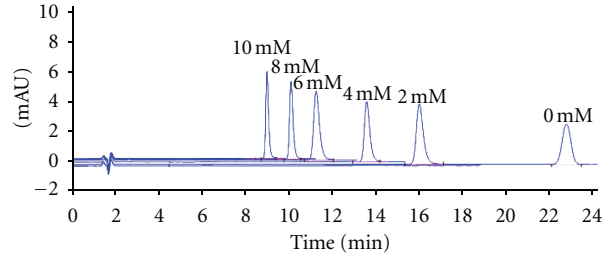


Figure 3: Effect of β-cyclodextrin concentration on the retention time of atenolol on YMC ODS-AQ C18 column using 50 mM NaH_2PO_4, pH—6.8: methanol (92 : 8, v/v) as the mobile phase at a flow rate of 1.0 mL/min at 25°C.

It is not unusual to find this huge difference in the apparent stability constants using two different techniques [30]. Phase solubility studies are conducted at a much higher concentration levels as compared to chromatographic conditions, thus it is not significant to compare the results obtained in the present work. As is evident from Table 2, pH plays a significant role in atenolol-β-CD inclusion complex formation. The formation of inclusion complex is related to the hydrophobic nature of the drug. The *pKa* of atenolol at 25°C is 9.6 and it remains in an unionized form at this pH, while under acidic pH the amino group is protonated and this has negative influence in the complex formation as β-CD has a nonpolar cavity, resulting in a lower value of apparent formation constant. The K value of $179.47\ M^{-1}$ at pH 6.8 could have further increased in the alkaline pH due to the basic nature of atenolol. However, in the alkaline pH range the silica-based stationary phase tends to hydrolyse, which can affect the column efficiency and hence was not studied in the present work. The formation constants evaluated at pH 3.0, 4.0, and 5.0 were 134.36, 141.78, and $166.76\ M^{-1}$, respectively. This experiment indicates that pH of the mobile phase was more important in retention mechanism and therefore in the inclusion process. The probable inclusion phenomena can be explained by the hydrophobic effect of the phenyl ring which would be inside the hydrophobic cavity of β-CD. The apparent formation constant values obtained for all four columns at different pH are also summarized in Table 2. The values obtained for apparent formation constants (K) at different temperatures are presented in Table 4. Inclusion complexes are generally less stable at higher temperature as reported previously [31]. At lower temperature the retention decreases with higher degree of complexation. Consequently, the concentration of free atenolol that can be adsorbed on the stationary phase is reduced.

3.3. Thermodynamic Parameters for Atenolol-β-CD Inclusion Complex Formation. Determination of thermodynamic parameters is indispensable in understanding the process of molecular recognition. To understand the mechanism of interaction of atenolol with β-CD (10 mM), the thermodynamic parameters were evaluated from the van't Hoff plots. The plot of $\ln K$ versus $1/T$ was linear with correlation coefficient "r" greater than 0.9985 (Figure 4). The enthalpy and entropy values calculated from the slope and intercept were

Table 4: Apparent formation constant (K) of atenolol and thermodynamic parameters at different temperatures in presence of β-CD. Column: YMC ODS-AQ C18 (150 × 4.6 mm, 5 μ). Mobile phase: 10 mM β-CD in 50 mM NaH_2PO_4, pH—6.8: methanol (92 : 8, v/v). Wavelength: 226 nm. Flow rate: 1.0 mL/min.

Temperature, K	Apparent formation constant, K (M^{-1})	ΔG^0 (kJ/mol)	ΔH^0 (kJ/mol)	ΔS^0 (kJ/K/mol)	$T\Delta S^0$ (kJ/mol)
288	324.44	−13.85	—	—	—
293	239.85	−13.35	−42.40	−0.0933	−29.05
298	179.47	−12.86	−42.12	−0.0982	−29.26
303	135.64	−12.37	−42.05	−0.0979	−29.68
308	103.54	−11.88	−41.92	−0.0975	−30.04
313	79.84	−11.40	−41.68	−0.0967	−30.28

All measurements were done in triplicate.

Table 5: Retention factors of atenolol and thermodynamic parameters at different temperatures in absence of β-CD. Column: YMC ODS-AQ C18 (150 × 4.6 mm, 5 μ). Mobile phase: 50 mM NaH_2PO_4, pH—6.8: methanol (92 : 8, v/v). Wavelength: 226 nm. Flow rate: 1.0 mL/min.

Temperature, K	Retention factor, k	ΔG^0 (kJ/mol)	ΔH^0 (kJ/mol)	ΔS^0 (kJ/K/mol)	$T\Delta S^0$ (kJ/mol)
288	14.62	−6.42	—	—	—
293	13.00	−6.25	−16.48	−0.0349	−10.23
298	11.62	−6.08	−16.35	−0.0350	−10.27
303	10.43	−5.91	−16.23	−0.0341	−10.32
308	9.40	−5.74	−16.14	−0.0337	−10.40
313	8.51	−5.57	−16.04	−0.0334	−10.47

All measurements were done in triplicate.

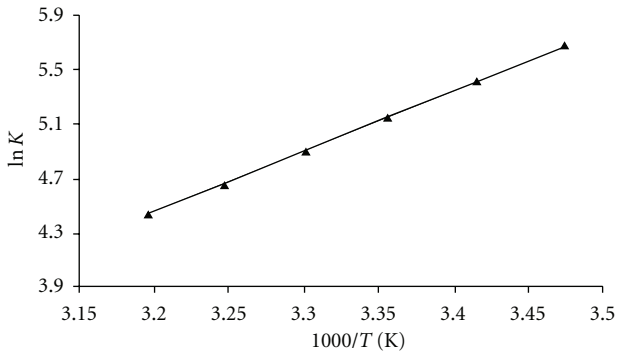


Figure 4: Van't Hoff plot ($\ln K$ versus $1/T$) for atenolol on YMC ODS-AQ C18 column in presence of 10 mM β-CD using 50 mM NaH_2PO_4, pH—6.8: methanol (92 : 8, v/v) as the mobile phase at a flow rate of 1.0 mL/min.

−42.12 kJ/mol and −29.26 J/mol/K, respectively, at 25°C (Table 4). The negative value for enthalpy indicates that the association between atenolol and β-CD is exothermic. This value is typical of interactions such as hydrophobic interactions as a result of displacement of water molecules from the cavity of β-CD, van der Waals interactions, formation of hydrogen bonds, and others [32]. The negative value of entropy can be related to a more ordered system due to decrease in translational and rotational degrees of freedom of the complexed species as compared to the free molecules. It is interesting to see that the magnitude of ΔH^0 is greater than $T\Delta S^0$ over the temperature range studied (Table 4). This further indicated that enthalpy played a greater role in complexation than entropy. The Gibbs free energy change (ΔG^0) for atenolol-β-CD inclusion complex at 25°C was −12.86 kJ/mol, indicating that the inclusion process is a spontaneous one.

3.4. Thermodynamic Parameters for Transfer of Atenolol from Mobile Phase to the Stationary Phase. A linear relationship ($r = 0.998$) was observed between $1/k$ and the absolute temperature in the absence of β-CD. This linear relationship suggests minimum conformational changes and that the retention mechanism is the same over the temperature range investigated. Zarzycki and Lamparczyk [33] have described an excellent linear relationship observed during the unmodified mobile phase with series of naphthalene derivatives. As evident from the values in Table 5 the magnitude of ΔH^0 was higher than $T\Delta S^0$ which can be attributed to favourable transfer of atenolol from the mobile phase to the stationary phase and is enthalpy driven. The negative ΔS^0 value suggests greater immobilization effect after the transfer and ordering of the chromatographic system. When atenolol was transferred from the mobile to the stationary phase, the solute-solvent interactions were replaced by van der Waals interactions, leading to negative enthalpy change. The retention mechanism of atenolol involved classical transfer of solute from mobile phase to stationary phase. The retention factor decreased with increase in temperature along with corresponding increase in free energy change from −6.42 to −5.57 kJ/mol for atenolol-stationary phase interaction.

3.5. Enthalpy and Entropy Compensation for the Inclusion Complex. The enthalpy-entropy compensation can work as

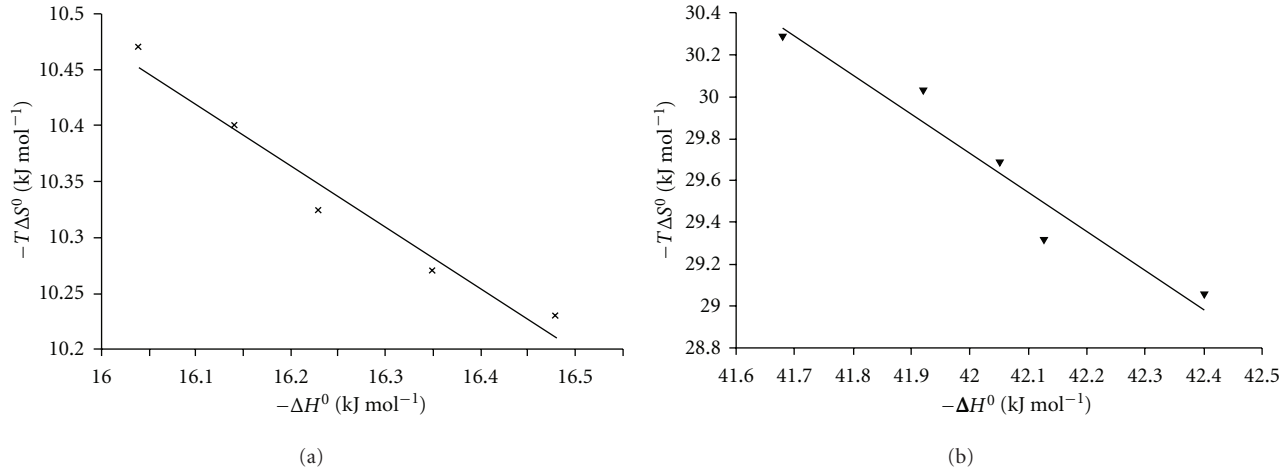


Figure 5: Enthalpy-entropy compensation plot ($T\Delta S^0$ versus ΔH^0) for atenolol in (a) absence and (b) presence of β-cyclodextrin on YMC ODS-AQ C18 column using 50 mM NaH_2PO_4, pH—6.8: methanol (92 : 8, v/v) as the mobile phase at a flow rate of 1.0 mL/min.

a tool in understanding molecular recognition between the analyte and β-CD [34]. The plots of $T\Delta S^0$ versus ΔH^0 show that both the parameters are linearly correlated in absence and presence of β-CD in Figures 5(a) and 5(b), respectively. The slope (α) of this linear plot indicates the extent to which the enthalpic gain is cancelled by the accompanying loss in entropy, which are affected when there is any interaction between atenolol and β-CD. Thus, only a fraction $(1-\alpha)$ of the enthalpic gain can contribute to the enhancement of complex stability. The intercept represents the inherent complex stability obtained at $\Delta H^0 = 0$, which means that the complex is stabilized even in the absence of enthalpic gain when $T\Delta S^0$ term is positive. The slope (α) and the intercept $T\Delta S^0$ of the regression equation can be related to the degree of conformational change and to the extent of desolvation upon complexation, respectively, [35]. The lower the value of the slope, the less will be the conformational change on account of complexation. Similarly, the higher the value of the intercept the greater is the desolvation, which favours complexation. The value of slope from $T\Delta S^0$ versus ΔH^0 plots changed from -0.55 in absence of β-CD (Figure 5(a)) to -1.71 in presence of β-CD (Figure 5(b)). This change in magnitude of the slope indicates that the drug may be partially inside the β-CD cavity. The NMR studies have also shown a chemical shift of δ 0.131–0.148 for phenyl protons upon complexation [29]. By extrapolating, the intercept at $\Delta H^0 = 0$ in presence of β-CD was -98.1 and was higher in magnitude compared to -19.43 in the absence of β-CD. This implies higher desolvation of atenolol on account of inclusion complex formation. Further, as is evident from the enthalpy and free energy values at different temperatures in Tables 4 and 5, atenolol-β-CD interaction is more favourable as compared to atenolol-stationary phase interaction.

4. Conclusion

The mechanism of retention in RP-HPLC and inclusion complex formation of atenolol with β-CD has been studied. The dependence of inclusion complex on pH and temperature was investigated and trends in thermodynamics parameters were determined over a wide range of column temperature. The variation observed can be explained in terms of two main physicochemical processes, solute inclusion in the β-CD in cavity and solute transfer from the mobile phase to stationary phase. It is evident from the apparent formation constants that the extent to which the inclusion complexation is favoured depends not only on the polarity and structure of the guest but also on the composition and pH of the mobile phase. The thermodynamic data proves the formation of atenolol-β-CD inclusion complex and that the process is essentially enthalpy driven. Further, the enthalpy-entropy compensation data supports the phenomena of molecular inclusion of atenolol in β-CD.

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References

[1] P. S. Shrivastav, S. M. Buha, and M. Sanyal, "Detection and quantitation of β-blockers in plasma and urine," *Bioanalysis*, vol. 2, no. 2, pp. 263–276, 2010.

[2] L. Dong and J. Huang, "Determination of atenolol in human plasma by pseudo reversed phase liquid chromatography-tandem mass spectrometry," *Chromatographia*, vol. 64, no. 9-10, pp. 583–586, 2006.

[3] F. Hirayama and K. Uekama, "Cyclodextrin-based controlled drug release system," *Advanced Drug Delivery Reviews*, vol. 36, no. 1, pp. 125–141, 1999.

[4] T. Loftsson and D. Duchêne, "Cyclodextrins and their pharmaceutical applications," *International Journal of Pharmaceutics*, vol. 329, no. 1-2, pp. 1–11, 2007.

[5] K. A. Connors, "The stability of cyclodextrin complexes in solution," *Chemical Reviews*, vol. 97, no. 5, pp. 1325–1357, 1997.

[6] S. E. Çelik, M. Özyürek, A. N. Tufan, K. Güçü, and R. Apak, "Spectroscopic study and antioxidant properties of the inclusion complexes of rosmarinic acid with natural and derivative cyclodextrins," *Spectrochimica Acta Part A*, vol. 78, no. 5, pp. 1615–1624, 2011.

[7] J. Chao, D. Meng, J. Li, H. Xu, and S. Huang, "Preparation and study on the novel solid inclusion complex of ciprofloxacin with HP-β-cyclodextrin," *Spectrochimica Acta Part A*, vol. 60, no. 3, pp. 729–734, 2004.

[8] Y. Wang, H. Chen, Y. Xiao et al., "Preparation of cyclodextrin chiral stationary phases by organic soluble catalytic "click" chemistry," *Nature Protocols*, vol. 6, no. 7, pp. 935–942, 2011.

[9] D. W. Armstrong, W. Li, C. D. Chang, and J. Pitha, "Polar-liquid, derivatized cyclodextrin stationary phases for the capillary gas chromatography separation of enantiomers," *Analytical Chemistry*, vol. 62, no. 9, pp. 914–923, 1990.

[10] Z. Juvancz and J. Szejtli, "The role of cyclodextrins in chiral selective chromatography," *Trends in Analytical Chemistry*, vol. 21, no. 5, pp. 379–388, 2002.

[11] H. Y. Aboul-Enein and I. Ali, *Chiral Separation by Liquid Chromatography and Related Technology*, vol. 90, chapter 10, Marcel Dekker, New York, NY, USA, 2003.

[12] L. M. Yuan, "Effect of mobile phase additive on chiral separation," *Separation and Purification Technology*, vol. 63, no. 3, pp. 701–705, 2008.

[13] E. Majid, K. B. Male, Y. M. Tzeng, J. O. Omamogho, J. D. Glennon, and J. H. T. Luong, "Cyclodextrin-modified capillary electrophoresis for achiral and chiral separation of ergostane and lanostane compounds extracted from the fruiting body of Antrodia camphorata," *Electrophoresis*, vol. 30, no. 11, pp. 1967–1975, 2009.

[14] A. Kwaterczak, K. Duszczyk, and A. Bielejewska, "Comparison of chiral separation of basic drugs in capillary electrophoresis and liquid chromatography using neutral and negatively charged cyclodextrins," *Analytica Chimica Acta*, vol. 645, no. 1-2, pp. 98–104, 2009.

[15] H. Dodziuk, "Molecules with holes—cyclodextrins," in *Cyclodextrins and Their Complexes: Chemistry, Analytical Methods, Applications*, H. Dodziuk, Ed., chapter 1, pp. 1–30, Wiley-VCH, Weinheim, Germany, 2006.

[16] Y. Wei, X. Lin, and C. Zhu, "Chiral separation of some β-blockers using electrophoresis with dual cyclodextrin systems," *Canadian Journal of Analytical Sciences and Spectroscopy*, vol. 50, no. 3, pp. 135–140, 2005.

[17] L. Gagyi, A. Gyéresi, and F. Kilár, "Role of chemical structure in stereoselective recognition of beta-blockers by cyclodextrins in capillary zone electrophoresis," *Journal of Biochemical and Biophysical Methods*, vol. 70, no. 6, pp. 1268–1275, 2008.

[18] C. Gazpio, M. Sánchez, I. X. García-Zubiri et al., "HPLC and solubility study of the interaction between pindolol and cyclodextrins," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 37, no. 3, pp. 487–492, 2005.

[19] R. Ficarra, P. Ficarra, M. R. di Bella et al., "Study of the inclusion complex of atenolol with β-cyclodextrins," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 23, no. 1, pp. 231–236, 2000.

[20] L. R. Snyder, J. W. Dolan, and P. W. Carr, "The hydrophobic-subtraction model of reversed-phase column selectivity," *Journal of Chromatography A*, vol. 1060, no. 1-2, pp. 77–116, 2004.

[21] L. R. Snyder, J. W. Dolan, and P. W. Carr, "A new look at the selectivity of reversed-phase HPLC columns," *Analytical Chemistry*, vol. 79, no. 9, pp. 3255–3262, 2007.

[22] http://ymc.co.jp/en/columns/ymc_pack_ods_a/.

[23] http://www.usp.org/USPNF/columnsUSPapproach.html.

[24] J. M. López-Nicolás, E. Núñez-Delicado, A. J. Pérez-López, A. C. Barrachina, and P. Cuadra-Crespo, "Determination of stoichiometric coefficients and apparent formation constants for β-cyclodextrin complexes of trans-resveratrol using reversed-phase liquid chromatography," *Journal of Chromatography A*, vol. 1135, no. 2, pp. 158–165, 2006.

[25] N. F. S. de Melo, R. Grillo, A. H. Rosa, and L. F. Fraceto, "Interaction between nitroheterocyclic compounds with β-cyclodextrins: phase solubility and HPLC studies," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 47, no. 4-5, pp. 865–869, 2008.

[26] Y. Matsui and K. Mochida, "Binding forces contributing to the association of cyclodextrin with alcohol in an aqueous solution," *Bulletin of the Chemical Society of Japan*, vol. 52, no. 10, pp. 2808–2814, 1979.

[27] I. Clarot, D. Cledat, S. Battu, and P. J. P. Cardot, "Chromatographic study of terpene derivatives on porous graphitic carbon stationary phase with β-cyclodextrin as mobile phase modifier," *Journal of Chromatography A*, vol. 903, no. 1-2, pp. 67–76, 2000.

[28] T. Loftsson, M. Másson, and M. E. Brewster, "Self-association of cyclodextrins and cyclodextrin complexes," *Journal of Pharmaceutical Sciences*, vol. 93, no. 5, pp. 1091–1099, 2004.

[29] R. Ficarra, P. Ficarra, M. R. di Bella et al., "Study of β-blockers/β-cyclodextrins inclusion complex by NMR, DSC, X-Ray and SEM investigation," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 23, no. 1, pp. 33–40, 2000.

[30] M. V. Rekharsky and Y. Inoue, "Complexation thermodynamics of cyclodextrins," *Chemical Reviews*, vol. 98, no. 5, pp. 1875–1917, 1998.

[31] S. Li and W. C. Purdy, "Cyclodextrins and their applications in analytical chemistry," *Chemical Reviews*, vol. 92, no. 6, pp. 1457–1470, 1992.

[32] C. Ravelet, A. Geze, A. Villet et al., "Chromatographic determination of the association constants between nimesulide and native and modified β-cyclodextrins," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 29, no. 3, pp. 425–430, 2002.

[33] P. K. Zarzycki and H. Lamparczyk, "Evidences for temperature-dependent mechanism of host-guest complexation," *Chromatographia*, vol. 48, no. 5-6, pp. 377–382, 1998.

[34] M. V. Rekharsky and Y. Inoue, "Microcalorimetry," in *Cyclodextrins and Their Complexes: Chemistry, Analytical Methods, Applications*, H. Dodziuk, Ed., chapter 8, pp. 199–230, Wiley-VCH, Weinheim, Germany, 2006.

[35] Y. Inoue and T. Wada, "Molecular recognition in chemistry and biology as viewed from enthalpy-entropy compensation effect," in *Advances in Supramolecular Chemistry*, G. W. Gokel, Ed., pp. 55–96, JAI Press, Greenwich, Conn, USA, 1997.