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Enantioselective Separation and Determination of Pirbuterol in Bulk Drugs and Formulations by HPLC

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A simple and rapid liquid chromatographic methods for enantioselective separation and determination of -(±)-enantiomers of pirbuterol in bulk drugs and formulations using UV and polarimetric detectors connected in series was developed. The enantiomers were tested on numerous commercial HPLC columns. Pirbuterol separation was determined on amylose chiralpak AD-H column. The mobile phase compositions were systematically studied to obtain the optimal chromatographic methods. Validation of methods in selected conditions shows that the chosen methods are selective and precise with linear response of detector for both pairs of enantiomers.

Key Words: β-Adrenoceptor agonists, Polysaccharide stationary phases, Chiral recognition, Polarimetric detector.

INTRODUCTION

There are two categories of antiasthma drugs, bronchodilators and antiinflammatory agents. Drugs used as bronchodilators include β2-adrenoceptor agonists, xanthines, cysteinyl-leukotriene receptor antagonists and muscarinic receptor antagonists. The β2-adrenoceptor agonists, their primary effect in asthma is to dilate the bronchi by a direct action on the β2-adrenoceptors on the smooth muscle. These drugs are usually given by inhalation of aerosol, powder or nebulised solution, but some may be given orally or by injection. Two categories of β2-adrenoceptor agonists are used in asthma.

Short-acting agents: Salbutamol and terbutaline, these are given by inhalation the maximum effect occurs within 0.5 h and the duration of action is 4-6 h. They are usually used on an as needed basis to control symptoms.

Larger-acting agents: Salmeterol are given by inhalation and the duration of action is 12 h. They are not used as needed but are given regularly twice daily as adjunctive they apply in patients whose asthma is inadequately controlled by gluco-corticoids. Other long acting agents are pirbuterol, formoterol, fenoterol and reprotelol.

Most of the chiral drugs available in the market are administered as racemate[1]. However, the great difference in pharmacological effects and pharmacokinetics between the two enantiomeric forms of many drugs is also well known[2,3]. Therefore, the pharmaceutical industry increasingly needs new analytical and preparative procedures capable of resolving and quantitation of drug enantiomers and the resolution of racemic mixtures is becoming a highly challenging area of separation technology.

Pirbuterol, (((1,1-dimethyl ethyl)amino)methyl)-3-hydroxyl-2,6-pyridine methanol) (PBT) are long-acting β-2-adrenoceptor agonist with bronchodilatory effects and rapid onset of action. It has been shown that single enantiomers of chiral drugs are often more potent or have less side effects compared to their racemate. Throughout the 20th century, β-adrenoceptor agonists have been developed and marketed as racemate. The pharmacological activity usually resides in the (R)-enantiomer. Despite claims for the opposite, there is so far no compelling evidence that the presence of the less active (S)-enantiomer is of any harm to the patient.

Isotachophoresis (ITP)[4,5], capillary gel electrophoresis (CGE)[6,7], micellar electrokinetic chromatography (MEKC)[8,9] and capillary electrochromatography (CEC)[10,11] methods are available for analysis of pirbuterol, but chiral HPLC method based on polysaccharide based stationary phases are limited, so we have tried to develop a method on polysaccharide based stationary phases. Most experiments quoted in the literature were performed with β-cyclodextrin; γ-cyclodextrin was less frequently used, basically because of its higher price.

EXPERIMENTAL

All reagents were of analytical-reagent grade unless stated otherwise. HPLC-grade diethylamine (DEA), *n*-hexane, 1-propanol, 2-propanol and ethanol were purchased from SD Fine Chem., (Mumbai, India). (±)-enantiomers of pirbuterol

were gifted from Neuland laboratories Ltd. (Hyderabad, India). All solutions were filtered through 0.45 µm membrane filters procured from Pall Pharmalab Filtration Pvt. Ltd. (Mumbai, India).

The HPLC system composed of LC-10AT VP pump, SPD-10A VP UV detector and SIL-10AD VP auto injector and SCL-10A VP system controller attached with thermostat (all from Shimadzu, Kyoto, Japan). Polarimetric detector (IBZ Messtechnik GmbH, Hanover, Germany) was connected to UV detector in series for identification of the enantiomers. Chiralcel OD-H (250 mm × 4.6 mm; particle size 5 µm), chiralcel OJ-H (250 mm × 4.6 mm; particle size 5 µm) and Chiralpak AD-H (250 mm × 4.6 mm; particle size 5 µm) (Daicel Chemical Industries, Tokyo, Japan) were used for separation. The chromatographic and the integrated data were recorded using HP-Vectra (Hewlett Packard, Waldron, Germany) computer system.

Chromatographic conditions: Chromatographic separation of enantiomers of pirbuterol achieved on Chiralpak AD-H (250 mm × 4.6 mm; particle size 5 µm) column. For pirbuterol the mobile phase consisting of *n*-hexane:ethanol: DEA (90:10:0.1 v/v/v) at 25 ºC were used. For pirbuterol the UV detector was kept at 283 nm. The flow rate was 1.0 mL/min and injection volume was 20 µL and total run time was 10 min.

Preparation of stock and standard solutions: Stock solutions of (±)-pirbuterol were prepared by dissolving 200.1 mg of (±)-pirbuterol weighed in 100 mL volumetric flasks, dissolved in 25 mL methanol and made up to the mark with the mobile phase. The stock solutions were wrapped with aluminum foil and kept in the refrigerator at 5 ºC. The specified concentration of enantiomer of pirbuterol were taken as 200 µg/mL for the analysis.

RESULTS AND DISCUSSION

The column, mobile phase selectivity, effect of diethylamine and column temperature on resolution and retention were studied for optimizing the LC conditions for separation of enantiomers of pirbuterol.

Method optimization of pirbuterol enantiomers

Column selectivity: Polysaccharide-based stationary phases chiralcel OD-H (cellulose *tris*-(3,5-dimethylphenylcarbamate)), chiralcel OJ-H (cellulose *tris*-(4-methylbenzoate) and Chiralpak AD-H (amylose *tris*-(3,5-dimethylphenylcarbamate)) were evaluated using 2-propanol, 1-propanol and ethanol as organic modifier in *n*-hexane. Chiralcel OD-H and chiralcel OJ-H columns have not shown any selectivity for pirbuterol enantiomers. Chiralpak AD-H has shown good selectivity and resolution using 1-propanol and ethanol as organic modifier (Table-1). The chiral recognition mechanism on these CSPs is generally due to the formation of solute-CSP complexes through inclusion of the enantiomers in to the chiral cavities in the higher order structures of the CSPs. CSPs with carbamate derivatives have shown better selectivity compared to triacetate derived CSPs. In general, the mechanism of chiral recognition on this type of phases is not well established and an empirical approach based on experimentation is always necessary. However, it is known to some extent that the chiral resolutions were achieved through different interactions with stationary phases. In case of CSPs with carbamate derivatives, the binding of the solutes to the CSPs is through interactions between the solutes and the polar carbamate groups on the CSPs[4,5]. The carbamate groups can interact with solutes through hydrogen bonding using C=O and NH groups and through dipole-dipole interaction using C=O moiety. In the present study the available functional groups on the solutes are OH and NH which can form hydrogen bonds with the C=O group on the CSPs. The solutes having aromatic functionalities could provide additional stabilizing effect to the solute-CSP complex by insertion of the aromatic ring into the chiral cavity. In the present case, this type of stabilization effect may be possible due to the presence of the aromatic functionality on the solutes. Chiralcel OD-H column did not show any selectivity for the pirbuterol enantiomers. Whereas Chiralpak AD-H column has shown excellent selectivity for the pirbuterol enantiomers (Table-1). These differences in chiral recognition mechanism could be attributed to the different configurations of the glucose residues (a and β linkages) and higher order structures of chiral stationary phases of chiralcel OD-H and Chiralpak AD-H columns. So, Chiralpak AD-H column was chosen for further development.

Effect of organic modifier: The type and concentration of organic modifier was found to influence the retention and resolution of pirbuterol enantiomers. 2-Propanol has not shown any selectivity for pirbuterol enantiomers. While 1-propanol and ethanol has shown good selectivity for pirbuterol enantiomers, the selectivity and resolution of the pirbuterol enantiomers with 1-propanol and ethanol on Chiralpak AD-H column are shown in (Table-1). Both the organic modifiers have shown good selectivity for pirbuterol enantiomers. However, ethanol has shown better selectivity when compared to 1-propanol. This phenomenon could be explained by the

TABLE-1
SELECTIVITY OF PIRBUTEROL ENANTIOMERS ON CHIRALPAK AD-H COLUMN WITH DIFFERENT ORGANIC MODIFIERS AT 25 ºC

Organic modifier	K_1	K_2	α	R_s
(A) Chiralpak AD-D				
n-Hexane/1-propanol = 95/5 + 0.5 % diethylamine	8.31	10.37	0.81	4.66
n-Hexane/1-propanol = 93/7 + 0.1 % diethylamine	4.41	4.79	0.93	1.09
n-Hexane/1-propanol = 90/10 + 0.1 % diethylamine	1.89	2.10	0.93	0.96
n-Hexane/1-propanol = 80/20 + 0.1 % diethylamine	–	–	–	–
n-Hexane/ethanol = 93/7 + 0.1 % diethylamine	2.89	4.03	0.77	4.69
n-Hexane/ethanol = 90/10 + 0.1 % diethylamine	1.75	2.34	0.82	4.17
n-Hexane/ethanol = 85/15 + 0.1 % diethylamine	0.82	1.06	0.88	1.60
n-Hexane/ethanol = 80/20 + 0.1 % diethylamine	0.55	0.68	0.91	1.45

difference in the steric bulkiness around the hydroxyl moiety of the mobile phase modifier. The lower alcohols could be inserted in to the cavity of the CSP more easily than bulkier alcohols. The insertion of the mobile phase modifier into the cavities of the CSP could induce changes in the dominant chiral recognition mechanism leading to formation of more stable diastereomeric complexes of the enantiomers causing better separation. As 2-propanol is a bulkier alcohol has not shown any separation for pirbuterol enantiomers. 1-Propanol is less bulky than 2-propanol has shown good separation for pirbuterol enantiomers while ethanol least bulky alcohol has shown excellent separation for pirbuterol enantiomers. The effect of concentration of ethanol and 1-propanol was studied. On decreasing the concentration of organic modifier, the retention factors as well as resolutions were increased. Using ethanol, sharp peaks with higher sensitivity (higher detections limits) and lower retention were observed. Thus, ethanol was chosen as an organic modifier. At 10 % of ethanol in *n*-hexane, resolution > 4.0 was obtained. Further decreasing of ethanol concentration led to peak broadening and higher retentions. As a compromise for higher resolution and lower retention, 10 % of ethanol in *n*-hexane was chosen for analysis.

Effect of concentration of diethylamine: To minimize peak tailing diethylamine was added to mobile phase. Diethylamine was not having much effect on retention factors. But on increasing the diethylamine concentration, peak shapes were sharpen and tailing was reduced. But increasing the diethylamine concentration from 0.1-0.3 %, increased the baseline noise and decrease the peak intensity. As a compromise 0.1 % of diethylamine was chosen as optimum for pirbuterol.

Effect of column temperature: The effect of column temperature on resolution and retention of pirbuterol enantiomers was studied in the range 298-313 K (25-40 ºC) on Chiralpak AD-H column. On increasing the temperature, retentions (Fig. 1) as well as resolutions were decreased (Table-2). Under thermodynamically equilibrium conditions, free energy accompanying the separation of two enantiomers related to retention factors by the following equation:

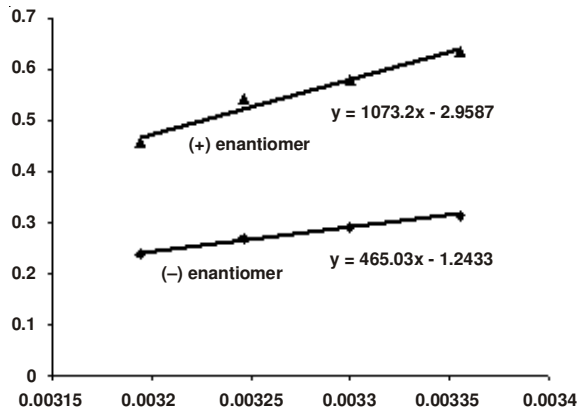

Fig. 1. Van't Hoff plots of the pirbuterol enantiomers at 10 % of ethanol on Chiralpak AD-H column

$$\Delta G^\circ = -RT \ln k \quad (1)$$

where k is the retention factor, R the gas constant and T is the temperature in K. An expansion of eqn. 1 to involve the enthalpy (H) and entropy (S) terms yield.

TABLE-2
EFFECT OF TEMPERATURE ON SELECTIVITY AND RESOLUTION OF PBT ENANTIOMERS ON CHIRALPAK AD-H COLUMN

Temperature (ºC)	k_1	k_2	α	R_s
25	1.68	2.24	0.81	4.10
30	1.65	2.20	0.82	4.02
35	1.62	2.13	0.83	3.82
40	1.52	2.06	0.84	3.54

$$\ln k = \frac{-\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (2)$$

Van't Hoff plots were drawn for logarithm of retention factor (ln k) *versus* inverted temperature (1/T) in K for the two isomers, which yielded straight lines 275 × -0.415 and 527 × -0.958 for (+)-pirbuterol and (–)-pirbuterol enantiomers, respectively. ΔHº and ΔSº for the two enantiomers were obtained from slope and intercept of the straight lines, respectively. The change in free energy accompanying the separation of two enantiomers was given by

$$\Delta\Delta G^\circ = \Delta\Delta H^\circ - T\Delta\Delta S^\circ \quad (3)$$

The enthalpy change (ΔΔHº), entropy change (ΔΔSº) and Gibb's free energy change (ΔΔGº) accompanying the separation were recorded in Table-3. The data indicated that the separation of pirbuterol enantiomers was an enthalpy driven process.

Optimized conditions: Thus, a mobile phase containing *n*-hexane:ethanol:diethylamine (90:10:0.1 v/v/v) was chosen for the separation of pirbuterol enantiomers on Chiralpak AD-H column maintained at 25 ºC. The flow rate was kept at 1.0 mL/min throughout the analysis. The chromatographic separation of (+)-pirbuterol, (–)-pirbuterol in the optimized conditions using UV detector and polarimetric detector. The method was validated in terms of accuracy, precision and linearity as per ICH guidelines.

Validation of pirbuterol enantiomers

System suitability: The solution of pirbuterol-(±) (50 µg/mL) prepared in the mobile phase was used for system suitability studies. The Chiralpak AD-H column was stabilized for 0.5 h in the optimized conditions and three replicate injections were made. The system was deemed to be suitable if resolution between the two pirbuterol enantiomers is not less than 4.0 and tailing factor is not more than 1.47 (at 10 % base).

Precision: Precision of the method was tested by preparing six individual solutions of pirbuterol-(±) and making triplicate injections for each solution. The RSD % of the assay was less than 0.43 %. Inter- and intra-day assay precision was performed by analyzing the solutions for five times in a day for three consecutive days. The RSD % of the assay was less than 0.63 % for the both isomers.

Linearity: Calibration graphs are drawn in the range of 50-400 µg/mL of pirbuterol enantiomers by preparing fresh solutions every day for 3 days. Curves were linear with r^2 > 0.9999 and the regression equations for (+)-pirbuterol and (–)-pirbuterol were Y = 14240× – 2035 and Y = 14300× - 2018, respectively.

Accuracy: Accuracy was determined by spiking pirbuterol solution at five levels in the range 50-150 % with

TABLE-3
THERMODYNAMIC DATA CALCULATED FROM THE VAN'T HOFF PLOTS OF PIRBUTEROL ENANTIOMERS

Enantiomer	ΔH° (KJ mol^{-1})	$\Delta\Delta H^\circ$ (KJ mol^{-1})	ΔS° (J K^{-1} mol^{-1})	$\Delta\Delta S^\circ$ (J K^{-1} mol^{-1})	$\Delta\Delta G^\circ$ (KJ mol^{-1})
-(+)-Pirbuterol	-2.286	-2.0977	-3.451	-4.51	-0.753 (298 K)
-(–)-Pirbuterol	-4.384		-7.964		

ΔH° = Slope × R, ΔS° = Intercept × R, $\Delta\Delta G^\circ = \Delta\Delta H^\circ - T\Delta\Delta S^\circ$. Enantiomeric separation of PBT onChiralpak AD-H column.

TABLE-4
ASSAY OF PIRBUTEROL IN BULK DRUGS AND FORMULATIONS

S. No.	Concentration of pirbuterol (mg/mL)				
	Number of injection	Taken	Recovered	Recovered (%)	RSD (%)*
I	1	0.0987	0.0985	99.86	0.31
	2	0.0987	0.0983		
	3	0.0987	0.0989		
II	1	0.0495	0.0490	100.12	0.99
	2	0.0495	0.0498		
	3	0.0495	0.0499		
III	1	0.0247	0.0249	100.26	1.29
	2	0.0247	0.0244		
	3	0.0247	0.0250		
I	1	0.0984	0.0987	100.13	0.47
	2	0.0984	0.0989		
	3	0.0984	0.0980		
II	1	0.0490	0.0498	101.09	0.53
	2	0.0490	0.0494		
	3	0.0490	0.0499		
III	1	0.0246	0.0243	100.26	1.42
	2	0.0246	0.0247		
	3	0.0246	0.0250		

*n = 3 injections.

respective to specified level (200 µg/mL) and analyzing the each solution in triplicate for three days. Percentage recoveries were between 99.57 and 100.61 %.

Robustness: Robustness of the method was checked by making small deliberate changes in the operating parameters. Variation of 0.5 % of ethanol did not affect the resolution except that retentions were changed. The effect of temperature has been studied by analyzing sample at 25 ± 1 ºC. The resolution remained still above 4. The effect of flow rate was studied by analyzing the samples with 0.9 and 1.1 mL/min flow rates. In both the cases resolution was above 4. The effect of diethylamine was studied by adding 0.09 and 0.11 % diethylamine to the mobile phase and it has not any effect on resolution and retentions.

LOD and LOQ: Limit of detection (LOD) and limit of quantification (LOQ) were calculated using signal/noise (S/N) ratio method. LOD is taken as a concentration of analyte where S/N was 3 and it was found to be 0.90 µg/mL for both the enantiomers. LOQ is taken as concentration of analyte where S/N is 10 and it was found to be 2.35 µg/mL for both the enantiomers.

Conclusion

Separation and determination of pirbuterol enantiomers was studied on polysaccharide stationary phases. Chiralpak AD-H column has shown excellent selectivity for pirbuterol enantiomers. The effect of organic modifiers and temperature on resolution and retention of enantiomers have been evaluated to optimize the mobile phase composition. The enantiomeric separation was found to be an enthalpy driven processes for pirbuterol. The methods were validated with respect to accuracy, precision, linearity, LOD, LOQ and robustness. The developed methods are quite simple, rapid, sensitive and enantioselective and could be of use for determination of enantiomeric purity of pirbuterol in bulk drugs and formulations (Table-4).

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