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ENANTIOSEPARATION OF BISOPROLOL FUMARATE BY TLC AND HPTLC USING (+)-10-CAMPHORSULPHONIC ACID AS A CHIRAL SELECTOR

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ABSTRACT

The enantiomeric separation of Bisoprolol fumarate into its enantiomers was achieved by TLC and HPTLC on silica gel plate using optically pure (+)-10-camphorsulphonic acid as a chiral selector in mobile phase and triethyl amine–methanol–1-pentanol (0.14:9.9:0.18, v/v/v) as the solvent system. Spots were located in UV chamber. The detection limit was 8 µg for TLC and 50 ng for HPTLC for both the isomers. The effect of concentration of chiral selector on separation has been studied and satisfactory results were obtained followed by frequent resolution of the enantiomers using these techniques. The procedure was applied successfully to resolve commercially available formulation of bisoprolol fumarate.

Keywords: Bisoprolol fumarate, (+)-10-camphorsulphonic acid, Chiral separation, HPTLC, TLC

INTRODUCTION

Bisoprolol fumarate, (±)-1-[p-(2-isopropoxyethoxy methyl) phenoxy]-3-(isopropylamino)-2-propanol fumarate, is β_1-selective adrenoreceptor antagonist without membrane stabilizing activity or intrinsic sympathomimetic activity. It is well known that two optical isomers of almost all chiral compounds have different biological effects. Bisoprolol is marketed as a racemic mixture. The only S (-) isomer is responsible for most β_1-

blocking activity while R (+) isomer is inactive. Thus both isomers should be considered as different drugs and need to be separated [1, 2].

Analysis of enantiomeric purity of drugs is very important during production and storage of drugs. The countries involved with high production of drugs and having advanced pharmaceutical technologies, require a full documentation of enantiodifferentiating procedures and detailed pharmacological activity of pure enantiomeric forms for the registration of a new chiral drug [3].

In this paper we represent the resolution of (±)-bisoprolol fumarate using (+)-10-camphorsulphonic acid as chiral selector in mobile phase by TLC and HPTLC. Generally high performance liquid chromatography (HPLC) used for resolution of racemic compound but TLC provides direct resolution of enantiomers of a variety of compounds and is used for its several advantages, which include parallel separation of samples, high-throughput screening, static and sequential detection for identification and integrity of the total sample, besides being simple and less expensive [4–9]. While HPTLC provide better resolution, sensitivity, excellent accuracy and precision and lower detection limit.

MATERIALS

(±)-Bisoprolol fumarate was obtained from E-MERCK Ltd. (Bombay, India) Market formulation were obtained BISELECT 5 (INTAS Pharmaceuticals Ltd.) and CONCOR 5 (E-MERCK) commercially. Silica Gel GF_{254} with fluorescent indicator was from Spectrochem Pvt Ltd. (Mumbai, India). Pre-coated silica gel GF_{254} 20 × 20 cm aluminium sheets were from Merck. (+)-10-camphorsulphonic acid was from spectrochem pvt. Ltd. (Mumbai, India). Other reagents were used of analytical grade.

The Polarimeter, model Autopol IV automatic polarimeter, was used for the measurement of optical rotation of separated isomers. Spectrophotometric measurements were made on a Shimadzu 1700 double beam UV Visible spectrophotometer with a fix slit width of 1 nm coupled HP 7540 computer loaded with Shimadzu UV PC software of version 2.0. The HPTLC of CAMAG TLC Scanner 3“Scanner 3_120520”S/N 120520(1.14.21) was used.

METHOD

1. Preparation of plates

TLC: Thin-layer plates (20 × 10 cm × 0.5 mm) were prepared by spreading slurry of silica gel GF_{254} (50 gm) in distilled water (100 ml). The plates were dried in air and activated in oven at 110 ºC for 30 min.

HPTLC: Precoated silica gel GF_{254} plates were used.

2. Chromatographic method

TLC: The sample solution containing racemic bisoprolol fumarate (1 mg/ml) was prepared in methanol and applied to the plates at 10 µl level. Chromatograms were developed at room temperature for 20 min. in triethyl amine–methanol–1-pentanol (0.14:9.9:0.18, v/v/v) containing 35 mM of (+)-10-camphorsulphonic acid in a glass chamber, pre-equilibrated with the solvent system for 20 to 25 min. The developed plates were dried at room temperature. For the commercially available formulation the solution of bisoprolol fumarate was prepared by weighing accurately dry powder containing equivalent of 10 mg of bisoprolol fumarate, which was centrifuged after addition of 10 ml of methanol at 3000 rpm for 10 mints. The supernant was collected and same chromatographic procedure was applied.

HPTLC: The sample solution containing racemic bisoprolol fumarate (100 ng/µl) was prepared in methanol and applied by Linomat 5 type applicator to the pre-coated silica gel GF_{254} plate at 1, 2, 3, 4, 5, 6 µl levels and same chromatographic method was applied as TLC.

3. Visualization

TLC**:** The detection of the test samples was carried out by UV chamber at short wavelength (254 nm). The chiral separation factor (α) of the two separated spots was calculated as the ratio of the higher R_f – value and the lower R_f – value for the two enantiomers. The photograph of actual chromatogram was shown in Figure 1.

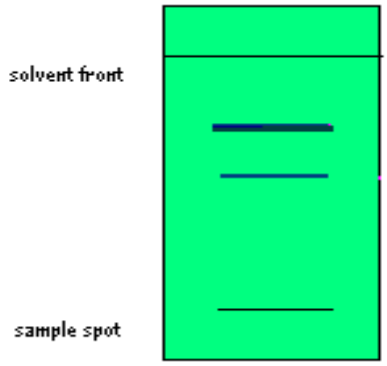

Figure-1: Photograph of actual chromatogram.

HPTLC: The detection of test samples was carried out by CAMAG TLC SCANNER at 224 nm which was obtained by scanning. The chiral separation factor (α) was calculated according to above mentioned procedure. Two separate peak of (+) and (-) bisoprolol fumarate at 224 nm was shown in Figure 2.

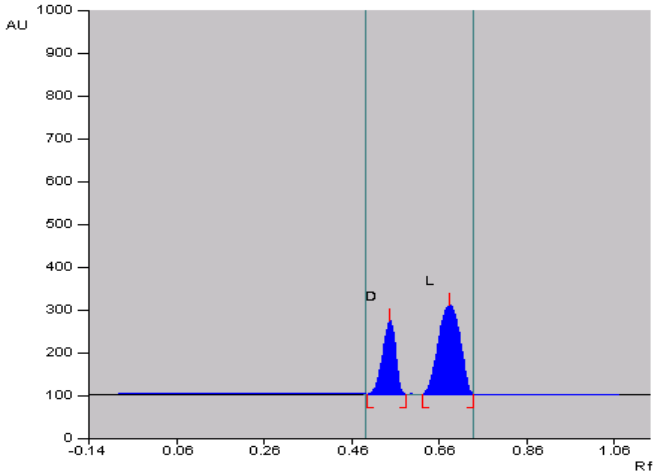

Figure-2: Two separate peak of (+) and (-) bisoprolol fumarate at 224nm.

4. Optical rotation study of separated spots obtained in TLC

The two separated spots were marked and scrapped off silica, dissolved in chloroform separately, centrifuged at 3000 rpm for 10 min. Supernant was collected separately, chloroform was evaporated and dry residues were collected. The dry residues (100 mg) dissolved in methanol and optical rotation was measured using polarimeter**.**

5. Calibration plots of separated (+) and (-) bisoprolol fumarate

The sample solutions were prepared by takining 10 mg of (+) and (-) bisoprolol fumarate separately. The solutions were diluted to get final concentration of 100 µg/ml of both (+) and (-) isomers of bisoprolol fumarate. Using the

stock solutions mentioned above, various aliquots were taken to get linear calibration plots between the range of 5 – 30 µg/ml for both the isomers, which were measured spectrophotometrically.

RESULTS AND DISCUSSION

Generally the combination mixture of the following solvents like acetonitrile, methanol, water, triethyl amine, hexane, 1-pentanol and 2-propanol used for the separation of β-blockers by HPLC [10-12] and TLC[13]. In the present studies, solvent mixtures from these reports were systematically modified to get successful chiral separation. The effect of mobile phase systems on enantiomeric resolution with TLC and HPTLC was shown in Table 1.

Table-1: The effect of mobile phase system on enantiomeric resolution in both TLC and HPTLC.

Mobile phase system [trietyl amine: methanol: 1-pentanol(v/v/v)]	hR_F values of separated isomers (TLC)		Separation factor (α) (TLC)	hR_F values of separated isomers (HPTLC)		Separation factor (α) (HPTLC)
	d(+)	l(-)		d(+)	l(-)	
0.2:9:0.2	60	65	1.08	51	60	1.17
0.2:9.9:0.2	65	71	1.09	53	63	1.19
0.14:9.9:0.18	64	74	1.16	55	69	1.25
0.14:9.9:0.24	68	74	1.09	59	70	1.18
0.24:9 .9:0.18	62	68	1.10	52	62	1.19

Good separation was obtained using mixture of triethyl amine–methanol–1-pentanol (0.14:9.9:0.18, v/v/v). The hR_F ($R_F \times 100$) value for the resolved (+) isomer of bisoprolol fumarate was 64 by TLC and 55 by HPTLC and (-) isomer of bisoprolol fumarate was 74 by TLC and 69 by HPTLC. The separation factor (α) was 1.16 by TLC and 1.25 by HPTLC. All the results were average of five identical runs.

The effect of concentration of the chiral selector on resolution of enantiomers of bisoprolol fumarate showed that best resolution was at 35 mM of chiral selector. Above or below 35 mM of chiral selector no resolution was observed.

The formation of diastereomers was obtained by reaction of hydroxyl group of bisoprolol fumarate (fig.3) and polar group of chiral selector(Figure 4)[14-17]. Triethyl amine was competing for ion-pair formation with the counter ion ((+)-10-camphorsulphonic acid). Thus diastereomers were interacted differently with the stationary phase [18-20] to give satisfactorily good resolution.

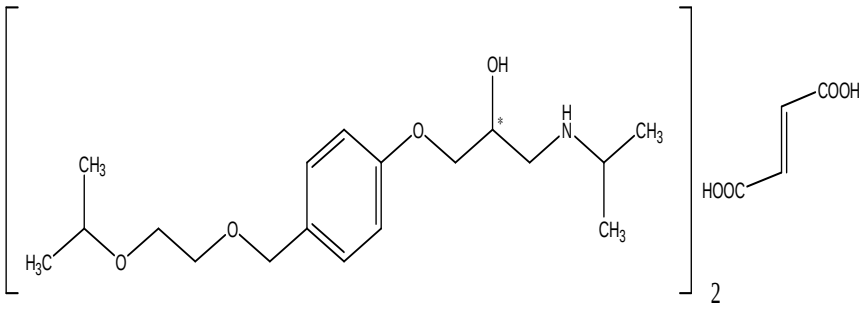

Figure-3: Structure of (±)-Bisoprolol fumarate.

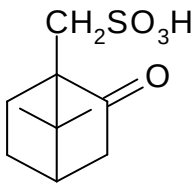

Figure-4: Structure of (+)-10-camphorsulphonic acid.

The specific optical rotations of two separated spots at hR_F – 64 and hR_F – 74 were +0.8 and -1.9 respectively. This is in good agreement between the reported specific optical rotation of bisoprolol fumarate (between -2 and +2ºc)[21]. Therefore, diastereomers of the type (+) bisoprolol-(+)-10-camphorsulphonic acid and (-) bisoprolol-(+)-10-camphorsulphonic acid was separated by solubilizing in choloroform.

The calibration ranges for both the isomers were 5-30 µg/ml. The recovery studies were carried out and the results were listed in table 2 for both TLC and HPTLC. Both the methods were validated and various validation parameters were represented in table 3. The concentration of (+) and (-) bisoprolol fumarate in market formulation were determined by TLC and HPTLC was shown in table 4.

Table-2: Results of recovery studies for both TLC and HPTLC.

Methods	Brand name	Isomers	Conc. of pure separated isomers from formulation	Conc. of pure separated isomers from bulk drug added	Conc. applied	Conc. found	%recovery
TLC	BISELECT5	d(+)	0.10mg/ml	0.08mg/ml	18µg	17.6µg	95%
		l(-)	0.10mg/ml	0.08mg/ml	18µg	17.7µg	96.25%
		d(+)	0.10mg/ml	0.10mg/ml	20µg	19.8µg	98%
		l(-)	0.10mg/ml	0.10mg/ml	20µg	19.5µg	95%
		d(+)	0.10mg/ml	0.12mg/ml	22µg	21.5µg	95.83%
		l(-)	0.10mg/ml	0.12mg/ml	22µg	21.6µg	96.66%
	CONCOR 5	d(+)	0.10mg/ml	0.08mg/ml	18µg	17.9µg	98.75%
		l(-)	0.10mg/ml	0.08mg/ml	18µg	17.6µg	95%
		d(+)	0.10mg/ml	0.10mg/ml	20µg	19.7µg	97%
		l(-)	0.10mg/ml	0.10mg/ml	20µg	19.8µg	98%
		d(+)	0.10mg/ml	0.12mg/ml	22µg	21.7µg	97.5%
		l(-)	0.10mg/ml	0.12mg/ml	22µg	21.5µg	95.83%
HPTLC	BISELECT5	d(+)	100ng/µl	80ng/µl	180ng	178.9ng	98.62%
		l(-)	100ng/µl	80ng/µl	180ng	179.2ng	99%
		d(+)	100ng/µl	100ng/µl	200ng	200.09ng	100.09%
		l(-)	100ng/µl	100ng/µl	200ng	199.4ng	99.4%
		d(+)	100ng/µl	120ng/µl	220ng	219.7ng	99.75%
		l(-)	100ng/µl	120ng/µl	220ng	220.04ng	100.03%
	CONCOR 5	d(+)	100ng/µl	80ng/µl	180ng	179ng	98.75%
		l(-)	100ng/µl	80ng/µl	180ng	179.3ng	99.12%
		d(+)	100ng/µl	100ng/µl	200ng	200.09ng	100.09%
		l(-)	100ng/µl	100ng/µl	200ng	199.6ng	99.6%
		d(+)	100ng/µl	120ng/µl	220ng	219.8ng	99.83%
		l(-)	100ng/µl	120ng/µl	220ng	220.04ng	100.03%

Table-3: Validation parameters for TLC and HPTLC.

PARAMETERS	ISOMERS	TLC	HPTLC
λmax	d	223.8 nm	224nm
	l	223.8 nm	224nm
Regression equation	d	Y= 0.0419x+0.0047	Y=1.449x+35.155
	l	Y= 0.0421x+0.011	Y=0.829x+30.853
Regression coefficient	d	0.9997	0.9900
	l	0.9999	0.9920
Precision	d	RSD<2	RSD<2
	l	RSD<2	RSD<2
Limit of detection	d	0.487µg/ml	17ng/µl
	l	0.231µg/ml	33ng/µl
Limit of quantification	d	0.922µg/ml	50ng/µl
	l	0.771µg/ml	90ng/µl

Table-4: Results of marketed formulation analysis.

Methods	Brand name	Conc./spot	Isomers	Conc. found	%lable claim
TLC	BISELECT 5	20µg	d(+)	6.2µg	30%±0.92
			l(-)	13.66µg	68.3%±0.99
	CONCOR 5	20µg	d(+)	6.12µg	30.6%±0.92
			l(-)	13.67µg	68.35%±0.99
HPTLC	BISELECT 5	200ng	d(+)	60.6ng	30.3%±0.94
			l(-)	138.9ng	69.4%±0.99
	CONCOR 5	200ng	d(+)	61.4ng	30.7%±0.94
			l(-)	138.9ng	69.4%±0.99

CONCLUSION

The TLC and HPTLC methods resolved the d and l isomers clearly and they were found to be 30:70 (d:l) in pure drug as well as in formulations. Both the techniques are versatile, flexible, simple, direct and economical compared to the other chromatographic techniques for routine enantiomeric purity analysis of bisoprolol fumarate. TLC, compared with the HPTLC, was found to be very simple, easy to carry out and less expensive and HPTLC was found to show better resolution, excellent accuracy and lower detection limit. Finally both the developed methods were successfully utilized for enantiomeric resolution of bisoprolol fumarate in pure as well as commercial formulations.

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