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*ORIGINAL RESEARCH*

# Direct TLC resolution of atenolol and propranolol into their enantiomers using three different chiral selectors as impregnating reagents

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ABSTRACT: Direct resolution of racemic atenolol and propranolol into their enantiomers was achieved by normal phase TLC on silica gel plates impregnated with optically pure L-tartaric acid, (*R*)-mandelic acid and (–)-erythromycin as chiral selectors. Different solvent systems were worked out to resolve the enantiomers. Spots were detected using iodine vapour. The TLC method was validated for linearity, limit of detection and limit of quantification. The influence of pH, temperature and concentration of chiral selector was studied. Copyright © 2008 John Wiley & Sons, Ltd.

KEYWORDS: enantiomeric separation; pharmaceutical preparation; atenolol; propranolol; L-tartaric acid; (*R*)-mandelic acid; (–)-erythromycin

## INTRODUCTION

The title molecules are chiral hydroxyl amine-containing compounds and belong to a commonly known group of *β*-blockers. They are synthetically produced and most of them are marketed as racemic mixtures. The pharmacological action is largely confined to the levo isomers (Burger, 1970), while some metabolites of D-form show signs of toxicity. Some of their clinical applications include the treatment of hypertension, arrhythmia and angina pectoris. However, *β*-blockers are known to have several side effects such as gastrointestinal irritation, tiredness, dizziness, depression, parethiasis, muscle aching, asthmatic wheezing and many others (Meyers *et al.*, 1980). The two enantiomers should be considered as different drugs and a clear picture of their pharmacodynamic and pharmacokinetic profile is likely to emerge only when the fate of each enantiomer is established. There is a strong need to develop rapid and reliable methods of enantiomeric resolution that can be useful in control of enantiomeric purity or monitor the stereoselective synthesis.

Direct enantiomeric resolution of racemic atenolol, propranolol and metoprolol by TLC has been reported from this laboratory using certain chiral selectors as impregnating reagents; these include L-lysine and L-arginine (Bhushan and Thiongo, 1998), L-aspartic acid (Bhushan and Arora, 2003) and Cu(II)-L-arginine (Bhushan and Gupta, 2006). Separation of enantiomers of *β*-blockers has also been achieved on TLC plates impregnated with ammonium-D-10-camphor sulfonate (Huang *et al.*, 1997), on LiChrosorb Diol high-performance TLC plates with dichloromethane and a chiral counter ion, *N*-benzyloxycarbonylglycyl-L-proline, as mobile phase additive (Tivert and Beckman, 1993), and by use of a molecularly imprinted polymer (Suedee *et al.*, 1999, 2001). Direct resolution of a variety of compounds on impregnated TLC plates has been reported by Bhushan and Martens (1997, 1998, 2001, 2003, 2007).

Direct enantiomeric resolution of one or more *β*-blockers has been achieved by HPLC using cyclodextrin CSP (Armstrong *et al.*, 1992), Pirkle-type CSP (Ohwa *et al.*, 1990); cellulose triphenyl carbamate derivatives (Okamoto *et al.*, 1986), immobilized $\alpha_1$-acid glycoprotein (Hermansson, 1985; Enquist and Hermansson, 1990), ovomucoid bonded silica (Haginaka *et al.*, 1990) and DBD-COCl as fluorogenic derivatizing reagent on cellulose chiral column (Yang *et al.*, 1987). Certain other reports on direct enantiomeric resolution of propranolol include the use of Chiracel OD (Aboul-Enein *et al.*, 1996), cyclodextrin bonded CSP (Ching *et al.*, 2000), chiral crown ether-based CSP (Steffeck *et al.*, 2002), and membranes based on chiral derivatized polysulfone (Gumi *et al.*, 2005).

The antibiotocs like vancomycin and erythromycin seem to have many of the useful enantioselectivity properties of proteins and other polymeric selectors. The literature reveals that vancomycin has been used as a chiral impregnating reagent in TLC for direct enantiomeric resolution of dansyl-DL-amino acids (Bhushan and Thiongo, 2000), and racemic verapamil (Bhushan

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and Gupta, 2005), while erythromycin has been successfully used as a chiral impregnating reagent in TLC for direct enantiomeric resolution of dansyl-DL-amino acids (Bhushan and Parshad, 1996).

In search of new suitable chiral selectors for enantiomeric resolution of racemic atenolol and propranolol, thin-layer plates were impregnated with L-tartaric acid, (*R*)-mandelic acid and (–)-erythromycin, in the present study. To the best of the author's knowledge there has been no report on the enantiomeric resolution of atenolol and propranolol with these chiral selectors.

## EXPERIMENTAL

**Materials and apparatus.** Racemic atenolol and propranol were obtained from ICI (India) Madras, India. The racemic drugs were purified by recrystallization with MeOH before subjecting to enantiomeric resolution. Optically pure isomers of atenolol and propranolol and (–)-erythromycin were obtained from Sigma-Aldrich (St Louis, MO, USA) and (*R*)-mandelic acid was from Merck (Darmstadt, Germany). Silica gel G, with 13% calcium sulfate as binder, having chloride, iron and lead impurities up to 0.02% and showing pH 7.0 in a 10% aqueous suspension, was from E. Merck (Bombay, India). Other reagents and chemicals used were of analytical reagent grade and were obtained from SISCO Research Laboratory (Bombay, India), BDH (Central Drug House, New Delhi, India) and E. Merck (Bombay, India). L-Tartaric acid was taken in free form. A polarimeter (model Krüss P3002, Germany), a UV spectrophotometer (model Hitachi U 2001) and a pH meter (Cyberscan 510) were used.

**Preparation of thin-layer plates and solutions.** Impregnated thin-layer plates (10 × 20 cm × 0.5 mm) were prepared by spreading a slurry of silica gel G (25 g) in distilled water (50 mL containing 0.5% chiral selector), with a Stahl-type applicator. A few drops of ammonia were added to the slurry to maintain pH. The plates were activated for 8–10 h at 60°C. Since erythromycin is sparingly soluble in water, it was first dissolved in 10 mL of ethanol and made up to 50 mL with water; the silica gel slurry was made in this solution to prepare erythromycin-impregnated plates.

The solutions of racemic atenolol (25 mM) and propranolol (25 mM) and their pure isomers were prepared in methanol in the same concentration.

Erythromycin (1 g) was dissolved in a minimum volume of chloroform by warming to 40°C. After filtration, the solution was kept at –15°C. The crystals were collected by filtration and dried under vacuum; these were nearly colourless (0.9 g), m.p. 135–140°C, resolidifying and melting at 190–193°C.

**Chromatography.** Solutions (5–10 μL) of racemic atenolol, propranolol and that of their pure (*S*)-isomer were spotted on impregnated plates with the help of Hamilton syringe. The spots of each racemic and single enantiomer were applied side-by-side on the plate. The chromatograms were developed in rectangular glass chambers pre-equilibrated with the solvent system for 15 min. After development, the plates were dried at 60°C for 10 min and spots were located in an iodine chamber. Various solvent systems and their combinations were evaluated for enantiomeric resolution of both atenolol and propranolol on three different impregnated plates.

## RESULTS AND DISCUSSION

Extensive experiments were carried out using methanol, acetonitrile, dichloro methane, ethanol, chloroform, butan-1-ol, acetic acid, water and their different combinations to work out successful solvent system for enantioseparation of racemic atenolol and propranolol. Only the finally successful solvent combinations showing the best separation along with $hR_f$ ($R_f \times 100$) values are reported in Table 1. The results are the averages of at least five identical runs. It was observed that the (*S*)-isomer eluted before the (*R*)-isomer in all cases. However, the effect of a change in concentration of the impregnating reagent, pH and temperature was studied for the successful systems only.

Erythromycin is a complex (14-membered ring) antibiotic, characterized by a molecular structure containing a large lactone ring linked with amino sugars through glycosidic bonds. It is a therapeutically useful wide-range antibiotic produced by a strain of

**Table 1. $hR_f$ values of enantiomers of atenolol and propranolol resolved on three different impregnated plates**

| Compound in racemic form | Impregnating reagent | Solvent system | Composition | $hR_F$ Racemic R | $hR_F$ Racemic S | $hR_F$ Pure S | $R_S$[a] |
|---|---|---|---|---|---|---|---|
| Atenolol | L-tartaric acid | A | 3:3:4 | 28 | 52 | 52 | 3.1 |
| | (*R*)-Mandelic acid | B | 5:5:0.5 | 68 | 85 | 85 | 2.6 |
| | (–)-Erythromycin | C | 2:1 | 56 | 81 | 81 | 3.3 |
| Propranolol | L-Tartaric acid | B | 8:1:0.5 | 60 | 86 | 86 | 3.5 |
| | (*R*)-mandelic acid | B | 5:5:0.5 | 72 | 90 | 90 | 2.8 |
| | (–)-erythromycin | C | 2:1 | 62 | 91 | 91 | 3.8 |

A = $CH_3CN$-$CH_3OH$-$CH_2Cl_2$; B = $CH_3CN$-$CH_3OH$-HOAc; C = $C_2H_5OH$-$CHCl_3$.
[a] $R_S$ = resolution; solvent front = 8 cm.



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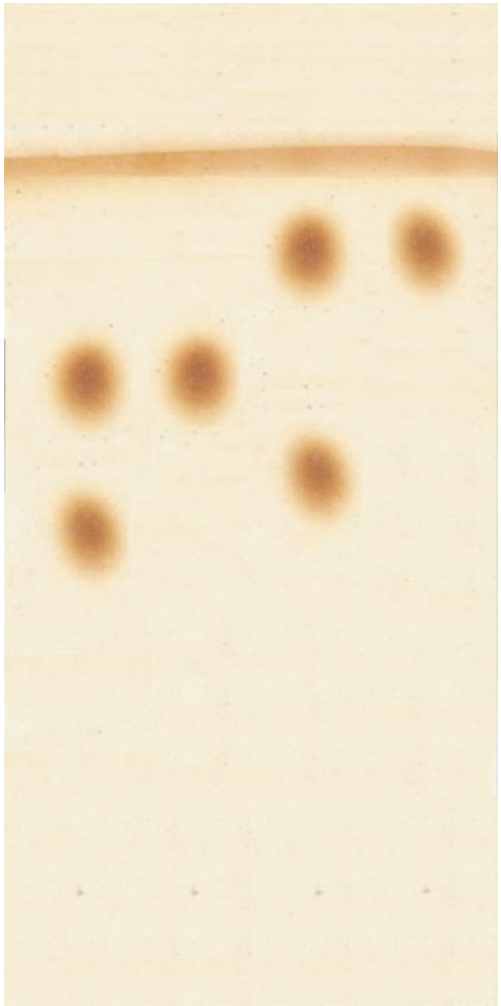

**Figure 1.** Photograph of the chromatogram showing resolution of racemic atenolol and propranolol on (–)-erythromycin impregnated plate. From left to right: spot 1, lower for (*R*)-atenolol and upper for (*S*)-atenolol; spot 2, pure (*S*)-atenolol; spot 3, lower for (*R*)-propranolol and upper for (*S*)-propranolol; spot 4, pure (*S*)-propranolol; mobile phase, EtOH-$CHCl_3$ (2:1, v/v); run time, 10 min; solvent front, 8 cm; detection, iodine vapour. This figure is available in colour online at www.interscience.wiley.com/journal/bmc

*Streptomyces erythreus* and contains one methoxyl, two N-methyl and eight or more (18%) C-methyl groups. Of the erythromycins, only erythromycin-A is certified by USFDA; it is basic in nature ($pK_a$ = 8.6) and has a specific rotation $[\alpha]_D^{25} = -78°$ ($c$ = 1.9 in ethanol).

A photograph of the actual chromatogram showing the resolution of racemic atenolol and propranolol on plates impregnated with erythromycin is shown in Fig. 1. The solvent system ethanol–chloroform (2:1, v/v) was found to be successful for both the racemates. The two spots were located by exposure to iodine vapours; these were marked and iodine was allowed to evaporate off. The spots representing the two enantiomers for propranolol were cut (from several plates, nearly 30), combined and eluted with ether to remove erythromycin; these were dried at room temperature and then eluted with water containing a drop of dilute HCl, by warming. The combined solution for each was filtered, dried by lyophilization and dissolved in ethanol. The solutions were examined using a polarimeter. Each was found to be optically pure, e.g. $[\alpha]_D^{25} = +26°$ ($c$ = 1 in ethanol); the specific rotation values were in agreement with the literature. Thus, diastereomers of the type (*R*)-propranolol-(–)-erythromycin and (*S*)-propranolol-(–)-erythromycin formed *in situ* were separated. Since erythromycin is insoluble in water and had already been removed with ether, only the enantiomers of propranolol went into solution. Similar confirmations were made for separation of enantiomers of atenolol. Enantioseparation may be possible via $\pi$–$\pi$ complexation, hydrogen bonding, inclusion in a hydrophobic pocket, dipole stacking, steric interactions or combinations thereof. Accordingly, the resolutions are significantly affected by variations in the solution environment, which in turn tend to affect the chiral antibiotic, which is ionizable, contains hydrophobic and hydrophilic moieties and is somewhat flexible.

When an impregnated plate was developed without spotting any of the racemic samples, the surface of the stationary phase appeared uniformly light blue under UV irradiation. This suggested that erythromycin used as impregnating agent was immobilized on the thin layer. On the other hand, experiments were conducted with plates not impregnated with erythromycin but spotted with the racemic analytes and developed under the identical experimental conditions; both the racemates gave a single spot. This further confirmed that impregnation with erythromycin was playing a role as a necessary requirement for enantiomeric resolution.

The $\beta$-blockers can exist as protonated ammonium cations and, consequently, these compounds can form diastereomers, *in situ*, with the anionic compounds. Both tartaric acid and mandelic acid used in the present studies exist in the anionic form under the experimental conditions. Thus, under the experimental conditions, Coulombic interactions occurred that favored formation of diastereomers and hence enantioresolution. The resolution can be considered as based on ion exchange mechanism and is different from the principal of ligand exchange TLC reported earlier for $\beta$-blockers on Cu (II)-L-arginine impregnated plates (Bhushan and Gupta, 2006). The photographs of actual chromatograms showing resolution of racemic atenolol and propranolol on plates impregnated with L-tartaric acid and (*R*)-mandelic acid are shown in Figs 2–4.

The polarimetric and UV studies showed that the enantiomers were present in the ratio of 1:1 in the sample. The concentrations of the separated enantiomers (through cutting of spots and elution, as described above) were determined by calibration plot and optical rotation measurement.



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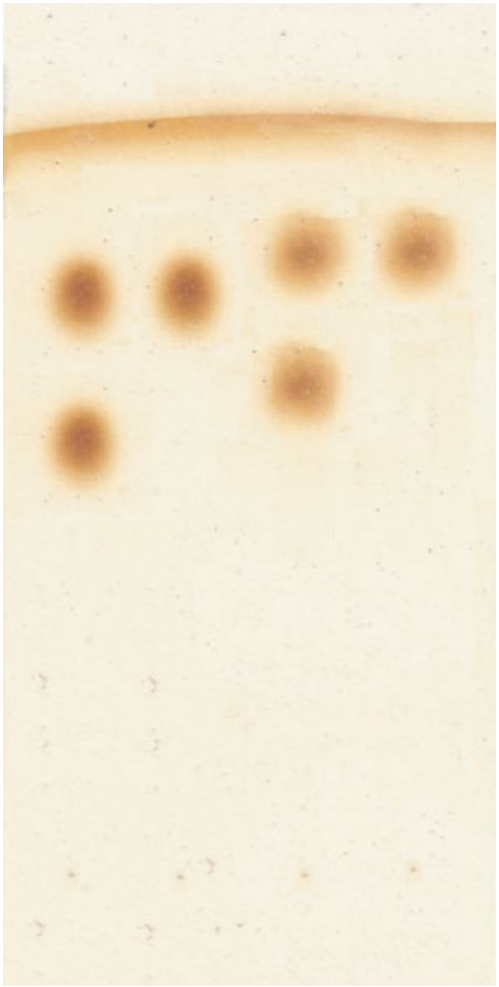

**Figure 2.** Photograph of the chromatogram showing resolution of racemic atenolol and propranolol on (*R*)-mandelic acid impregnated plate. From left to right: spot 1, lower for (*R*)-atenolol and upper for (*S*)-atenolol; spot 2, pure (*S*)-atenolol; spot 3, lower for (*R*)-propranolol and upper for (*S*)-propranolol; spot 4, pure (*S*)-propranolol; mobile phase, $CH_3CN$-$CH_3OH$-HOAc (8:1:0.5, v/v); run time, 10 min; solvent front, 8 cm; detection, iodine vapour. This figure is available in colour online at www.interscience.wiley.com/journal/bmc

**Figure 3.** Photograph of the chromatogram showing resolution of racemic atenolol on L-tartaric acid impregnated plate. From left to right: spot 1, lower for (*R*)-atenolol and upper for (*S*)-atenolol; spot 2, pure (*S*)-atenolol; mobile phase, $CH_3CN$-$CH_3OH$-$CH_2Cl_2$ (3:3:4, v/v); run time, 15 min; solvent front, 8 cm; detection, iodine vapour. This figure is available in colour online at www.interscience.wiley.com/journal/bmc

## Effect of concentration of impregnating reagent

A study of the effect of concentration of chiral impregnating reagent on the resolution of (*R*,*S*)-atenolol and propranolol into their enantiomers showed that the best resolution was achieved at 0.5% for all the three chiral selectors. When the concentration was decreased to 0.4, 0.3, 0.2 and 0.1%, there appeared tailing of the spots or a single spot in all the solvent combinations, as mentioned in Table 1. An increase in the concentration of chiral selector up to 0.6% resulted in an 8-shaped spot on plates impregnated with erythromycin while tailing or single spot was observed for others. Further increase in concentration up to 0.8% (by a factor of 0.1% each time) showed a single spot.

## Effect of temperature and pH

Earlier studies on chromatographic resolution of enantiomers of different compounds (Bhushan and Martens, 2001, 2003; Bhushan and Parshad, 1996), including the $\beta$-blockers (Bhushan and Thiongo, 1998; Bhushan and Arora, 2003; Bhushan and Gupta, 2006), have shown that chiral interactions are affected by temperature and pH. In the present case, it was observed that the best resolution occurred at 16 ± 2°C on the plates impregnated with L-tartaric acid, and at 18 ± 2°C on the plates impregnated with (*R*)-mandelic acid and (–)-erythromycin, and there was no resolution on further increase in temperature. Experiments at 35°C showed tailing of spots while for a decrease in temperature up to 10°C no resolution was observed.

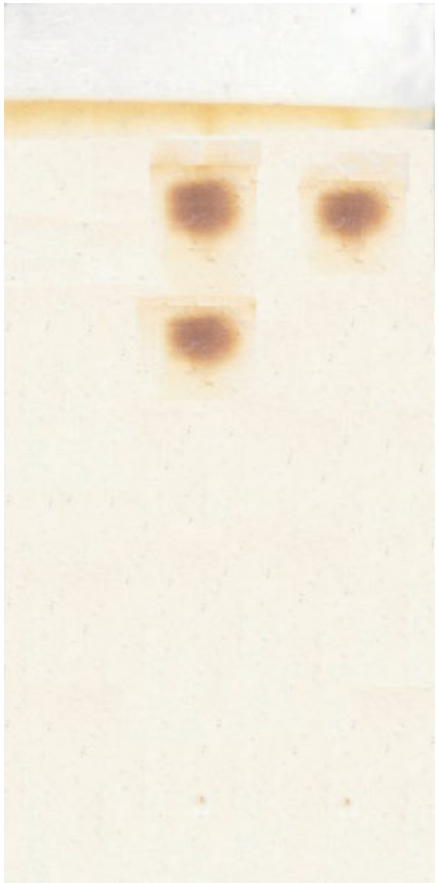

**Figure 4.** Photograph of the chromatogram showing resolution of racemic propranolol on L-tartaric acid impregnated plate. From left to right: spot 1, lower for (*R*)-propranolol and upper for (*S*)-propranolol; spot 2, pure (*S*)-propranolol; mobile phase, $CH_3CN$-$CH_3OH$-HOAc (5:5:0.5, v/v); run time, 15 min; solvent front, 8 cm; detection, iodine vapour. This figure is available in colour online at www.interscience.wiley.com/journal/bmc

It was observed that enantiomeric resolution was observed in the pH range of 6–7 in case of all the chiral selectors. Resolution of atenolol and propranolol into their enantiomers was observed at pH 6.0 on the plates impregnated with L-tartaric acid, and at pH 6.5 for (*R*)-mandelic acid and (–)-erythromycin impregnated plates.

Lowering of pH might result into neutral tartaric and mandelic acids molecules while at higher pH the analytes might exist as neutral molecules, providing no sites for coulombic interactions and no enantioresolution is observed under the changed conditions. Similarly, an increase or decrease in temperature and pH might be expected to affect the spatial or ionic interactions between erythromycin and the isomers of atenolol and propranolol because erythromycin is a large macro molecule having hydrophobic and hydrophilic moieties and ionizable groups and thus result in failure of enantiomer separation under changed conditions.

## Method development and validation

**Linearity.** Linearity was evaluated on the basis of correlation between absorbance and concentration of recovered analytes from the plate. The calibration parameters for linearity are shown in Table 2. The result shows that the linearity is in the range.

**Accuracy and precision.** The repeatability and reproducibility of the proposed method were assayed by six replicate measurements of the samples. The precision was represented as the relative standard deviation (RSD). The detection limits of atenolol and propranolol were defined as the concentration given at a signal-to-noise (S/N) ratio of 3. Table 3 shows precision expressed as RSD for *R*-isomers. The RSD was below 5% for both intra- and inter-assay precision.

The method was successful in resolving as little as 1.5 μg of atenolol and propranolol using (–)-erythromycin as chiral impregnating agent. L-Tartaric acid and (*R*)-mandelic acid were able to resolve 2.2 and 2.5 μg of atenolol and propranolol, respectively.

The reported method is successful for direct enantiomeric resolution of atenolol and propranolol and is valid for quantitative resolution of these drugs as well.

**Table 2. Calibration data of atenolol and propranolol**

| Chiral selector | Compound | Calibration curve[a] | Range (mg/mL) | Linearity, $r$[b] | Detection limit[c], μg/mL |
|---|---|---|---|---|---|
| L-Tartaric acid | *R*-atenolol | $y = 0.099x + 0.467$ | 10–50 | 0.998 | 2.2 |
| | *S*-atenolol | $y = 0.099x + 0.092$ | | 0.999 | |
| | *R*-propranolol | $y = 0.099x + 0.494$ | 10–50 | 0.999 | 2.2 |
| | *S*-propranolol | $y = 0.100x + 0.472$ | | 0.999 | |
| *R*-Mandelic acid | *R*-atenolol | $y = 0.203x + 0.089$ | 5–25 | 0.999 | 2.5 |
| | *S*-atenolol | $y = 0.200x + 0.679$ | | 0.999 | |
| | *R*-propranolol | $y = 0.201x + 0.428$ | 5–25 | 0.999 | 2.5 |
| | *S*-propranolol | $y = 0.201x + 0.381$ | | 0.999 | |
| (–)-Erythromycin | *R*-atenolol | $y = 0.204x + 1.032$ | 5–25 | 0.998 | 1.5 |
| | *S*-atenolol | $y = 0.201x + 0.775$ | | 0.999 | |
| | *R*-propranolol | $y = 0.198x + 0.802$ | 5–25 | 0.999 | 1.5 |
| | *S*-propranolol | $y = 0.201x + 0.277$ | | 0.999 | |

[a] $x$ sample concentration, μg/mL; $y$ = absorbance; [b] $r$ = correlation coefficient; [c] detection limit at S/N = 3.



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**Table 3. Accuracy and precision of the method for determination of (*R*)-atenolol and (*R*)-propranolol (*n* = 6)**

| | | L-Tartaric acid | | | (*R*)-Mandelic acid | | | (–)-Erythromycin | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Analysis | Concentration | Mean ± SD | RSD (%) | Recovery (%) | Mean ± SD | RSD (%) | Recovery (%) | Mean ± SD | RSD (%) | Recovery (%) |
| | | (*R*)-atenolol | | | | | | | | |
| Intra | 10 | 9.77 ± 0.102 | 1.05 | 97.7 | 9.88 ± 0.53 | 0.54 | 98.8 | 9.91 ± 0.04 | 0.40 | 99.1 |
| | 20 | 19.59 ± 0.183 | 0.93 | 97.9 | 19.79 ± 0.09 | 0.47 | 98.9 | 19.72 ± 0.12 | 0.63 | 98.6 |
| | 30 | 29.83 ± 0.076 | 0.25 | 99.4 | 29.82 ± 0.08 | 0.26 | 99.4 | 29.53 ± 0.21 | 0.71 | 98.4 |
| Inter | 10 | 9.57 ± 0.19 | 2.0 | 95.7 | 9.66 ± 0.15 | 1.5 | 96.6 | 9.84 ± 0.71 | 0.72 | 98.4 |
| | 20 | 19.46 ± 0.24 | 1.2 | 97.3 | 19.78 ± 0.98 | 0.49 | 98.9 | 19.52 ± 0.21 | 1.0 | 97.6 |
| | 30 | 29.22 ± 0.34 | 1.1 | 97.4 | 29.64 ± 0.16 | 0.54 | 98.8 | 29.29 ± 0.31 | 1.0 | 97.6 |
| | | (*R*)-propranolol | | | | | | | | |
| Intra | 10 | 9.89 ± 0.04 | 0.49 | 98.9 | 9.58 ± 0.18 | 1.9 | 95.8 | 9.19 ± 0.36 | 3.9 | 91.9 |
| | 20 | 19.78 ± 0.09 | 0.49 | 98.9 | 19.41 ± 0.26 | 1.3 | 97.0 | 19.25 ± 0.33 | 1.7 | 96.2 |
| | 30 | 29.65 ± 0.15 | 0.52 | 98.8 | 29.23 ± 0.34 | 1.1 | 97.4 | 29.68 ± 0.14 | 0.4 | 98.9 |
| Inter | 10 | 9.64 ± 0.16 | 1.6 | 96.4 | 9.52 ± 0.21 | 2.2 | 95.2 | 9.05 ± 0.42 | 4.6 | 90.5 |
| | 20 | 19.43 ± 0.25 | 1.3 | 97.1 | 19.04 ± 0.42 | 2.2 | 95.2 | 19.21 ± 0.35 | 1.8 | 96.0 |
| | 30 | 28.91 ± 0.48 | 1.6 | 96.3 | 29.09 ± 0.40 | 1.3 | 96.9 | 29.50 ± 0.22 | 0.7 | 98.3 |

It has potential applications in quality control in bulk and pharmaceutical formulations. Besides being simple, rapid and economical, the method has an advantage over other indirect methods that require chiral derivatization prior to chromatography or other expensive experimental set-up.

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