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Short communication

Enantiospecific assay of citadiol—A key intermediate of escitalopram by liquid chromatography on Chiralpak AD-H column connected with UV and polarimetric detectors in series

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Abstract

A simple, rapid, selective and reproducible LC method for separation and quantitative determination of citadiol (CTD), a key intermediate of escitalopram has been developed. An optimum resolution >3.0 was achieved on Chiralpak AD-H (250 mm × 4.6 mm); 5 μm column connected with UV and polarimetric detectors in series. The effects of organic modifiers, viz., methanol, ethanol, *n*-propanol and 2-propanol on enantioselectivity were evaluated. The limits of detection (LOD) and quantification (LOQ) were 0.02 μg/ml, 0.03 μg/ml and 0.07 μg/ml, 0.10 μg/ml for *R*-CTD and *S*-CTD enantiomers, respectively. The linearity of the method was studied in the range of 0.07–300 μg/ml and 0.1–300 μg/ml for *R*-CTD and *S*-CTD, respectively and the r^2 was ≥0.9999. The inter- and intra-day assay precision was less than 0.74% (%R.S.D.) and the recoveries were in the range 99.68–100.72% with %R.S.D. <0.49%.

Keywords: Citadiol; Escitalopram; Antidepressant; Chiralpak AD-H; Enantiomeric purity; Resolution; Polarimetric detection

1. Introduction

Citalopram is one of the widely used antidepressants of the selective serotonin reuptake inhibitors (SSRI) for treatment of various affective disorders [1]. It is active not only against depression, but also anxiety, panic, obsessive compulsive disorder pathological laughing and crying [2]. It's pharmacological activity is mainly due to the *S*-(+)-citalopram while *R*-(−)-citalopram is considered to be inactive [2]. Although most of the old formulations were made of racemates, the recent ones contain only the active *S*-(+)-citaloparm known as escitalopram. Recently we have developed an LC method for separation of citalopram enantiomers on a polysaccharide-based Chiralcel OD-H column, for determining the enantiomeric purity in bulk drugs [3]. The synthetic route for preparation of escitalopram involves the resolution of racemic citadiol ((*RS*)-CTD) [4]. This is one of the critical steps of the process, as the escitalopram has the stereochemistry of *S* at the chiral center. This was generally resolved using (+)- or (−)-di-*p*-toluoyl tartaric acid as shown in Fig. 1. Since it is a controlling step of the reaction process, (*R*)-CTD can be carried over as an impurity affecting the enantiomeric purity of escitalopram. Thus the purity of escitalopram finally depends on purity of (*S*)-CTD. In the literature, methods for determination of enantiomeric assay of (*S*)-CTD and its enantiomer (*R*)-CTD are not available. The present work describes the separation and quantitative determination of enantiomers of CTD on a polysaccharide-based, Chiralpak AD-H column connected with UV and polarimetric detectors in series. The effects of different organic modifiers on the separation of enantiomers of CTD have been studied.

2. Experimental

2.1. Materials and reagents

All reagents were of analytical-reagent grade unless stated otherwise. HPLC-grade *n*-hexane, *n*-propanol, 2-propanol, ethanol, methanol and triethylamine (TEA) were from S.D. Fine Chem. (Mumbai, India) and (*RS*)-CTD, *R*-CTD and *S*-CTD gifted by Hygro Chemical Pharmtek Pvt. Ltd. (Hyderabad,

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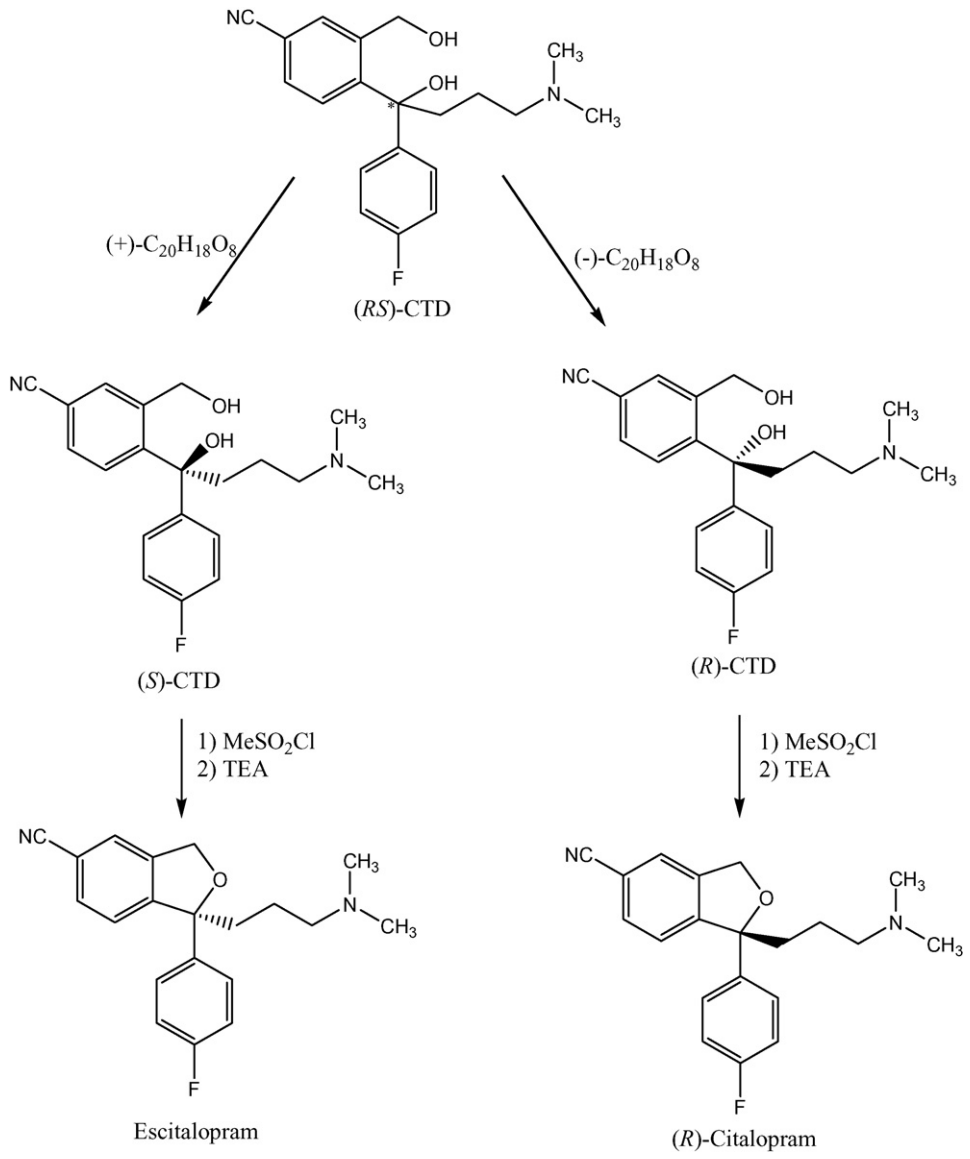

Fig. 1. Chemical resolution of (*RS*)-CTD in the synthesis of escitalopram.

India) were used. All the solutions were filtered through 0.45 μm membrane filters purchased from M/s Pall Pharmalab Filtration Pvt. Ltd. (Mumbai, India).

2.2. Apparatus

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, Hannover, Germany) was connected with UV detector in series for identification of the enantiomers. Chiralcel OD-H (250 mm × 4.6 mm); 5 μm with Chiralcel OD-H (1 cm × 4.6 mm); guard column, Chiralpak AD-H (250 mm × 4.6 mm); 5 μm with Chiralpak AD-H (1 cm × 4.6 mm); guard column (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.

2.3. Chromatographic conditions

Chromatographic separation was achieved on Chiralpak AD-H (250 mm × 4.6 mm); 5 μm column attached with Chiralpak AD-H (1 cm × 4.6 mm) guard column with mobile phase consists of *n*-hexane, ethanol, and TEA in the ratio (80:20:0.1 v/v/v) at 25 °C. The flow rate was 1.0 ml/min and the detector wavelength was kept at 240 nm. The sample injection volume was 20 μl and the analysis run time was 10 min. Polarimetric detector was used for identification of the enantiomers.

2.4. Preparation of stock and standard solutions

Stock solutions were prepared by dissolving 502 mg of (*RS*)-CTD, 504 mg of *R*-CTD and 498 mg of *S*-CTD precisely

Table 1
The selectivity of CTD enantiomers on Chiralpak AD-H with mobile phases containing different organic modifiers

Mobile phase	k'_1	α	Rs
n-Hexane:2-propanol:TEA (v/v/v)			
(95:05:0.1)	11.66	1.04	0.00
(90:10:0.1)	4.84	1.00	0.00
n-Hexane:*n*-propanol:TEA (v/v/v)			
(95:05:0.1)	8.42	1.19	2.98
(90:10:0.1)	3.53	1.06	0.54
n-Hexane:ethanol:TEA (v/v/v)			
(90:10:0.1)	3.62	1.41	3.99
(85:15:0.1)	2.21	1.37	3.34
(80:20:0.1)	1.25	1.45	3.12
(75:25:0.1)	1.01	1.26	1.29
n-Hexane:2-propanol:methanol:TEA (v/v/v/v)			
(80:10:10:0.1)	1.42	1.42	2.93
(80:15:05:0.1)	1.83	1.07	0.47

k'_1: retention factor of (+)-CTD. α: selectivity. Rs: resolution.

weighed in respective100 ml volumetric flasks, dissolved in 5 ml methanol first and made up to the mark with mobile phase. The flasks were wrapped with aluminum foil and kept in the refrigerator at 5 °C. The specified concentration of each enantiomer was 100 μg/ml for analysis. The sample and standard solutions for calibration were prepared by diluting appropriate volumes of stock solutions in 25 ml volumetric flasks with mobile phase. Standards were prepared in the range 20–300 μg/ml for calibration.

3. Results and discussion

Polysaccharide based columns such as Chiralpak AD-H and Chiralcel OD-H were tried for separation of enantiomers with different mobile phase combinations of 2-propanol, *n*-propanol, ethanol and methanol as organic modifiers in *n*-hexane. TEA (0.1%) was added to reduce the tailing of peaks. The chiral recognition mechanism on these CSPs is generally due to the formation of solute–CSP complexes [5,6]. Chiralcel OD-H column has shown little separation without baseline resolution for the citadiol enantiomers. With 2-propanol (5%) and *n*-propanol (5%), it showed a selectivity of 1.10 with resolution <1.22 and high retention. As Chiralpak AD-H column has shown excellent separation of citadiol enantiomers (Table 1), it was chosen for further development.

When 2-propanol was used as an organic modifier, the enantiomers were not separated on Chiralpak AD-H column. Where as *n*-propanol has shown little selectivity for separation of the enantiomers. But excellent separation was obtained when ethanol and methanol were used as organic modifiers (Table 1). As the size of the alcohol modifier decreased, the resolution was increased. This phenomenon could be explained by the difference in the steric bulkiness around the hydroxyl moiety of the mobile phase modifier. The lower alcohols could be readily incorporated in to the cavities of the CSP than bulkier ones. This type of incorporation of the mobile phase modifier into the

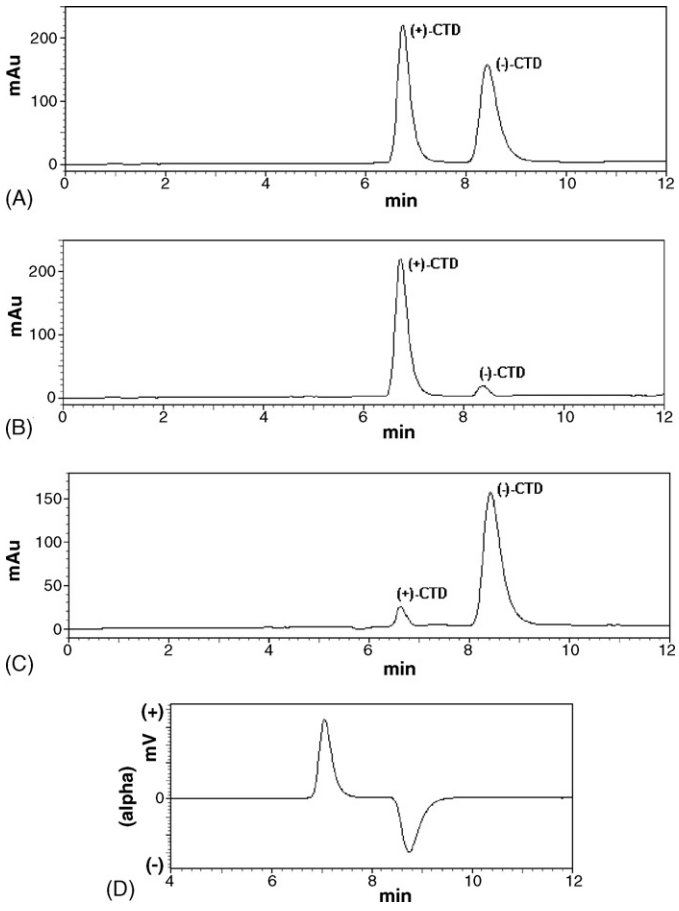

Fig. 2. Typical chromatograms of CTD enantiomers on Chiralpak AD-H column with *n*-hexane:ethanol:TEA (80:20:0.1 v/v/v) as mobile phase at 25 °C: (A) (*RS*)-citadiol, (B) (*R*)-(+)-citadiol, (C) (*S*)-(−)-citadiol using UV detector and (D) (*RS*)-citadiol using polarimetric detector.

chiral cavities of the CSP may change the steric environment on the surface of the CSP. According to Wenslow and Wang, it is possible that, compared to the shape/size of the chiral cavities incorporated with 2-propanol, the shape/size of the chiral cavities incorporated with lower alcohols (ethanol or methanol) was more favorable to the chiral interaction between the solutes and the CSP, resulting in increased separation of solute enantiomers [7]. Finally, ethanol was chosen as organic modifier for separation of CTD enantiomers. As the concentration of ethanol in the mobile phase was decreased, retention factors as well as resolutions were increased (Table 1). As a compromise for higher resolution and lower retention, 20% ethanol was chosen for optimum separation.

Thus a mobile phase containing *n*-hexane:ethanol:TEA (80:20:0.1 v/v/v) was chosen for separation of CTD enantiomers on Chiralpak AD-H column at 25 °C. The flow rate was kept at 1.0 ml/min throughout the analysis. The chromatographic separation of (*RS*)-CTD, *S*-(−)-CTD and *R*-(+)-CTD in the optimized conditions using UV and polarimetric detectors is shown in Fig. 2. Different batches of CTD were analyzed and the results are recorded in Table 2. The method was validated in terms of accuracy, precision and linearity as per ICH guidelines.

Table 2
The results of analysis of different batches of CTD by HPLC

Sample	Taken (mg)	(*S*)-CTD		(*R*)-CTD	
		Found (mg)	Recovery (%)	Found (mg)	Recovery (%)
(*RS*)-CTD-I	50	24.97	49.94	25.03	50.06
(*RS*)-CTD-II	50	25.91	50.38	24.81	49.62
(*S*)-CTD-III	50	49.55	99.10	0.45	0.90
(*S*)-CTD-IV	50	49.75	99.30	0.35	0.70
(*S*)-CTD-V	50	49.76	99.52	0.24	0.48

4. Method validation

4.1. System suitability

The solution of (*RS*)-CTD (20 μg/ml) prepared in the mobile phase was used for system suitability studies. The Chiralpak AD-H column was equilibrated for 30 min under the optimized conditions and three replicate injections were made. The system was deemed to be suitable if resolution between the two CTD enantiomers is not less than 3.0. The number of theoretical plates for *R*-(+)-CTD and *S*-(−)-CTD were 2560 and 2900 and tailing factors were 1.18 and 1.24 (at 10% base), respectively.

4.2. Precision

Precision of the method was tested by preparing six individual solutions of (*RS*)-CTD and *S*-CTD and making triplicate injections for each. The %R.S.D. of the assay was <0.55%. Inter- and intra-day assay precision was performed by analyzing the solutions for five times in a day for three consecutive days. The %R.S.D. of the assay were <0.74% for both the enantiomers.

4.3. Linearity

Calibration graphs were drawn in the range of 0.07–300 μg/ml and 0.1–300 for *R*-CTD and *S*-CTD, respectively. The curves were linear with $r^2 \geq 0.9999$ and the regression equations for *R*-CTD and *S*-CTD were $y = 40017x - 15217$ and $39857x - 12992$, respectively.

4.4. Accuracy

The accuracy of the method was determined by spiking CTD solution at five levels in the range 50–150% with respective to specified level (100 μg/ml) and analyzing the each solution in triplicate for 3 days. The percentage recoveries were between 99.68 and 100.72% with R.S.D. <0.49%.

4.5. Robustness

Robustness of the method was checked by making small deliberate changes in the operating conditions. Variation of 1.0% of ethanol did not affect the resolution except that retentions were changed. The effect of temperature was studied by analyzing sample at 25 ± 1 °C. The resolution remained still above 3.0. The effect of flow rate was studied by analyzing the samples with 0.9 ml/min and 1.1 ml/min flow rates. In both the cases resolution was above 3.0.

4.6. LOD and LOQ

Limits of detection (LOD) and quantification (LOQ) were calculated using signal/noise (S/N) ratio method. LOD was taken as the concentration of the analyte where S/N was 3 and it was found to be 0.02 μg/ml and 0.03 μg/ml for *R*-CTD and *S*-CTD, respectively. LOQ was taken as the concentration of the analyte where S/N is 10 and it was found to be 0.07 μg/ml and 0.10 μg/ml for *R*-CTD and *S*-CTD, respectively.

5. Conclusions

Direct resolution of citadiol enantiomers on two different polysaccharide based chiral stationary phases, viz., Chiralcel OD-H and Chiralpak AD-H was studied. Chiralpak AD-H shown better resolution compared to Chiralcel OD-H column. Baseline separation with resolution >3.0 was achieved between the two enantiomers within 10 min. Lower alcohols showed better enantioselectivity on Chiralpak AD-H column. The effect of organic modifiers on resolution and retention of enantiomers was evaluated and the mobile phase composition was optimized. The method was validated with respect to accuracy, precision, linearity, and robustness as per ICH guidelines. The developed method is quite simple, rapid, sensitive and enantioselective and could be of use for evaluation of enantiomeric purity of citadiol during the production of escitalopram in a pharmaceutical unit.

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