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Short communication

Development and validation of a liquid chromatographic method for determination of enantiomeric purity of citalopram in bulk drugs and pharmaceuticals

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Abstract

A simple, rapid and robust LC method for enantiospecific separation and determination of citalopram in drugs and pharmaceuticals was developed using UV and polarimetric detectors connected in series. Baseline separation with resolution ≥ 3.0 was achieved within 20 min on Chiralcel OD-H (250 mm × 4.6 mm) 5 μm column using a mobile phase containing of *n*-hexane:2-propanol:triethylamine (TEA) (95:05:0.1 v/v/v) at a flow rate of 1.0 ml/min at 25 °C. Effects of 2-propanol, triethylamine and temperature on enantioselectivity and resolution of the enantiomers were evaluated. Clopidogrel hydrogen sulphate was used as an internal standard (IS) for quantitative determinations using UV detector at 240 nm. Polarimetric detector was used for identification of enantiomers. The limits of detection (LOD) and quantification (LOQ) were 0.5 and 1.3 μg/ml respectively for both the enantiomers. The linearity of the method was in the range of 50–600 μg/ml with $r^2 > 0.9999$. The inter- and intra-day assay precision was less than 0.63% (%R.S.D.) and recoveries were in the range 99.38–100.41%. The method was validated and found to be suitable for determination enantiomeric purity of citalopram in bulk drugs and pharmaceutical formulations.

Keywords: Citalopram; Cellulose tris-(3,5-dimethylphenylcarbamate); Enantiomeric purity; Resolution; Polarimetric detection

1. Introduction

Citalopram (CIT) (Fig. 1) is one of the widely used antidepressants of the selective serotonin reuptake inhibitors (SSRI) for the treatment of various affective disorders [1]. It is active not only against major depression, but also anxiety, panic, obsessive compulsive disorder pathological laughing and crying [2,3]. Its pharmacological effect is mainly due to the *S*-(+)-CIT enantiomer while *R*-(−)-CIT considered to be inactive [2]. Several of the pharmaceutical formulations of CIT were in the form of racemates while the recent ones contain only the active *S*-(+)-CIT enantiomer named Escitalopram. Owing to the pharmacological and toxicological differences between these stereoisomers, it is quite important to develop a stereo specific assay for quality assurance of drugs and pharmaceuticals.

A thorough literature search has revealed that the work on stereo selective separation and determination of racemic citalopram and its application to pharmaceutical formulations was scarce in the literature A few CE methods for the determination of CIT enantiomers in biological fluids using sulphated β-cyclodextrin [4,5] and carboxymethyl-γ-cyclodextrin [6] as a chiral selectors were developed. HPLC was also used for determining the CIT enantiomers in biological matrices using different types of chiral columns. Most of these methods were in the reverse phase mode where polar solvents and buffers were used as mobile phases. Using Cyclobond I 2000 β-acylated column [7,8] CIT enantiomers were determined in biological fluids where the enantiomers were not resolved to baseline. Protein (α-acid glycoprotien) Chiral AGP and vancomycin-based columns with typical ion-pair reagents as mobile phase buffers were used in reverse phase mode for determining the two enantiomers in human plasma [9–11]. Polysaccharide-based stationary phases are quite popular with wide recognition for direct resolution of enantiomers. Chiralcel OD column was used for determination of CIT enantiomers in human plasma [12], which involves the analysis time of 40 min. To the best our knowledge, there were

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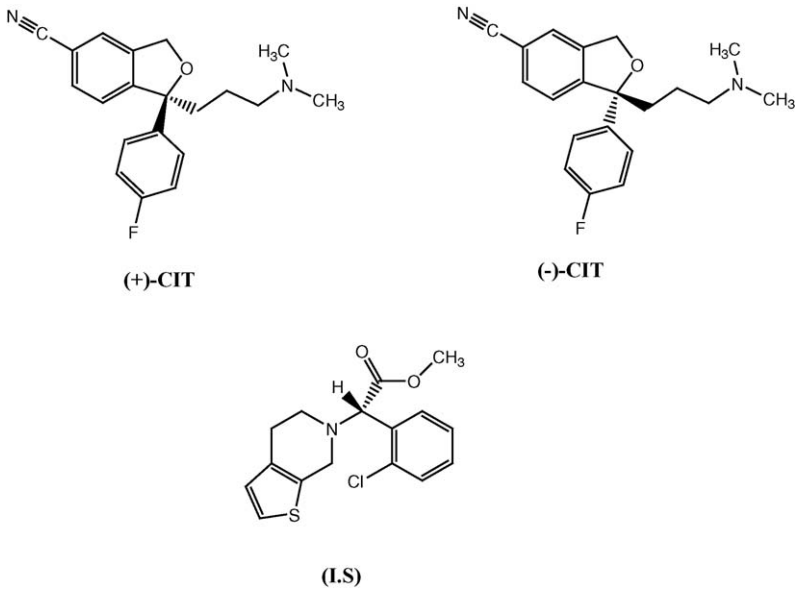

Fig. 1. Structural representation of citalopram enantiomers and *S*-clopidogrel (internal standard).

no validated LC methods for determination of the enantiomeric purity of citalopram in drugs and pharmaceuticals. Recently, Nevado et al. have developed and validated a CE method using carboxymethyl-γ-cyclodextrin as a chiral selector. In the present investigation, we report the development and validation of a normal-phase LC method using polysaccharide-based (Chiralcel OD-H) column for the first time for determination of enantiomeric purity of CIT (*RS*) and *S*-CIT in drugs and pharmaceuticals. The effects of organic modifiers, viz., ethanol, 2-propanol and temperature on resolution and retention of CIT enantiomers have studied and the mobile phase composition has been optimized.

2. Experimental

2.1. Materials and reagents

All reagents were of analytical-reagent grade unless stated otherwise. HPLC-grade *n*-hexane, 2-propanol, ethanol and triethyl amine (TEA) were purchased from S.D. Fine Chem. (Mumbai, India) and CIT (*RS*), *S*-CIT and clopidogrel hydrogen sulphate (CPG) were gifted by Hygro Chemical Pharmtek Pvt. Ltd. (Hyderabad, India). All 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 to UV detector in series for identification of the enantiomers. Chiral columns 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 Chiralcel OD-H (250 mm × 4.6 mm) 5 μm column attached with Chiralcel OD-H (1 cm × 4.6 mm) guard column with mobile phase consists of *n*-hexane, 2-propanol and TEA in the ratio (95:05:0.1 v/v/v) at 25 °C. The flow rate was 1.0 ml/min and detector wavelength was kept at 240 nm for monitoring the separation. Injection volume was 20 μl and total run time was 20 min. Polarimetric detector was used in series with UV detector for identification of the enantiomers.

2.4. Preparation of stock and standard solutions

Stock solutions of CIT (*RS*), *S*-CIT and internal standard were prepared by dissolving 601.0 mg of CIT (*RS*), 599.23 mg of *S*-CIT and 600.35 mg CPG precisely weighed in respective100 ml volumetric flasks and dissolved in 5 ml methanol first and made up to the mark with mobile phase. The stock solutions were wrapped with aluminum foil and kept in the refrigerator at 5 °C. The specified concentration of each enantiomer and CPG were 300 μg/ml for analysis. Standards were prepared in the range 50–600 μg/ml for calibration. Aliquot volumes of 0.417, 0.833, 1.667, 2.5, 3.33, 4.17 and 5.0 ml were taken from CIT (*RS*) stock solution separately into 25 ml volumetric flasks and 1.125 ml of CPG stock solution was added to each flask and diluted up to the mark with mobile phase to get standard solutions 50, 100, 200, 300, 400, 500 and 600 μg/ml of each enantiomer with 300 μg/ml of internal standard (CPG).

3. Results and discussion

3.1. Method optimization

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

3.2. Column selectivity

Two different polysaccharide-based stationary phases were (i) Chiralcel OD-H (cellulose tris-(3,5-dimethylphenylcarbamate)) and (ii) Chiralpak AD-H (amylose tris-(3,5-dimethylphenylcarbamate)) columns were evaluated using 2-propanol and ethanol as organic modifier in *n*-hexane. Table 1 shows the selectivity and resolution of CIT enantiomers on both the columns. It is clear from Table 1, that the enantiomers were retained long on Chiralpak AD-H, using ethanol compared to 2-propanol. However, both the organic modifiers could not yield good resolution with lower retention for CIT enantiomers on Chiralpak AD-H column. In case of Chiralcel OD-H column good enentioselectivity and resolution were found with lower retention. So Chiralcel OD-H column was chosen for further optimization.

3.3. Effect of concentration of TEA

To minimize peak tailing TEA was added to mobile phase. TEA was not having much effect on retention factors. But on increasing the TEA concentration, peak shapes were sharpen and tailing was reduced. But increasing the TEA concentration from 0.05 to 0.3%, increased the baseline noise and decreased the peak intensity. As a compromise 0.1% of TEA was chosen as optimum.

Table 1
The selectivity of CIT enantiomers on Chiralcel OD-H and Chiralpak AD-H columns with different organic modifiers at 25 °C and 0.1% TEA

Organic modifier	k'_1	k'_2	α	R_s
(A) Chiralcel OD-H				
% 2-Propanol				
5	3.48	4.49	1.29	3.05
7	3.11	3.84	1.23	2.27
10	2.25	2.75	1.22	1.91
15	1.55	1.79	1.15	1.40
% Ethanol				
2.5	3.40	3.83	1.13	1.58
5	2.50	2.75	1.22	1.26
10	1.41	1.51	1.07	0.69
15	1.21	1.27	1.05	0.51
(B) Chiralpak AD-H				
% 2-Propanol				
5	4.10	4.65	1.13	2.28
7	3.10	3.49	1.13	2.01
10	1.97	2.20	1.12	1.61
15	1.44	1.60	1.11	1.25
% Ethanol				
2.5	4.71	5.23	1.11	2.41
5	3.46	3.99	1.15	1.93
10	2.05	2.28	1.11	1.31
15	1.54	1.65	1.07	0.84

k'_1: retention factor of (−)-CIT; k'_2: retention factor of (+)-CIT; R_s: resolution; α: selectivity.

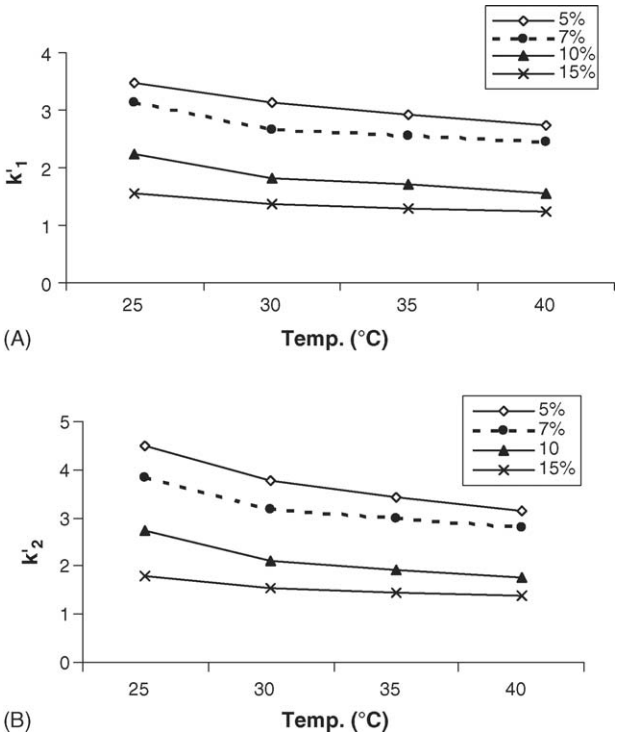

Fig. 2. The effect of temperature and 2-propanol on retention of (A) (−)-CIT and (B) (+)-CIT on Chiralcel OD-H Column. *Note*: The values in the inserted box represent the percentage composition of 2-propanol in the mobile phase.

3.4. Effect of organic modifier

The type and concentration of organic modifier was found to influence the retention and resolution of CIT enantiomers. The effect of 2-propanol and ethanol as organic modifiers on resolution of CIT enantiomers was investigated on Chiralcel OD-H column (Table 1). It is clear from the data that the selectivity and resolution of CIT enantiomers were good with 2-propanol when compared to ethanol. So 2-propanol was chosen for the separation. The effect of concentration of 2-propanol was studied. On decreasing the concentration of 2-propanol, retention factors (Fig. 2) as well as resolution (Table 2) were increased. At 5% of 2-propanol in *n*-hexane resolution >3.0 was obtained. Further decrease of 2-propanol concentration led to peak broadening and higher retentions. As a compromise for higher resolution and lower retention, 5% of 2-propanol in *n*-hexane was chosen for analysis.

3.5. Effect of column temperature

The effect of column temperature on resolution and retention of CIT enantiomers was studied in the range 298–313 K (25–40 °C) on Chiralcel OD-H column. On increasing the temperature, retentions (Fig. 2) as well as resolutions were decreased

Table 2
The effect of temperature, 2-propanol and TEA on resolution of CIT enantiomers on Chiralcel OD-H Column

% TEA	Temperature (°C)	Resolution (% 2-propanol) (*R*'s)			
		5	7	10	15
0.05	25	2.78	2.08	1.88	1.35
	30	2.44	1.87	1.62	1.23
	35	2.29	1.76	1.56	1.20
	40	2.14	1.65	1.37	1.17
0.1	25	3.05	2.27	1.91	1.40
	30	2.59	2.07	1.75	1.25
	35	2.41	1.89	1.60	1.21
	40	2.22	1.73	1.46	1.19
0.2	25	2.84	1.95	1.89	1.37
	30	2.51	1.84	1.73	1.23
	35	2.32	1.76	1.55	1.20
	40	2.18	1.64	1.41	1.16
0.3	25	2.92	2.03	1.90	1.39
	30	2.54	1.88	1.75	1.24
	35	2.35	1.77	1.59	1.21
	40	2.21	1.68	1.42	1.17

(Table 2). Under thermodynamically equilibrium conditions, free energy accompanying the separation of two enantiomers related to retention factors by the following equation:

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

where k' is the retention factor, R the gas constant and T is the temperature in K. An expansion of Eq. (1) to involve the enthalpy (H) and entropy (S) terms yield:

$$\ln k' = (-\Delta H^\circ / RT) + (\Delta S^\circ / R) \quad (2)$$

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 $2137.2x - 5.695$ and $1455.5x - 3.6472$ for (+)-CIT and (−)-CIT enantiomers, respectively. ΔH° and ΔS° 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 ($\Delta\Delta H^\circ$), entropy change ($\Delta\Delta S^\circ$) and Gibb's free energy change ($\Delta\Delta G^\circ$) accompanying the separation were recorded in Table 3. The data indicated that the separation of CIT enantiomers was an enthalpy driven process.

Thus, a mobile phase containing *n*-hexane:2-propanol:TEA (95:05:0.1 v/v/v) was chosen for the separation of CIT enantiomers on Chiralcel OD-H column maintained at 25 °C. The flow rate was kept at 1.0 ml/min throughout the analysis. Clopidogrel hydrogensulphate (IS) was used as an internal standard for quantification of each enantiomer. The chromatographic separation of CIT (±*RS*), *S*-(+)-CIT and IS in the optimized conditions using UV detector and polarimetric detector is shown in Fig. 3. The method was validated in terms of accuracy, precision and linearity as per ICH guidelines.

Table 4
The system suitability data using Chiracel OD-H column with *n*-hexane:2-propanol:TEA (95:05:0.1 v/v/v) as mobile phase at 25 °C

Compound	k'	α	R_s	A_s	N
S-(+)-CIT	3.48	1.29	3.05	1.21	18000
R-(−)-CIT	4.49			1.23	18000
CPG (IS)	2.26			1.08	21000

k': retention factor; α: selectivity; R_s: resolution; A_s: tailing factor; N: theoretical plates/meter.

4. Method validation

4.1. System suitability

The solution of CIT-(*RS*) (50 μg/ml) prepared in the mobile phase was used for system suitability studies. The Chiralcel OD-H column was stabilized for 30 min in the optimized conditions and three replicate injections were made. The system was deemed to be suitable if resolution between the two CIT enantiomers is not less than 3.0 and tailing factor is not more

Table 3
Thermodynamic data calculated from the Van't Hoff plots for the enantiomers of CIT

Compound	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})
(−)-CIT	−12.101	−5.668	−30.32	−17.03	−0.593 (298 K)
(+)-CIT	−17.769		−47.35		

ΔH° = slope × R; ΔS° = Intercept × R; $\Delta\Delta G^\circ = \Delta\Delta H^\circ - T\Delta\Delta S^\circ$.

Table 5
The results of analysis of bulk drugs and pharmaceutical formulations

Formulation	Claimed value (mg)	(−)-CIT		(+)-CIT	
		Found (mg)	Assay (%)	Found (mg)	Assay (%)
Bulk-I	50	24.955	49.91	25.045	50.09
Bulk-II	50	24.825	49.65	25.175	50.35
Bulk-III	50	0.135	0.27	49.865	99.73
Bulk-IV	50	0.21	0.42	49.791	99.58
Brand-I	40	19.932	49.83	20.068	50.17
Brand-II	40	19.964	49.91	20.036	50.09
Brand-III	40	19.981	49.95	20.019	50.15

than 1.21 (at 10% base). System suitability data was given in Table 4.

4.2. Precision

Precision of the method was tested by preparing six individual solutions of CIT-(*RS*) and *S*-CIT and making triplicate injections for each solution. The %R.S.D. 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 %R.S.D. of the assay were less than 0.63% for the both isomers.

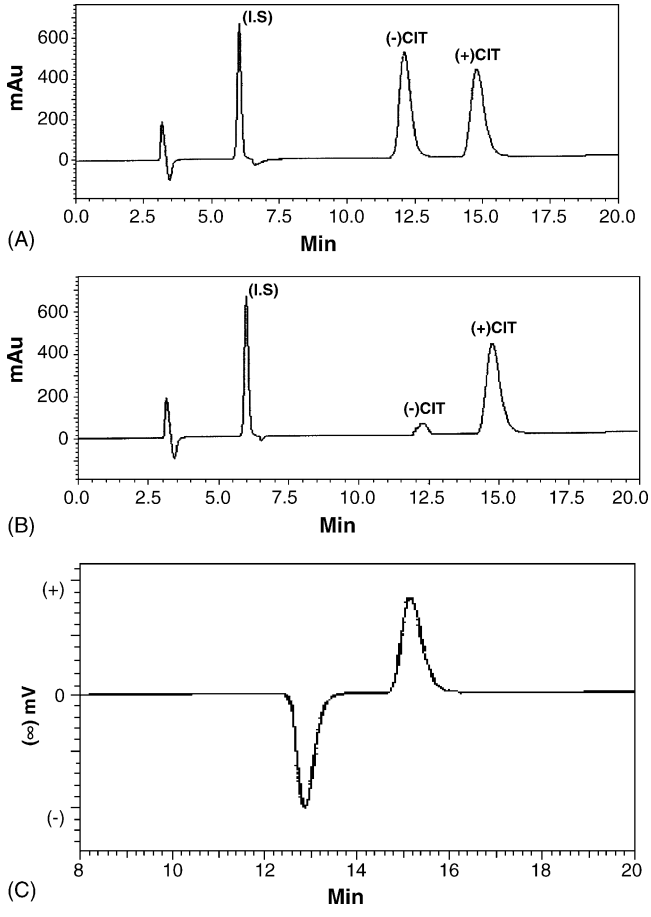

Fig. 3. Typical chromatograms showing the separation of CIT enantiomers and the internal standard (IS) on Chiralcel OD-H column with *n*-hexane:2-propanol:TEA (95:05:0.1 v/v/v) as mobile phase at 25 °C using UV detector: (A) (*RS*)-Citalopram, (B) (*S*)-Citalopram and (C) using polarimetric detector.

4.3. Linearity

Calibration graphs are drawn in the range of 50–600 μg/ml of CIT enantiomers by preparing fresh solutions every day for 3 days. Curves were linear with $r^2 > 0.9999$ and the regression equations for *R*-(−)-CIT and *S*-(+)-CIT were $y = 35170x - 4297$ and $35325x + 560$, respectively.

4.4. Accuracy

Accuracy was determined by spiking CIT solution at five levels in the range 50–150% with respective to specified level (300 μg/ml) and analyzing the each solution in triplicate for three days. Percentage recoveries were between 99.38 and 100.41%.

4.5. Robustness

Robustness of the method was checked by making small deliberate changes in the operating parameters. Variation of 0.5% of 2-propanol did not affect the resolution except that retentions were changed. The effect of temperature has been 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 and 1.1 ml/min flow rates. In both the cases resolution was above 3.0. The effect of TEA was studied by adding 0.09% and 0.11% TEA to the mobile phase and it has not any effect on resolution and retentions.

4.6. 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 0.5 μg/ml for both the enantiomers. LOQ is taken as concentration of analyte where S/N is 10 and it was found to be 1.3 μg/ml for both the enantiomers.

4.7. Assay in bulk drugs and pharmaceutical dosage forms

Twenty weighed tablets of citalopram (equivalent to 40 mg of citalopram) were ground to powder and an equivalent of 300 mg

of active ingredient dissolved in methanol was taken in 100 ml volumetric flask, ultra sonicated for about 10 min and made up to the mark with the methanol and supernant liquid was collected. Sample solutions were prepared by diluting appropriate volumes of supernant solution in 25 ml volumetric flasks with mobile phase. The proposed LC method was applied to analyze different tablet formulations of citalopram hydrobromide. The two enantiomers were very well separated under the developed conditions and there was no interference from the excipients in determining the ratio of enantiomers. Three pharmaceutical formulations and four bulk drugs were analyzed. The results are given in the Table 5. From these results, it could be seen that the developed method is quite simple, rapid and reliable for determination of enantiomeric purity of citalopram in bulk drugs pharmaceutical formulations.

5. Conclusions

Separation and determination of citalopram enantiomers on two different polysaccharide-based chiral stationary phases Chiralcel OD-H and Chiralpak AD-H was studied. Chiralcel OD-H column yielded better resolution over Chiralpak AD-H column. Baseline separation with resolution greater than 3.0 achieved between the two enantiomers within 20 min. The effect of organic modifiers and temperature on resolution and retention of enantiomers have been evaluated and the mobile phase composition has been optimized. The enantiomeric separation was found to be an enthalpy driven process. 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 determination of enantiomeric purity of citalopram in bulk drugs and pharmaceuticals.

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