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Original Article

CHIRAL SEPARATION OF CITALOPRAM BY REVERSED PHASE HPLC USING SULFATED BETA CYCLODEXTRIN AS CHIRAL MOBILE PHASE ADDITIVE

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ABSTRACT

Objective: To develop a simple and cost effective chiral HPLC method for the separation of citalopram (CIT) enantiomers using chiral mobile phase additives (CMPAs).

Methods: Sulfated beta cyclodextrin (S-β-CD) was synthesized in our laboratory and was evaluated as a CMPA. The parameters affecting the resolution were optimized. CIT enantiomers were resolved on an achiral Kromasil C8 column (150 mm × 4.6 mm, 5 µm) using methanol and 20 mM KH_2PO_4 (pH 3) containing 12 mM S-β-CD (35:65) as the mobile phase with a flow rate of 1 ml/min at 240 nm. Chiral resolution capacity of synthesized S-β-CD was compared to the marketed product. The method using synthesized S-β-CD as CMPA was validated and applied for the quantitative determination of CIT enantiomers in bulk drug and tablet formulation.

Results: Synthesized S-β-CD gave a better resolution than the marketed form. This method was validated as per ICH guidelines and was found to comply with the standard norms. A good linearity was observed in the concentration range of 1-30 µg/ml with R^2= 0.9993 for both enantiomers. The limit of detection and limit of quantification was 0.0272 and 0.0824 µg/ml for the R-enantiomer and 0.0303 and 0.0920 µg/ml for the S-enantiomer respectively.

Conclusion: A rapid and cost effective RP-HPLC method was developed and validated as per ICH guidelines to separate the CIT enantiomers. The method could be successfully applied for the quantitative determination of CIT enantiomers in bulk drug samples and pharmaceutical formulations.

Keywords: Enantiomeric resolution, Citalopram enantiomers, Citalopram enantiomeric purity, Chiral additive.

INTRODUCTION

Citalopram, (CIT) chemically known as ((RS)-1-[3-(dimethylamino) propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile), is a potent and highly selective serotonin reuptake inhibitor that is primarily prescribed for the treatment of depression and other central nervous system diseases, such as anxiety disorder, panic disorder, obsessive–compulsive disorder, social phobia or post-traumatic stress disorder [1]. The drug was initially marketed in its racemic form. Preclinical studies have postulated that escitalopram (SCIT) is more efficacious than racemic CIT and about 150-times more potent than the R-citalopram (RCIT), which was initially assumed to be pharmacologically inactive, but is currently known to counteract the action of the SCIT without causing pharmacokinetic interactions [2-4]. Hence SCIT is now also marketed as a single enantiomer. The structure of SCIT is shown in fig. 1.

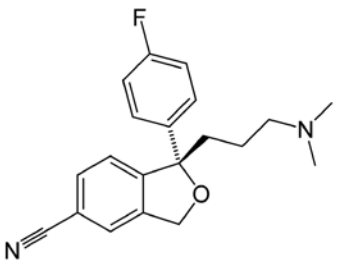

Fig. 1: Structure of escitalopram

The US Food and Drug Administration and other regulatory agencies have made it mandatory for the manufacturers to investigate each enantiomer of the chiral drug individually [5]. According to the International Conference on Harmonization (ICH) guidelines, chiral identity, enantiomeric impurity and chiral assay tests may be needed in drug substance and product specifications [6].

Chiral HPLC is a widely used technique for the determination of optical purity in bulk drugs and formulations. Chiral HPLC methods may either be direct, which utilize chiral stationary phases (CSPs) or chiral mobile phase additives (CMPAs), or indirect, which involve derivatization of samples. HPLC methods based on CMPAs offer the advantages of flexibility with a wide range of possible additives available and lower cost compared to the equivalent CSP. The use of chiral additives in the mobile phase provides a good alternative to expensive chiral columns because enantiomeric separation can be carried out using conventional achiral columns which have higher efficiencies and are less expensive compared to chiral columns [7]. Chiral HPLC is more challenging since enantiomers elute together, except in a chiral environment.

Cyclodextrins and their derivatives are one of the most widely used chiral additives in the mobile phase, which provide a cost effective alternative to relatively expensive CSPs [8, 9]. Cyclodextrins separate the enantiomers utilizing the phenomenon of host guest complexation. The affinity of the analyte for cyclodextrin is due to the hydrophobic interaction between the analyte and the cyclodextrin cavity and the hydrogen bonding between the analyte and the functional groups on the cyclodextrin ring. Derivatization of cyclodextrin not only increases the aqueous solubility but also changes the chiral recognition and hydrogen bonding capabilities of the native cyclodextrin.

There are reports of separation of CIT enantiomers using liquid chromatography, the majority of which employ CSPs [10-15] and beta cyclodextrin (β-CD) as CMPA on a cyanopropyl column [16]. Capillary electrophoretic methods have been reported where chiral selectors were added to the background electrolyte [17-19]. Enantioselective extraction of SCIT using a chiral imprinted polymer is also reported [20]. Semi-preparative separation of SCIT has been established with a CSP using supercritical fluid chromatography [21].

Sulfated beta cyclodextrin (S-β-CD), an anionic derivative of β-CD, is known to provide better resolving power for cationic enantiomers. It is a popular chiral additive in capillary electrophoresis (CE) but there are fewer reports of its application as a chiral additive in HPLC [22, 23]. However, there are no reports of RP-HPLC methods using S-β-CD as CMPA for separating CIT enantiomers. One of the major challenges in chiral HPLC method development is to develop a cost effective method. In the current investigation, the first attempt was made to resolve CIT enantiomers on RP-HPLC using S-β-CD as CMPA. S-β-CD was synthesized in our laboratory at a cost approximately 40 times lesser than that of the marketed product and was evaluated as a CMPA to resolve CIT enantiomers. The method was optimized, validated and applied to the pharmaceutical formulation. Resolution capability of marketed S-β-CD (S-β-CD1) was compared with that of S-β-CD synthesized in our laboratory (S-β-CD2).

MATERIALS AND METHODS

Chemicals and reagents

Citalopram HBr and Escitalopram oxalate were received as gift samples from Shri C. B. Patel Research Centre, Mumbai, India. Sulfated beta cyclodextrin sodium salt (degree of substitution 7-11) was purchased from Sigma Aldrich, Mumbai, India and was also synthesized in our laboratory [unpublished observations]. β-CD was purchased from Jay Chem Marketing, Mumbai, India and hydroxypropyl beta cyclodextrin (HP-β-CD) was received as a gift sample from Gangwal Chemicals Pvt. Ltd., Mumbai, India. Escitalopram oxalate tablets (S citadep 5mg) manufactured by Cipla Ltd. India, was used for assay studies. Orthophosphoric acid (OPA) and HPLC grade methanol was purchased from S. D. Fine-Chemicals, Mumbai, India. KH_2PO_4 and acetonitrile of HPLC grade were purchased from Merck, Mumbai, India. All other chemicals were of analytical reagent grade and used without further purification. Quartz double distilled water was used to prepare the mobile phase and diluents. Mobile phase was filtered through 0.45 µm nylon filter before use.

Instrumentation

FTIR spectra were recorded using Jasco V-460 plus FTIR spectrophotometer. KBr disc method was used to record the spectra.

Enantiomeric separation was carried out on Jasco-1500 series HPLC system comprising of an isocratic pump, a rheodyne injector with a fixed loop of 20 µl and UV detector. Borwin software was used for data processing. The measurements were carried out under isocratic elution at ambient temperature. Kromasil C8 column (150 mm × 4.6 mm, 5 µm) was used for the study. The flow rate was kept at 1 ml/min and the injection volume was 20 µl. Detection was carried out at 240 nm.

Preparation of stock and standard solution

Stock solutions of racemic CIT and SCIT were prepared by dissolving 50 mg of the drug in 50 ml methanol separately. Aliquots of 5 ml of the above solutions were diluted to 100 ml with methanol to provide 50 µg/ml and these were further used for analysis with proper dilutions. Diluent used for final dilutions was methanol: 20 mM KH_2PO_4 (65:35).

Preparation of mobile phase

The mobile phase was prepared by mixing methanol and 20 mM KH_2PO_4 containing 12 mM S-β-CD at pH 3 (adjusted with 10% OPA) in the ratio of 35:65. The mobile phase was filtered through 0.45 µm nylon filter and sonicated for 5 min before use.

Assay of tablet formulation

Twenty tablets of escitalopram oxalate were weighed and crushed to a fine powder using a mortar and pestle. Powder equivalent to one tablet was accurately weighed and transferred to a 50 ml standard volumetric flask. Methanol: water (80:20, 40 ml) was added to the flask and sonicated for 30 min and volume was made up using the same solvent. An aliquot of the solution was filtered through 0.45 µm nylon filter and transferred to a 10 ml standard volumetric flask to yield a concentration of 20 µg/ml.

RESULTS AND DISCUSSION

FT-IR analysis of sulfated beta cyclodextrin

FTIR spectrum of S-β-CD1 was compared to that of S-β-CD2 (fig. 2). S-β-CD marketed by Sigma Aldrich mentions the degree of –OH groups substituted by the sulfate group to be 7–11 mol per mol beta cyclodextrin. The remaining hydroxyl groups will show O–H stretching at 3475 cm^{-1} and O–H bending at 1642 cm^{-1}, which is clearly seen in both the spectra. A weak band at 2948 cm^{-1} is observed due to the stretching vibrations of C–H bond of $–CH_2$ groups. Band at 1158 cm^{-1} is observed due to the absorption of C–O stretching of C–OH bond and band at 941 cm^{-1} is attributed to the C–O stretch of the glucose ring. Bands at 1233 cm^{-1} and 1057 cm^{-1} are attributed to asymmetric stretching vibrations of S–O of S=O and S–OH respectively [24]. All these bands were observed in both the spectra. Based on these similarities in the IR spectra of both the compounds it can be said that both the compounds are identical. Hence the study was carried out using the synthesized S-β-CD.

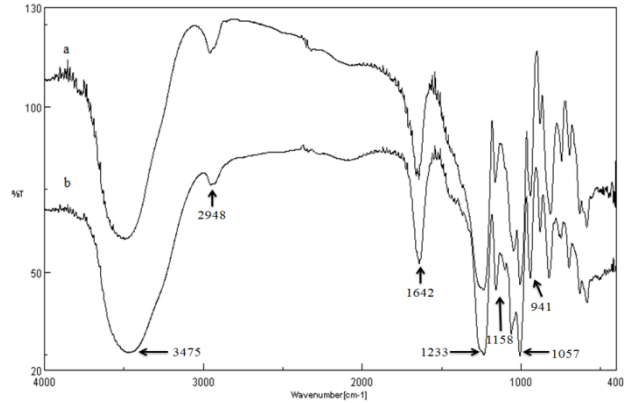

Fig. 2: FT-IR spectra of Sulfated beta cyclodextrin a. S-β-CD1 b. S-β-CD2

HPLC Method development

For the method development, 10 µg/ml of racemic CIT was used. To achieve best separation between the enantiomers, various parameters affecting the resolution were evaluated.

Effect of β-CD and its derivatives on resolution

β-CD, HP-β-CD and S-β-CD2 were evaluated as chiral additives for enantiomeric resolution of CIT. No resolution was obtained with β-CD (10-15 mM) and HP-β-CD (10-20 mM) as chiral additives. Baseline resolution was obtained only with S-β-CD2 at a concentration of 10 mM and above. This could be because the chiral recognition between the enantiomers and the charged cyclodextrins arise from several potential interactions, including electrostatic or ion pairing interactions as well as hydrophobic interactions and inclusion complexation [23]. Influence of S-β-CD2 in the concentration range of 8-15 mM on the resolution was investigated in the mobile phase containing 20 mM phosphate buffer of pH 3 and 35 % methanol. As shown in fig. 3, with an increase in the concentration of chiral additive in the mobile phase, resolution increases, and retention time decreases. However, at 15 mM concentrations of S-β-CD2 in the mobile phase, the enantiomers eluted early, thereby showing a loss in resolution. Hence, 12 mM concentration of S-β-CD2 in the mobile phase was observed to be the optimum concentration giving a resolution of 2.6 with a run time of 16 min.

Effect of pH on resolution

Chiral recognition is based on the formation of a stable inclusion complex and hydrogen bonding interaction with the guest enantiomer and pH plays an important role in maintaining the stability of the complex. The influence of mobile phase pH in the range of 3-5 with 12 mM chiral additive and 35% methanol was investigated. As shown in

fig. 4, with increase in pH of the mobile phase, retention time increased and resolution decreased. It was observed that the band broadened at higher pH values, resulting in lower resolution. Hence pH 3 was found to be the optimum pH for the analysis.

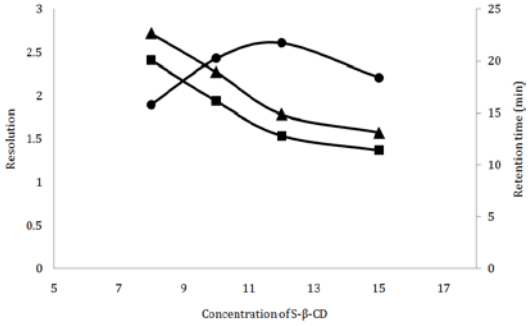

Fig. 3: Effect of concentration of S-β-CD2 on resolution and retention time of CIT enantiomers. ●: Resolution of CIT enantiomers. ■: Retention time of RCIT. ▲: Retention time of SCIT. Chromatographic conditions: Mobile phase: Methanol: 20 mM KH_2PO_4 containing S-β-CD2, pH 3 (35:65); Column: Kromasil C8 (150 mm × 4.6 mm, 5 µm); Flow rate: 1 ml/ min; λ: 240 nm (n=3)

Effect of organic component on resolution

Methanol and acetonitrile were evaluated as an organic component in the mobile phase for their effect on resolution.

As seen in table 1, a resolution of 2.6 was obtained with a mobile phase containing 35% methanol. When methanol was replaced with acetonitrile in the same ratio, it was found that the enantiomers eluted early leading to the loss in resolution. When the ratio of acetonitrile was reduced, a resolution of >2 was observed only with longer retention times. As methanol showed good resolution in a shorter run time it was preferred as the organic component in the mobile phase.

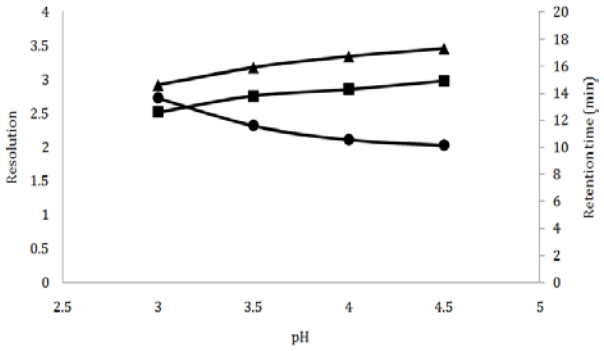

Fig. 4: Effect of pH on resolution and retention time of CIT enantiomers. ●: Resolution of CIT enantiomers. ■: Retention time of RCIT. ▲: Retention time of SCIT. Chromatographic conditions: Mobile phase: Methanol: 20 mM KH_2PO_4 containing 12 mM S-β-CD2 (35:65); Column: Kromasil C8 (150 mm × 4.6 mm, 5 µm); Flow rate: 1 ml/ min; λ: 240 nm (n=3)

Table 1: Effect of methanol and acetonitrile on resolution. Other chromatographic conditions: Mobile phase: 20 mM KH_2PO_4 containing 12 mM S-β-CD2, pH 3; Column: Kromasil C8 (150 mm × 4.6 mm, 5 µm); Flow rate: 1 ml/ min; λ: 240 nm (n=3)

Organic component	% Composition in mobile phase	Retention time (min); %RSD		Resolution
		RCIT	SCIT	
Methanol	40	8.9; 0.14	10.1; 0.42	1.8
	35	12.5; 0.26	14.7; 0.57	2.6
	30	23.4; 0.04	26.7; 0.24	3.1
Acetonitrile	35	6.8; 0.41	7.4; 0.30	0.9
	25	16.4; 0.20	18.1; 0.10	1.9
	20	22.8; 0.04	24.2; 0.15	2.2
	15	28.7; 0.41	30.2; 0.08	2.9

Effect of type and concentration of buffer on resolution

Ammonium acetate, triethylammonium acetate (TEAA) and KH_2PO_4 buffers were evaluated as an aqueous component in the mobile phase. The mobile phase containing ammonium acetate was unable to resolve the enantiomers. While in case of TEAA, broad peaks were observed, which resulted in lower resolution. Mobile phase with KH_2PO_4 buffer gave sharper peaks and good resolution. Further, the effect of 5, 10 and 20 mM phosphate buffer at pH 3 was investigated in mobile phase containing 12 mM S-β-CD2 and 35% methanol. It was observed that as KH_2PO_4 concentration in mobile phase increased, the peaks were sharper leading to better resolution. Hence, further studies were carried out using 20 mM KH_2PO_4.

The optimized mobile phase for the enantiomeric separation of CIT was methanol and 20 mM KH_2PO_4 containing 12 mM S-β-CD2 at pH 3 (35:65) which showed resolution of 2.6 within a run time of 16 min. S-β-CD1 was also evaluated as CMPA using the optimized conditions. As shown in fig. 5a, S-β-CD1 gave lower resolution of 1.45. Thus, S-β-CD2 was found to be a better CMPA than S-β-CD1 since it gave better resolution and lower retention times. A representative chromatogram using S-β-CD2 is shown in fig. 5b.

Method validation

After optimization of the parameters affecting the chromatographic separation of CIT enantiomers, the method was validated for linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and robustness as per ICH guidelines.

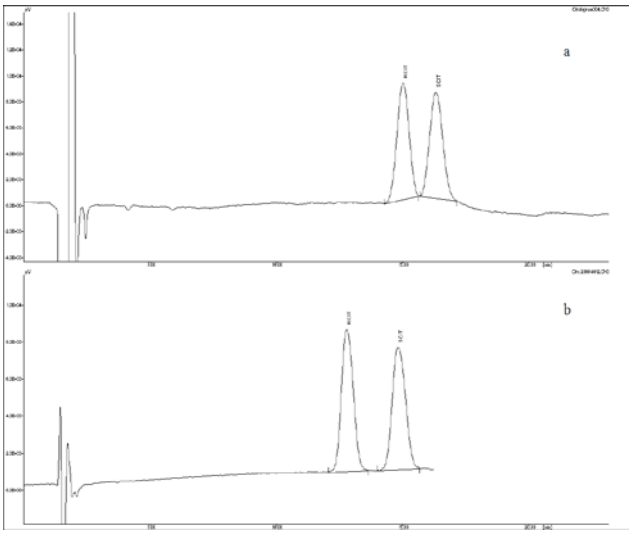

Fig. 5: Chromatogram of enantioseparation of CIT under optimized conditions. a. Using S-β-CD1 b. Using S-β-CD2. Chromatographic conditions: Mobile phase: Methanol: 20 mM KH_2PO_4 containing 12 mM S-β-CD2, pH 3 (35:65); Column: Kromasil C8 (150 mm × 4.6 mm, 5 µm); Flow rate: 1 ml/ min; λ: 240 nm

Linearity

Peak area (y) was plotted against concentration (x) for both enantiomers. A good linearity was observed in the concentration range of 1 to 30 µg/ml of racemic CIT with a linear regression equation y = 45556x-1695.7 for RCIT and y = 44746x+75.636 for SCIT with correlation coefficient (R^2) of 0.9993 for both enantiomers.

LOD & LOQ

LOD and LOQ were calculated based on standard deviation (SD) of response and slope. A series of dilute solutions of racemic CIT in the range of 0.25 – 1.25 µg/ml was injected and their response was plotted against concentration. The linear regression equation was found to be y = 48976x-33.438 and y = 48184x+149.56 for RCIT and SCIT, respectively. R^2 was found to be 0.9981 and 0.9977 for RCIT and SCIT, respectively. LOD and LOQ were calculated to be 0.0272 and 0.0824 µg/ml for RCIT and 0.0303 and 0.0920 µg/ml for SCIT, respectively.

Precision

Inter-day and intra-day precision was assessed by preparing racemic samples with 5, 10 and 15 µg/ml concentrations (2.5, 5 and 7.5 µg/ml concentrations for each enantiomer) and analyzing them on two different days. Relative standard deviations (RSD) were calculated for the said concentration and were found to be less than 2% (table 2).

Table 2: Precision of CIT enantiomers in bulk drug samples (n=3)

Concentration of racemic CIT (µg/ml)	Intraday precision (µg/ml) mean±SD; %RSD		Interday precision (µg/ml) mean±SD; %RSD	
	RCIT	SCIT	RCIT	SCIT
5	2.57±0.01; 0.48	2.55±0.03; 1.02	2.54±0.02; 0.92	2.58±0.03; 1.27
10	4.94±0.04; 0.74	4.96±0.06; 1.13	5.00±0.05; 1.03	4.98±0.04; 0.80
15	7.35±0.04; 0.58	7.38±0.04; 0.51	7.30±0.06; 0.76	7.29±0.03; 0.38

Table 3: Accuracy of citalopram enantiomers in bulk drug samples (n=3)

Actual concentration of SCIT (µg/ml)	Recovered Concentration of SCIT (µg/ml) mean±SD	Mean % Recovery
2.5	2.49±0.01	99.66
5	5.06±0.06	101.19
7.5	7.42±0.01	98.96

Accuracy

Accuracy of the method was determined by recovery studies. 2 µg/ml solutions of racemic CIT were spiked with SCIT to obtain a concentration of 2.5, 5, and 7.5 µg/ml for SCIT. The results are shown in table 3.

Robustness

Robustness is the ability of the method to remain unaffected by small, deliberate changes in the parameters such as flow rate, pH and mobile phase composition. Chromatographic resolution of 10 µg/ml solutions of racemic CIT was used to evaluate the robustness of the method under altered conditions (pH±0.1 units, % methanol±1 ml, flow rate±0.1 ml/min). Different batches of synthesized S-β-CDs were also evaluated. In all the cases, accuracy and precision were unaffected and the resolution was found to be >2.2.

Application of the method

The validated HPLC method was used for the quantitative analysis of CIT in bulk drug samples and commercial tablets. The assay chromatograms of bulk drug sample and tablet formulation are shown in fig. 6a and 6b, respectively. Assay of SCIT in the marketed formulation and bulk drug is presented in table 4 and 5, respectively.

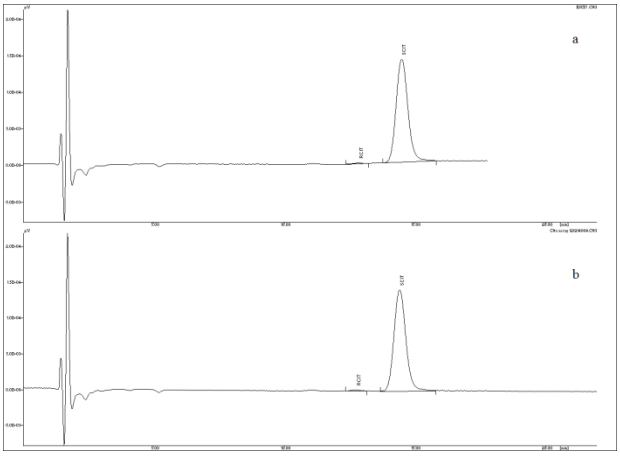

Fig. 6: Assay of Escitalopram in a. bulk drug b. formulation. Chromatographic conditions: Mobile phase, methanol: 20 mM KH_2PO_4 containing 12 mM S-β-CD2 pH 3 (35:65,v/v); Kromasil C8 (150 mm × 4.6 mm, 5 µm) column; flow rate 1 ml/ min; λ: 240 nm

Table 4: Assay of SCIT in tablet formulation (n=3)

Formulation	Label claim (mg)	Amount estimated (mg) mean±SD		% Assay±% RSD		Resolution
		SCIT	RCIT	SCIT	RCIT	
S citadep	5	5.07±0.03	$0.04±3.7 \times 10^{-4}$	101.45±0.7	0.86±0.04	2.61

Table 5: Assay of SCIT in bulk drug sample (n=3)

Concentration of SCIT (µg/ml)	Recovered concentration (µg/ml) mean±SD		% Assay±% RSD		Resolution
	SCIT	RCIT	SCIT	RCIT	
10	9.93±0.02	0.11±0.001	99.3±0.24	1.11±1.13	2.59

CONCLUSION

A cost effective, simple and rapid RP-HPLC method was developed and validated for the enantiomeric separation of CIT on an achiral C8 column using synthesized S-β-CD as chiral mobile phase additive. The method was optimized for various parameters affecting resolution of the enantiomers. Resolution of 2.6 was achieved between CIT enantiomers within a run time of 16 min. The developed method was successfully applied to the quantitative determination of CIT enantiomers in bulk drug and tablet formulation.

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CONFLICT OF INTERESTS

The authors have declared no conflict of interest

REFERENCES

1. Vaswani M, Linda FK, Ramesh S. Role of selective serotonin reuptake inhibitors in psychiatric disorders: A comprehensive review. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:85-102.
2. Mørk A, Kreilgaard M, Sánchez C. The R-enantiomer of citalopram counteracts escitalopram-induced increase in extracellular 5-HT in the frontal cortex of freely moving rats. Neuropharmacol 2003;45:167-73.
3. Zhong H, Haddjeri N, Sánchez C. Escitalopram, an antidepressant with an allosteric effect at the serotonin transporter—a review of current understanding of its mechanism of action. Psychopharmacol (Berl) 2012;219:1-13.
4. Leonard B, Taylor D. Escitalopram—translating molecular properties into clinical benefit: reviewing the evidence in major depression. J Psychopharmacol 2010;24:1143-52.
5. Rauws AG, Groen K. Current Regulatory (draft) Guidance on chiral medicinal products: Canada, EEC, Japan, United States. Chirality 1994;6(2):72-5.
6. Branch SK. Guidelines from the International Conference on Harmonisation (ICH). J Pharm Biomed Anal 2005;38:798-805.
7. Guan J, Yang J, Bi Y, Shi S, Yan F, Li F. Determination of the enantiomeric impurity in S-(-)pantoprazole using high performance liquid chromatography with sulfobutylether-beta-cyclodextrin as chiral additive. J Sep Sci 2008;31(2):288-93.
8. Rocco A, Fanali S. Enantiomeric separation of acidic compounds by nano-liquid chromatography with methylated-beta-cyclodextrin as a mobile phase additive. J Sep Sci 2009;32(10):1696-703.
9. Tong S, Zhang H, Shen M, Ito Y, Yan J. Enantioseparation of mandelic acid derivatives by high performance liquid chromatography with substituted β-cyclodextrin as chiral mobile phase additive and evaluation of inclusion complex formation. J Chromatogr B: Anal Technol Biomed Life Sci 2014;962:44-51.
10. Rochat B, Amey M, Baumann P. Analysis of enantiomers of citalopram and its demethylated metabolites in plasma of depressive patients using chiral reverse-phase liquid chromatography. Ther Drug Monit 1995;17(3):273-9.
11. Carlsson B, Norlander B. Optimization and characterization of the chiral Separation of citalopram and its demethylated metabolites by response-surface methodology. Chromatographia 2001;53(5-6):266-72.
12. Haupt D. Determination of citalopram enantiomers in human plasma by liquid chromatographic separation on a chiral-AGP column. J Chromatogr B Biomed Appl 1996;685(2):299-305.
13. Kosel M, Eap CB, Amey M, Baumann P. Analysis of the enantiomers of citalopram and its demethylated metabolites using chiral liquid chromatography. J Chromatogr B Biomed Sci Appl 1998;719(1-2):234-8.
14. Rochat B, Amey M, Van Gelderen H, Testa B, Baumann P. Determination of the enantiomers of citalopram, its demethylated and propionic acid metabolites in human plasma by chiral HPLC. Chirality 1995;7(6):389-95.
15. Gupta VK, Ali I, Agarwal S. Enantiomeric analysis of citalopram in human plasma by SPE and chiral HPLC method. Int J Electrochem Sci 2011;6:5639–48.
16. El-Gindy A, Emara S, Mesbah MK, Hadad GM. Liquid chromatography determination of citalopram enantiomers using beta-cyclodextrin as a chiral mobile phase additive. J AOAC Int 2006;89(1):65-70.
17. Berzas NJJ, Guiberteau CC, Villaseñor LMJ, Rodríguez RV. Enantiomeric determination, validation and robustness studies of racemic citalopram in pharmaceutical formulations by capillary electrophoresis. J Chromatogr A 2005;1072(2):249-57.
18. Mandrioli R, Fanali S, Pucci V, Raggi MA. Enantiomeric separation of citalopram and its metabolites by capillary electrophoresis. Electrophoresis 2003;24(15):2608-16.
19. Kumar AP, Park JH. Azithromycin as a new chiral selector in capillary electrophoresis. J Chromatogr A 2011;1218(9):1314-7.
20. Unceta N, Gómez-Caballero A, García D, Díaz G, Guerreiro A, Piletsky S, *et al*. Enantioselective extraction of (+)-(S)-citalopram and its main metabolites using a tailor-made stir bar chiral imprinted polymer for their LC-ESI-MS/MS quantitation in urine Samples. Talanta 2013;116:448-53.
21. Li M, Bao Z, Su B, Xing H, Yang Y, Ren Q. Enantiomeric separation of citalopram base by supercritical fluid chromatography. J Sep Sci 2013;36(18):3093–100.
22. Kenley KN, Qiqing Z, Kavita M, Nik C. Effect of sulfobutylether beta-cyclodextrin modifier on selectivity of reversed phase HPLC separations. J Liq Chromatogr Relat Technol 2012;35:2845–59.
23. Ameyibor E, Stewart JT. Resolution and quantitation of pentazocine enantiomers in human serum by reversed-phase high-performance liquid chromatography using sulfated β-cyclodextrin as chiral mobile phase additive and solid-phase extraction. J Chromatogr B Biomed Sci Appl 1997;703(1-2):273-8.
24. Wei M, Wang J, He J, Evans DG, Duan X. In situ FT-IR, in situ HT-XRD and TPDE study of thermal decomposition of sulfated β-cyclodextrin intercalated in layered double hydroxides. Microporous Mesoporous Mater 2005;78(1):53-61.