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Short communication

HPLC determination of sertraline in bulk drug, tablets and capsules using hydroxypropyl-β-cyclodextrin as mobile phase additive

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Abstract

A sensitive and stereospecific high-performance liquid chromatography (HPLC) method for determination of sertraline in bulk drug, tablets and capsules was developed. Chromatography resolution of the sertraline enantiomeric forms and *trans* diastereoisomers was performed on Alltima C18 (250 mm × 4.6 mm i.d., 5 μm) column with hydroxypropyl-β-cyclodextrin (HP-β-CD) as mobile phase additive. The composition of the mobile phase was 68:32 (v/v) aqueous 170 mM phosphate buffer, pH 3.0 (adjusted with 85% phosphoric acid) containing 18 mM HP-β-CD/acetonitrile at a flow rate of 1.0 ml min^{-1}. The UV detector was set at 225 nm. Calibration curves were linear ($r = 0.9999$, $n = 9$) in the range of 1–120 μg ml^{-1} for sertraline. Limit of detection and quantitation for sertraline was 0.029 and 0.097 μg ml^{-1}. The values of R.S.D. of repeatability and intermediate precision for bulk drug, tablets and capsules of sertraline hydrochloride were less than 1.0%.

Keywords: Sertraline; Enantiomer; Hydroxypropyl-β-cyclodextrin; Bulk drug; Tablets; Capsules

1. Introduction

Sertraline hydrochloride (*cis*-(1*S*,4*S*)-*N*-methyl-4 (3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenamine hydrochloride) is an antidepressant for oral administration. It is chemically unrelated to tricyclic, tetracyclic, or other available antidepressant agents. Sertraline hydrochloride is a novel drug substance belonging to the group of selective serotonin (5-hydroxitriptamine, 5-HT) reuptake inhibitors (SSRIs) in the brain [1]. During synthesis, it probably introduces *cis*-(1*R*,4*R*)-*N*-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenmine hydrochloride, *trans*-(1*S*,4*R*) and (1*R*, 4*S*)-*N*-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenemine hydrochloride (see Fig. 1). Therefore, the stereoselective determination of sertraline is important in order to assure therapeutic efficacy and safety.

Cyclodextrins [CDs; cyclic oligosaccharides composed of six, seven, or eight α-D-glucopyranose units (α-, β-, γ-CD, respectively)] form a family of excellent chiral selectors in high-performance liquid chromatography (HPLC) [2,3]. They are inherently chiral and undergo chiral interactions with analytes. CDs separate enantiomers utilizing the phenomenon of host–guest complexation, where a transient diastereomeric complex is formed between the CD and the analyte. Derivatization of the hydroxyl groups increases solubility and selectivity compared to the

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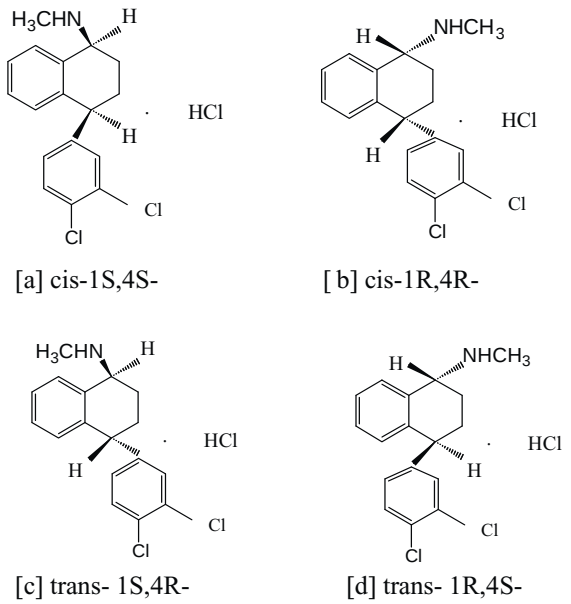

Fig. 1. Chemical structures of: (a) sertraline hydrochloride; (b) enantiomer of sertraline hydrochloride; (c) *trans* stereoisomers of sertraline hydrochloride; and (d) *trans* stereoisomers of sertraline hydrochloride.

native β-CD and the hydroxyl groups also undergo additional interactions with the analytes, thereby enhancing chiral recognition [4].

The determination of sertraline and its metabolites in biological fluids has been investigated by GC, GC-Mass, HPLC and CE [5–14], its enantiomeric separations with double CD have also been published [15]. Despite this, single CD as mobile phase additive on the separate of the sertraline enantiomers and *trans* diastereoisomers has not been reported.

This paper reports the use of hydroxypropyl-β-cyclodextrin (HP-β-CD) as chiral mobile phase additive to separate sertraline enantiomers and *trans* diastereoisomers. The developed method was successfully used in the determination of sertraline in bulk drug, tablets and capsules.

2. Experimental

2.1. Chemicals and reagents

All chemical were of analytical grade if not stated otherwise. Sertraline hydrochloride (*cis*-(1*S*,4*S*)-*N*-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenamine hydrochloride), *cis*-(1*R*,4*R*)-*N*-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenamine hydrochloride, *trans*-(1*S*,4*R*) and (1*R*, 4*S*)-*N*-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenamine hydrochloride, bulk drug, tablets (sertraline 55.96 mg, excipients q.s.p. 139.96 mg) and capsules (sertrsaline 55.96 mg, excipients q.s.p. 150.52 mg) of sertraline hydrochloride were a gift from Science & Technology Co. of Yangzhou Lianhuan (Jiangsu, China). HP-β-CD was purchased from NFTZ Thinker Chemical Co. Ltd (Zhejiang, China). 85% phosphoric acid, sodium phosphate and acetonitrile were purchased from Huaiyin-Bang Science & Technology Co. (Jiangsu, China). Water was deionized and bidistilled.

2.2. Apparatus and chromatographic conditions

The chromatograph used in this study consisted of a LC-10A pump (Shimadzu, Japan), a SIL-10A injection valve with a 20 μl loop, a SPD-10A UV-Vis detector operated at 230 nm. Data acquisition was performed using N2000 chromatography software from Zhejiang University (Zhejiang, China). The pH measurement was performed on a pH meter (Orion, model 818, Shanghai, China).

The separation of the analytes was achieved on an Alltima C18 (250 mm × 4.6 mm i.d., 5 μm) column (Alltech, USA). The mobile phase was consisted of 68:32 (v/v) aqueous 170 mM phosphate buffer, pH 3.0 (adjusted with 85% phosphoric acid) containing 18 mM HP-β-CD/acetonitrile, and delivered at a flow rate of 1.0 ml min^{-1}. The mobile phase was filtered through a 0.45 μm filter and sonicated prior to use. The column was operated at ambient temperature (24 ± 1 °C).

2.3. Preparation of stock and standard solutions

Standard stock solution of sertraline, the enantiomeric form, *trans*-(1*S*,4*R*) and *trans*-(1*R*,4*S*) diastereoisomers (1 mg ml^{-1}), were prepared by dissolving 50 mg standard sertraline, the enantiomeric form, *cis* and *trans* diastereoisomers, in 50 ml mobile phase. The sertraline stock solution was diluted with mobile phase to obtain the final concentration of sertraline 1.0, 10, 20, 30, 40, 50, 80, 100, 120 μg ml^{-1}.

Standard solution was prepared with the concentration of sertraline 20 μg ml^{-1}. This solution was used as the working standard for the determination of sertraline. Standard solution was found to be stable.

The mixture solution was prepared as follows: 1.0 mg ml^{-1} of sertraline hydrochloride was spiked with stock solution of *cis*-(1*R*,4*R*) enantiomer, *trans*-(1*S*,4*R*) and *trans*-(1*R*,4*S*) diastereoisomers in appropriate amounts.

2.4. Calibration procedure

Calibration curve of sertraline was conducted using the standard solutions described previously. Triplicate 20 μl injections were made of each standard solution and each calibration curve was fitted by linear regression according to the peak area against the corresponding concentration of sertraline.

2.5. Assay of sertraline in bulk drug

Solutions were prepared by transferring about 50 mg, accurately weighted, to a 50 ml volumetric flask with 50 ml of mobile phase to obtain a stock solution containing 1.0 mg ml^{-1}. The stock solution was diluted with mobile phase to obtain the final concentration of sertraline 20 and 100 μg ml^{-1}.

2.6. Assay of pharmaceutical preparations

Twenty tablets and capsules were weighted and finely pulverized, respectively. An appropriate of this powder, equivalent to 1.0 mg of sertraline was placed in 50 ml volumetric flask with 40 ml of mobile phase. The solution was sonicated for 3 min and diluted to volume with mobile phase. The aliquot was filtered through the 0.45 μm membrane and used for the analysis containing 20 μg ml^{-1} of sertraline. 20 μl sample was injected into the HPLC system as mentioned above.

3. Results and discussion

3.1. Method development

In order to achieve the separation of sertraline enantiomeric forms and diastereoisomers, the type and concentration of CDs were studied during optimization. Addition of β-CD (5–15 mM) to the mobile phase composed of 66:34 (v/v) aqueous 150 mM phosphate buffer, pH 3.0 (adjusted with 85% phosphoric acid)/acetonitrile could not resolve sertraline enantiomers. However, addition of HP-β-CD gave a better enantiomeric resolution of sertraline enantiomers and diastereoisomers. On the other hand, it was necessary to examine the influence of the concentration of HP-β-CD on the resolution of sertraline enantiomers and diastereoisomers. The results are given in Fig. 2 and the optimal concentration was achieved with 18 mM HP-β-CD.

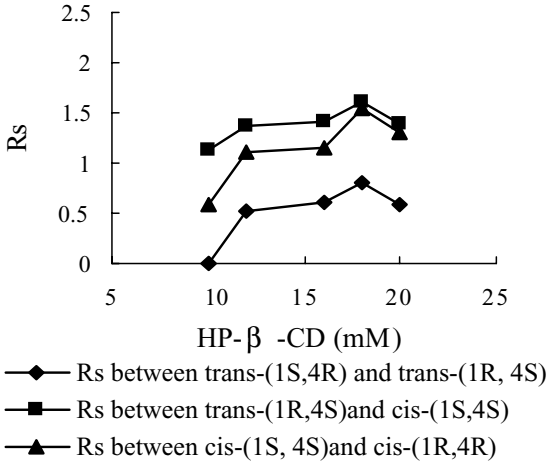

Fig. 2. Effect of concentration of HP-β-CD on the resolution of the enantiomeric form and *trans* diastereoisomers. Column: Alltima C18 (250 mm × 4.6 mm i.d., 5 μm). Mobile phase: 66:34 (v/v) aqueous 150 mM phosphate buffer, pH 3.0 (adjusted with 85% phosphoric acid)/acetonitrile. Flow rate: 1.0 ml min^{-1}. Injection volume: 20 μl. Analyte: *cis*-(1*S*,4*S*) enantiomer (sertraline hydrochloride) (20 μg ml^{-1}), *cis*-(1*R*,4*R*) enantiomer (40 μg ml^{-1}), *trans*-(1*S*,4*R*) isomer (20 μg ml^{-1}) and *trans*-(1*R*,4*S*) isomer (20 μg ml^{-1}). Wavelength used for UV detection: 225 nm.

The pH and the composition of mobile phases was found to be important for improvement of enantioselectivity. The buffer concentration on the resolution was investigated for sertraline enantiomers and diastereoisomers using 50–200 mM sodium phosphate at pH 3.0 with 18 mM HP-β-CD containing 34% acetonitrile. The results are described in Fig. 3. Better separation of sertraline enantiomers and diastereoisomers could be achieved by using mobile phases containing sodium phosphate from 150 to 200 mM, where the

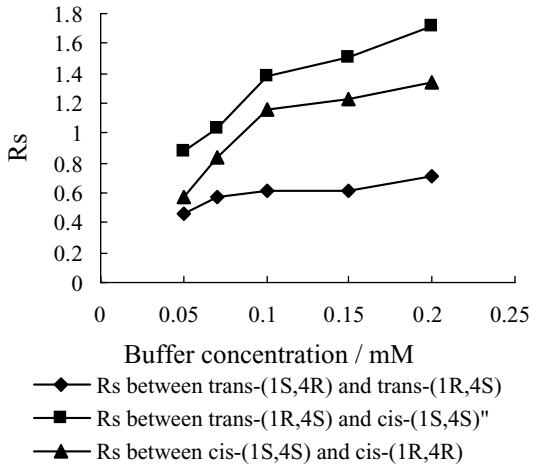

Fig. 3. Effect of concentration of buffer on the resolution of the enantiomeric form and *trans* diastereoisomers. Mobile phase: 66:34 (v/v) aqueous 18 mM hydroxypropyl-β-cyclodextrin (HP-β-CD), pH 3.0 (adjusted with 85% phosphoric acid)/acetonitrile. Other operating conditions as in Fig. 2.

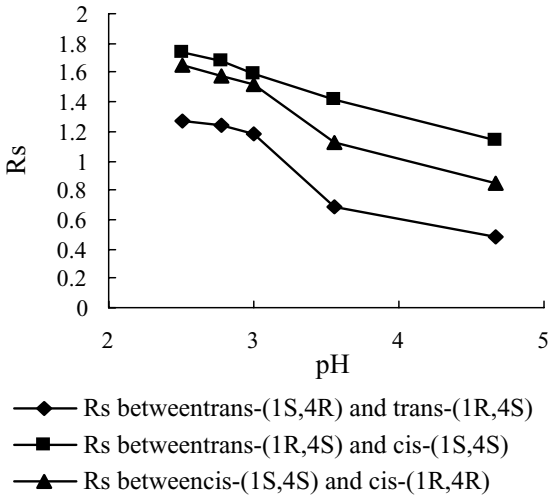

Fig. 4. Effect of pH on the resolution of the enantiomeric form and *trans* diastereoisomers. Mobile phase: 66:34 (v/v) aqueous 170 mM phosphate buffer with 18 mM hydroxypropyl-β-cyclodextrin (HP-β-CD)/acetonitrile. Other operating conditions as in Fig. 2.

resolution was about 0.62–0.71 for the separation of *trans* enantiomeric forms, 1.51–1.71 for *trans*-(1*R*,4*S*) diastereoisomer and sertraline, 1.23–1.34 for sertraline enantiomers.

The pH value of buffer on the enantioselectivity was examined by using 0.17 mM buffer solutions (pH 2.5–4.5) with 18 mM HP-β-CD containing 34% acetonitrile as the mobile phase as shown in Fig. 4. It is

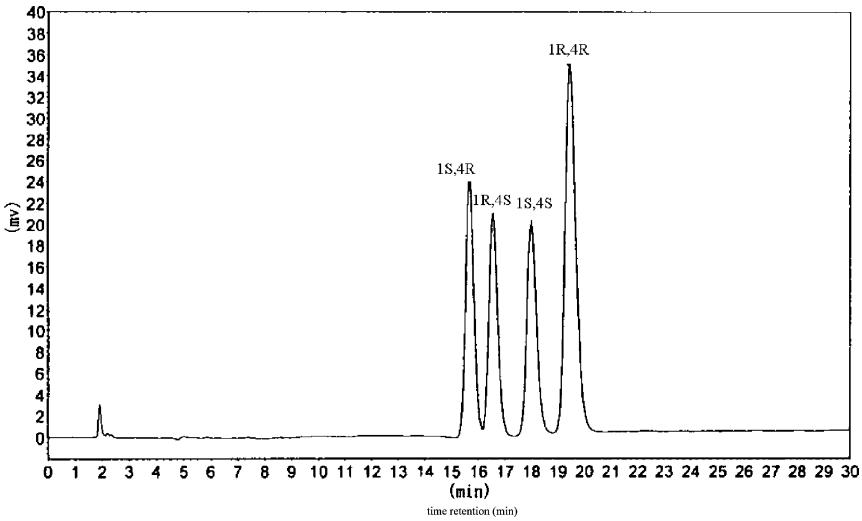

Fig. 5. Typical chromatogram showing the separation of sertraline hydrochloride (20 μg ml^{-1}), *cis*-(1*R*,4*R*) enantiomer (40 μg ml^{-1}), *trans*-(1*S*,4*R*) isomer (20 μg ml^{-1}) and *trans*-(1*R*,4*S*) isomer (20 μg ml^{-1}). Mobile phase: 68:32 (v/v) aqueous 170 mM phosphate buffer, pH 3.0 (adjusted with 85% phosphoric acid) with 18 mM hydroxypropyl-β-cyclodextrin (HP-β-CD)/acetonitrile. Other operating conditions as in Fig. 2.

observed that the resolution of the enantiomers and diastereoisomers is pH dependent. Better selectivity for sertraline enantiomers and diastereoisomers was achieved at pH 2.5–3.0.

Acetonitrile was used as the organic modifier. The concentration of acetonitrile used in these experiments was 25–40%. The selectivity for sertraline enantiomers and diastereoisomers was optimum at 32%, where the resolution was about 1.23 for the separation of *trans* enantiomeric forms, 1.90 for *trans*-(1*R*,4*S*) diastereoisomer and sertraline, 1.77 for sertraline enantiomers. The resolution decreased by increasing acetonitrile concentration.

Fig. 5 is the typical chromatogram with the mobile phase of 68:32 (v/v) aqueous 170 mM phosphate buffer, pH 3.0 (adjusted with 85% phosphoric acid) containing 18 mM HP-β-CD/acetonitrile at a flow rate of 1.0 ml min^{-1}. Fig. 6 is the chromatograms comparing the separation of unpurified sertraline hydrochloride with purified sertraline hydrochloride in bulk drug. 1.82% sertraline enantiomer and 0.13% diastereoisomers were detected in the unpurified sertraline. After purified, no sertraline enantiomer and diastereoisomers were detected.

3.2. Validation of the method

The stability of the standard solution of sertraline (19.21 μg ml^{-1}) was monitored by measuring the areas of response of 20 μl injection over a period of 7

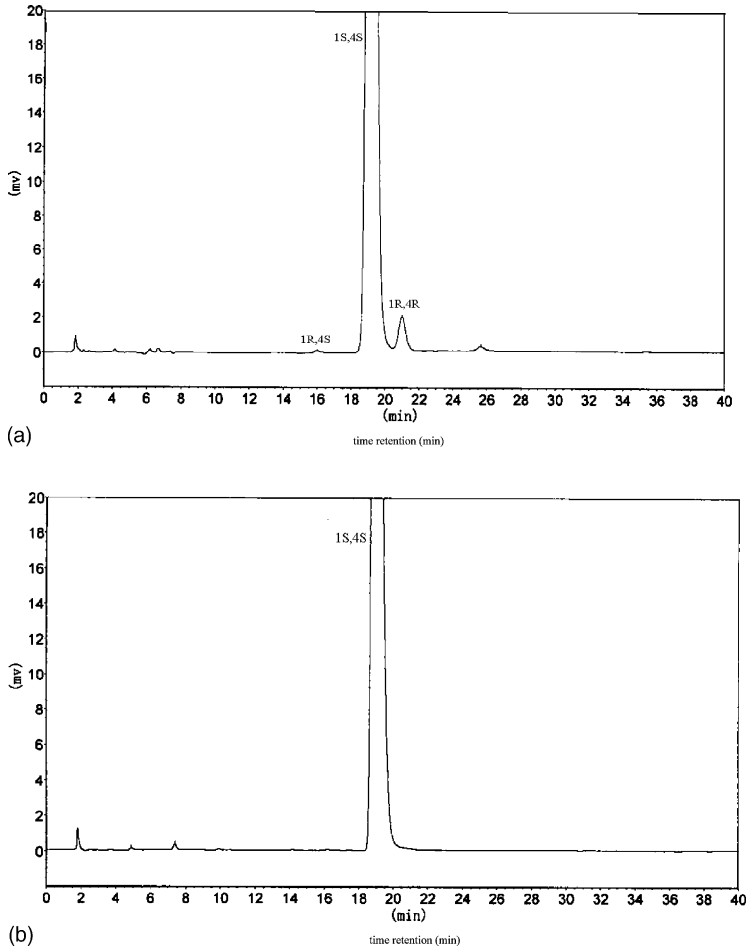

Fig. 6. Chromatograms of: (a) unpurified sertraline hydrochloride (100 μg ml^{-1}), (b) purified sertraline in bulk drug hydrochloride (100 μg ml^{-1}). Other operating conditions as in Fig. 5.

days: 0–7 days. The R.S.D. (%) values of sertraline was 0.52 within 7 days.

The linearity of sertraline in standard solutions was investigated at nine concentration levels. The calibration curve for sertraline was linear in the range 1–120 $\mu g\,ml^{-1}$. The representative linear equation relating y (peak area) $\times$ x (concentration $\mu g\,ml^{-1}$) was $y = 32439x - 42461$ ($r = 0.9999$). Limits of detection (LOD) (S/N 3:1) and limit of quantification (LOQ) (S/N 10:1) for sertraline were 0.029 and 0.097 $\mu g\,ml^{-1}$, respectively ($n = 6$).

The standard addition recoveries were carried out by adding a known amount of standard to the blank excipient at three different levels. Each level was repeated three times ($n = 3$) and the amounts of drug were found by the assay methods. The recovery was calculated by dividing the amount of found by the added, then multiplied by 100%. Results and statistical parameters were shown in Table 1.

Repeatability and intermediate precision of the HPLC method was studied. The repeatability was evaluated by preparing six real sample solutions for determination of the samples of the same batch number according the assay of pharmaceutical preparations. The R.S.D. (%) value of repeatability of sertraline was 0.45 for bulk drug, 0.48 for tablets and 0.50 for capsule, respectively. Two different analysts in two labs on two instruments performed intermediate precision experiments with separated mobile phase according to the assay of pharmaceutical preparations. Each real sample solution was assayed in triplicate times. The R.S.D. (%) value of intermediate precision was 0.54 for bulk drug, 0.61 for tablets and 0.67 for capsule, respectively. The results in Table 2 show that the method is precise and accurate.

Table 1
Accuracy evaluation in sertraline of tablet and capsule ($n = 3$)

	Amount of sertraline added (mg)	Amount founded (mg)	Recovery (%)	R.S.D. (%)
Tablet	45.25	44.89	99.21	0.60
	45.45	45.25	99.75	
	44.48	43.88	98.66	
	56.48	56.65	100.3	
	57.18	57.00	99.68	
	57.78	57.51	99.54	
	69.83	69.68	100.2	
	70.15	69.15	98.57	
	67.98	67.47	99.25	
Capsule	45.98	46.17	100.4	0.63
	46.05	45.86	99.59	
	45.15	44.61	98.80	
	56.41	56.20	99.62	
	56.05	56.33	100.3	
	56.11	55.80	99.45	
	69.28	68.32	98.62	
	64.79	64.49	99.33	
	67.43	67.25	99.74	

Table 2
The evaluation of intermediate precision ($n = 3$)

	Purity in bulk drug	Amount of sertraline (mg per tablet) (label claim 55.96 mg sertraline)	Amount of sertraline (mg per capsule) (label claim 55.96 mg sertraline)
Lab 1			
Analyst 1	98.77	55.79	56.29
	100.2	55.45	56.13
Analyst 2	99.45	55.98	55.62
	100.3	55.13	55.36
Lab 2			
Analyst 1	99.70	55.39	56.02
	99.76	55.86	55.92
Analyst 2	99.82	55.15	55.65
	98.90	55.23	55.24
Mean	99.62	55.50	0.58
R.S.D. (%)	0.54	0.61	0.67

Table 3
Analysis results for sertraline in bulk drug (D1, D2, D3), tablets (T1, T2, T3) and capsules (C1, C2, C3)

Sample	Theoretical amount (mg)	Experimental amount (mg)	Purity[a]	R.S.D. (%)
D1	–	–	99.28	0.48
D2	–	–	99.80	0.56
D3	–	–	99.97	0.53
T1	55.96	55.57	99.30	0.52
T2	55.96	55.75	99.62	0.76
T3	55.96	55.77	99.66	0.63
C1	55.96	55.64	99.43	0.84
C2	55.96	55.85	99.80	0.59
C3	55.96	55.62	99.39	0.65

[a] Mean of six determination for bulk drug, tablets and capsules.

3.3. Analysis of real samples

Different batches of sertraline in bulk drug, tablets and capsules were analyzed by HPLC. The results are listed in Table 3.

4. Conclusion

The HPLC method for resolution of the enantiomeric form and *trans* diastereoisomers was established and the assay of sertraline in bulk drug, tablets and capsules of sertraline hydrochloride was developed and validated. It was easy to perform, precise and accurate. The whole procedure may be extended to the applications on quality control of commercial products.

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