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STABILITY-INDICATING RP-HPLC METHOD VALIDATION OF SERTRALINE HYDROCHLORIDE AND ITS RELATED SUBSTANCES IN TABLETS

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ABSTRACT

In the present study, a high-performance liquid chromatographic method was validated to evaluate purity of Sertraline Hydrochloride in tablet dosage form. Sertraline Hydrochloride and two related substances, trans isomer and 2,3-dichloro isomer of Sertraline hydrochloride, were well resolved using a ZORBAX RX C_8 column (250 mm x 4.6 mm, 5 □) with the mobile phase consisting of 50mM KH_2PO_4 containing 0.1% octane sulfonic acid sodium salt (pH: 2.5 with Dil. H_3PO_4, 0.2% Triethylamine) and methanol (43:57, v/v). Flow rate was kept at 1.0ml/min and the detection was carried out by UV at 215nm. Resolution between any pair of isomers was found to be more than 2. The Limit of detection and Limit of quantification for both trans isomer and 2,3-dichloro isomer of Sertraline Hydrochloride were 10ng/ml and 30ng/ml, respectively. The developed and optimized method was applicable for routine determination of Sertraline Hydrochloride and its trans isomer and 2,3-dichloro isomer in tablet dosage form with sufficient selectivity, precision and accuracy.

Key words: *Trans isomer; 2,3-dichloro isomer; Sertraline Hydrochloride; High performance liquid chromatography.*

INTRODUCTION

Sertraline hydrochloride, (Cis-(1S,4S)-N-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenamine hydrochloride), used as an anti-depressant is administered orally[1,2]. Sertraline has two chiral centers and therefore a possibility of four stereoisomers (*cis* enantiomers and *trans* diasteromers). Sertraline is marketed as cis- 1S, 4S form. The probable introduction of other isomers cis-1R,4R; trans-1S,4R; trans-1R, 4S is possible during the synthesis of Sertraline.[1] In order to ensure the quality, efficacy and safety, it is mandatory to determine the unwanted isomers of Sertraline which are formed during the synthesis. Determination of Sertraline in biological samples has been reported in the literature using techniques namely GC, GC-MS, LC and CE.[3-11] Adam A.I., *et. al.,* 2001 reported a HPLC assay method of Sertraline in drug substance and tablets.[11] Although Yuzhu H., *et. al.,* 2004 reported Chromatography resolution of the Sertraline enantiomeric forms and *trans* diastereoisomers with Alltima C18 (250mm × 4.6mm i.d., 5ìm) column with Hydroxy Propyl- â-cyclodextrin (HP- â -CD) as mobile phase additive[12] and Marilyn X., *et. al.,* 2004, developed an analytical method for the quantitation of Sertraline hydrochloride stereoisomers by electrokinetic chromatography[13] but with through extensive search of literature it was concluded that there is not any simple HPLC method for the simultaneous determination and separation of Sertraline hydrochloride, trans isomer and 2,3-dicholoro isomer of Sertraline hydrochloride.

Our present study aims at the separation of Sertraline hydrochloride, trans isomer and 2, 3-dicholoro isomer of Sertraline hydrochloride, using a combination of ion-pair (IPs) reagent as additives by Liquid chromatography (LC) using an ODS column. The present method describes the study of comparative enantiomeric separation of sertraline using single ion-pair and a combination thereof. The method also illustrates the use of different buffers at different pH; IP at different concentrations; different columns packed with C_8 and ODS.

MATERIALS AND METHOD

Chemicals and reagents

All chemicals and reagents were of the highest purity. Sertraline hydrochloride and its isomer's standards were obtained from Torrent Pharmaceutical Limited, Gandhinagar as gift sample. Tablets used correspond to the "Sertraline hydrochloride film coated tablet 100 mg" was purchased from local market. HPLC-grade methanol and triethylamine were obtained from Ranbaxy Fine Chemicals Limited, Delhi, India. Water was purified using a Milli-Q system (Millipore, Tokyo, Japan). Other reagents and solvents were HPLC grade or the highest grade commercially available, and used without further purification.

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Chromatographic equipment

An HPLC system (LC-10A, Shimadzu, Kyoto, Japan) was composed of an autosampler (SIL 10ADvp), a pump (LC-10AD or LC-10ADvp), a column oven (CTO-10Acvp or CTO-10ASvp), a UV detector (SPD-10AV or SPD-10AVvp), SPD M-10AVP photo diode array detector and a data processor (CLASS-LC10 or CLASS-VP). Detection was performed at 215 nm, and the injection volume was 10µl throughout the work.

Chromatographic conditions

Separations were carried out on a Zorbax RX C_8 column (250 mm x 4.6 mm, 5 □, Agilant, USA) with the mobile phase consisting of 50mM KH_2PO_4 containing 0.1% Octane Sulfonic acid sodium salt (pH: 2.5 with Dil. H_3PO_4, 0.2% Triethylamine) and methanol (43:57, v/v). Flow rate was kept at 1.0ml/min with an analysis time of 40 minute and column oven temperature 35ºC. The sample temperature was kept at 15 ± 2° C in autosampler.

Some other octadecylsilica columns Symmetry C_{18} (250 x 4.6 mm i.d., 5 µ, Waters, Milford, USA), Thermo Hypersil BDS C_{18} (250 x 4.6 mm i.d., 5□m, Thermo, Runcorn, Great Britain) and X-Terra C_{18} (250 x 4.6 mm i.d., 5□, Waters, Milford, USA) were also used to select an appropriate stationary phase for the separation of Sertraline hydrochloride and its related substances. The analysis was performed using the same mobile phase and conditions.

The effect of change in type ion-pair reagent, change in flow rate, change in pH of buffer, change in column oven temperature, change in concentration of peak modifier and change in ratio of mobile phase were also studied during method development and optimization process.

Optimization studies for the separation of Sertraline hydrochloride and its related substances

Stock solutions of Sertraline hydrochloride, trans isomer and 2,3dichloro isomer of Sertraline hydrochloride were separately prepared by accurately weighing 11.2mg of each compound in a 100ml volumetric flask followed by dissolution in mobile phase. The stock solutions were prepared just before use, although they were stable for at least 24 h at 25 æ%C. Standard solutions (10 µg/ml each) of a mixture of Sertraline hydrochloride, trans isomer and 2,3dichloro isomer of Sertraline hydrochloride was prepared by diluting the stock solutions with mobile phase. A portion (10µl) of the solution was injected into the HPLC column.

Degradation of* Sertraline hydrochloride *in suspension

The placebo, API and tablets (equivalent Sertraline hydrochloride in tablet powder) were subjected to acid, base, oxidation, thermal and photo degradation. Sertraline hydrochloride solution (1000µg/ml) was prepared in 5N hydrochloric acid, and the suspension was refluxed for 4 h, and allowed to cool to room temperature. The mixture after keeping in hydrochloric acid was neutralized with 5N sodium hydroxide solution. Sertraline hydrochloride was suspended in 5N sodium hydroxide solution to get a concentration of 1000µg/ml, and the suspension was refluxed for 3 h, and allowed to cool to room temperature. The mixture after keeping in sodium hydroxide was neutralized with 5N hydrochloric acid. Sertraline hydrochloride (100 mg) was suspended in water (100 ml) in a clear glass bottle, and the suspension was exposed to light providing an overall illumination of 1.2 million lx h and an integrated UV energy of 200Wh/m^2 at 25°C. Oxidative stress was generated by suspending Sertraline hydrochloride in 30% H_2O_2 to get a solution of 1000µg/ml. the solution was kept at room temperature for 12 h afterward it was neutralized with sodium thio-bisulphate. Thermal degradation was carried out at 100°C for 72 hours. A blank was prepared accordingly for every degradation study. A portion (100µl) of all the above solutions was injected into the HPLC column.

Analytical procedure

Solutions (200µg/ml) of Sertraline hydrochloride, trans isomer and 2,3dichloro isomer of Sertraline hydrochloride were prepared in the mobile phase by dissolving known amounts of the components in the mobile phase. These solutions were adequately diluted to study the accuracy, precision, linearity and limit of detection and limit of quantization.

RESULT AND DISCUSSION

The aim of our work was method development for the simultaneous determination of all substances (Sertraline hydrochloride and its related substances) in one step. The method should be stability indicating, free of interference from excipients. The aim of method validation was to demonstrate the method suitability for its intended purpose as stated in ICH guidelines Q3A, Q3B and FDA stability guideline[14, 15]. The optimized method was validated by a standard procedure to evaluate adequate validation characteristics (accuracy, precision, linearity, selectivity, sensitivity-LOD, LOQ, robustness and stability).

Accuracy (% of recovery, % of R.S.D.) was investigated using placebo samples spiked with standard solutions of Sertraline hydrochloride, trans isomer and 2, 3dichloro isomer of Sertraline hydrochloride. Recovery samples were prepared by spiking stock solution of trans isomer of Sertraline hydrochloride and 2, 3-dichloro isomer of Sertraline hydrochloride in placebo and Sertraline hydrochloride preparation at three levels: LOQ, 0.10%, and 0.30% of sample concentration (LOQ, 0.2 and, 0.6µg/ml). Comparison of real sample concentration and determined concentration was calculated with the results from 97.07 to 100.29% for recovery, 0.92–1.77% for R.S.D.,

respectively. Repeatability was assessed by injecting a solution containing 100% (0.40µg/ml) specification level concentration of related substance Sertraline hydrochloride in the optimized chromatographic condition. The repeatability of the proposed method and the percentage RSD for intraday condition was found to be 2.18% (lesser than 5%) of 2,3 dichloro isomer of Sertraline hydrochloride, as the Trans isomer of Sertraline hydrochloride and any unknown impurity was below LOQ level in specification limit concentration. Selectivity was verified by injection of standard solution, placebo of pharmaceutical preparation and pharmaceutical preparation treated according to sample preparation procedure. No interferences were observed**.**

Linearity for trans isomer of Sertraline hydrochloride, 2,3-dichloro isomer of Sertraline hydrochloride were determined over the range of LOQ to 150% of specification or LOQ to 0.30% sample concentration. The mixed standard solution at LOQ, 0.05%, 0.08%, 0.10%, 0.12%, 0.15%, 0.20% and 0.30% of sample concentration (LOQ, 0.10, 0.16, 0.20, 0.24, 0.30, 0.40 and 0.60µg/ml for each component) were prepared by spiking from *Stock solutions*. Placebo stock solution was prepared and required volume was added to each preparation (Concentration of placebo in final solution shall be same as in sample preparation as per method). The calibration curves were prepared by the plot of concentration against the mean peak area.

The slope and the other statistical parameters of the calibration curves were calculated by linear regression analysis as shown in Table 1**.**

Table 1: *Linearity results for Sertraline hydrochloride and its isomers*

Name of component	Trans Isomer	Sertraline hydrochloride	2,3-Dichloro Isomer
r^2	0.9996	0.9996	0.9996
y-Intercept	-635.73	-688.05	-728.2
Slope of regression line	125236	129943	130230
Residual sum of square	348166.7	351045	363320.9
Equation for regression line	Y=125236X-635.73	Y=129943X-688.05	Y=130230X-728.2
Range	0.02%-0.30%	0.02%-0.30%	0.02%-0.30%

Limits of detection (LOD) and limits of quantification (LOQ), as a measure of method sensitivity, were provided for degradation products and impurity calculated by means of the method of signal-to-noise ratio. These limits are parameters of quantitative assays of low level compounds in the sample and they are used especially for the determination of impurities as in our case. The LOD and LOQ for Sertraline hydrochloride and its isomers were depicted in the Table 2**.**

Table 2: *LOD and LOQ values of Sertraline hydrochloride and its isomers*

Name of component	LOD		LOQ	
	Concentration (ng/ml)	% RSD	Concentration (ng/ml)	% RSD
Trans isomer	20	10.89	40	6.21
2,3-Dichloro isomer	20	9.75	40	3.85
Sertraline hydrochloride	20	5.45	40	5.37

Stability of the analytical solution determines the period of time a solution can be held before analysis without compromising accuracy. This delay is beyond that included in the method procedure and anticipated unexpected instrument delay. Sample preparation and standard preparation were injected in duplicate initially and then after keeping that for 24 hours Sample preparation and standard preparation were injected in duplicate initially and then after keeping that for 24 hours at bench top (H" 25°C) and in refrigerator (H" 5-8°C). The solution was stable for 24 hours at room temperature. The robustness as a measure of method capacity to remain unaffected by small, but deliberate, variations in method parameters was studied. System suitability parameters were measured so as to verify the system performance. All important characteristics including repeatability, peak resolution, theoretical plate number and peak asymmetry were measured and calculated using standard solution injection in six replicates. The results of method validation and system suitability test in comparison with the required limits could be seen in Table 3**.**

Table 3: *System suitability parameters for the RS method*

System Suitability Parameter	Observation		Acceptance criteria
	Component	Value	
Peak asymmetry (10%)	Trans isomer	1.36	?2.0
	Sertraline hydrochloride	1.51	
	2,3- Di Chloro isomer	1.28	
Capacity factor (k)	Sertraline hydrochloride	9.0	?5.0
Theoretical plates/meter	Sertraline hydrochloride	12061	?5000
% CV of 6 injection	Sertraline hydrochloride	0.76	% RSD should be less than 1.0
Resolution	Trans isomer	-	Should not be less than 2.0
	Sertraline hydrochloride	2.68	
	2,3- Di Chloro isomer	3.52	
S/N ratio	Sertraline hydrochloride	215	Should not be less than 50

CONCLUSIONS

The LC method described in the present study is very versatile, precise and accurate fro the determination of isomers of hydrochloride in the bulk drug and dosage forms. The method has been validated and the same can be extrapolated for the identification, quantitative analysis, homogeneity tests and stability tests of all compounds in a tablet dosage form.

REFERENCES

1. Jhonson BM, Anal Profile of Drug Substances & Excipients, Vol.24, Academic Press, London, 1996, p 443.
2. Davis R, *et al.* Pharmacoeconomics. 1997; 11: 378.
3. Snopek J, *et al.* J Chromatogr. 1988; 452: 571.
4. Szepesi J, *et al.* Cyclodextrins and Their Inclusion Complexes, Akademiai Kiado, Budapest, 1982.p33.

5. Kudo K, *et al.* Enantiomer. 2000; 5: 369

6. Tremaine LM, *et al.* J Chromatogr A. 1989; 496; 423.

7. Eap CB, *et al.* J Chromatogr Sci.1998; 36: 365.

8. Fouda HG, *et al.* J Chromatogr A. 1987; 417: 197.

9. Lacassie E, *et al.* J Chromatogr B. 2000; 742: 229.

10. Dallet P, *et al.* J Liq Chromatogr & Rel Tech. 2002; 25: 101.

11. Adam A.I. , *et al.* J Pharma. Biomed Anal. 2001; 26: 505.

12. Yuzhu H, *et al.* J Pharma. Biomed Anal. 2004; 34: 239.

13. Marilyn X, *et al.* J Chromatogr A 2004; 1052: 13.

14. ICH, "Impurities in New Drug Products", International Conference on Harmonisation, IFPMA, Geneva, 1996.p2.

15. ICH, "Impurities in New Drug substances", International Conference on Harmonisation, IFPMA, Geneva, 1996.p1.