Original Article

Estimation of sertraline by chromatographic (HPLC-$UV_{273\ nm}$) technique under hydrolytic stress conditions

Abstract

Purpose: In this paper, simple, specific and accurate RP-HPLC method was developed in order to study decomposition of sertraline (SRT) under the hydrolytic stress conditions (acid, neutral, alkaline and oxidative). **Materials and Methods:** The best separation of SRT and its degradation products were achieved on reverse phase LiChoCART with Purospher (RP-18e) column. The mobile phase was composed of methanol/water (75:25, v/v). The detection wavelength was 273 nm. The method was validated and response was found to be linear in the drug concentration range of 10–200 μg ml−1 with correlation coefficient of 0.998. **Results:** The RSD values for intra- and inter-day precision were < 0.65 and < 0.72%, respectively. Employing RP-HPLC method, degradation products were detected in the exposed samples. **Conclusion:** It was found that the susceptibility of SRT to hydrolytic decomposition increased in following manner: Neutral condition < alkaline condition < acid condition < oxidative condition.

Key words: Antidepressant, high-performance liquid chromatography, hydrolysis, limit of detection, limit of quantitation, sertraline, stability

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INTRODUCTION

Depression, a common mental disorder, is a chronic or recurrent illness that affects both economic and social functions of patients and can eventually lead to suicidal behavior. The selective serotonin reuptake inhibitors (SSRIs) are the most widespread class of second-generation antidepressant drugs and are in fact becoming the drug of first choice for the treatment of depression. Sertraline (SRT) [(1*S*,4*S*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine] [Figure 1] blocks the reuptake of serotonin at central synapses selectively and powerfully. SSRIs have a therapeutic efficacy similar to that of traditional, tricyclic antidepressants, but have a much more favorable side and toxic effect profile; furthermore, the former are also very useful in the treatment of depression-related disorders such as anxiety, panic and obsessive compulsive disorders.[1]

In order to achieve high level of safety and effectiveness of pharmacotherapy, the regulatory authorities escalate the requirements on the quality of pharmaceutical products. The investigations of stability of drugs represent an important issue in drug quality evaluation. Many environmental conditions such as heat, light, moisture, as well as the chemical susceptibility of substances to hydrolysis or oxidation can play extremely serious role in pharmaceutical stability.[2]

A stress testing of drug substance can help to identify the likely degradation products and to provide important information on drug's inherent stability. Consecutively, it can be a fundamental contribution to development and validation of stability-indicating analytical method used in monitoring of quality of pharmaceutical products. Independent of the final dosage form, forced drug degradation by exposure of the drug solution to acidic, alkaline, or oxidative

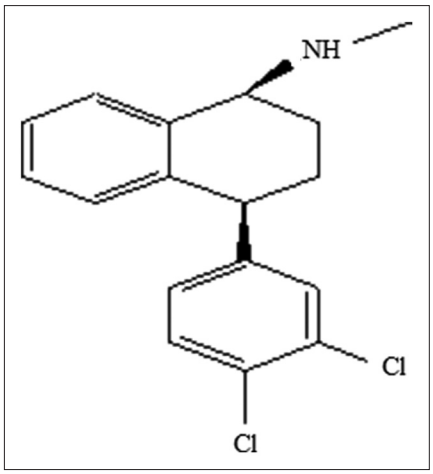

Figure 1: Chemical structure of sertraline (SRT)

conditions is useful to predict the potential hydrolytic degradation products. Hydrolysis (during wide range of pH) is one of the most common degradation chemical reactions. Since water, either as a solvent or in the form of the potential moisture in the air, contacts most pharmaceutical dosage forms to some degree; the potential for this degradation pathway exists for most drugs and excipients.[3]

Several methods have been reported for the determination of SRT in biological fluids and pharmaceutical formulations, including high-performance liquid chromatography (HPLC) with UV detection,[4,5] gas chromatography–mass spectrometry (GC–MS),[6,7] and specrophotometric method.[8] SRT has also been analyzed together with other antidepressants using HPLC techniques.[9] Also, SRT has been determined by liquid chromatography and tandem mass spectrometry (LC–MS/MS).[10]

Any systematic study about the behavior of SRT under the stress conditions, e.g., hydrolytic, is not available in the literature. The aim of this paper is to study hydrolytic stability of SRT and to develop reversed phase-HPLC (RP-HPLC) analytical method for determination of SRT in the presence of its hydrolytic degradation products. Moreover, the method can be helpful in an effort to assure the quality, safety, and effectiveness of pharmacotherapy.

MATERIALS AND METHODS

The following reagents were used: SRT (purity 99.8%) was kindly provided by Ranbaxy, Gurgaon, India. Methanol, acetonitrile, hydrogen peroxide, hydrochloric acid, and sodium hydroxide were purchased from Merck Chem., India. Ultra-pure water was obtained from Milli-Q system.

Instrumentation

A Shimadzu model HPLC equipped with quaternary LC-10AVP pumps, variable wavelength programmable UV detector SPD-10AVP column oven (Shimadzu), and SCL 10AVP system controller (Shimadzu), which consisted of Rheodyne injector fitted with a 20-µL loop, was used. The detector was set at 273.0 nm and peak areas were integrated automatically by computer using PC 1000 Software. An appropriate separation was achieved on a LiChroCART column (250 × 4.6 mm ID) with Purospher (RP-18e, 5 µm) as a stationary phase (Merck, Germany).

Method development

A variety of mobile phases were investigated in the development of an HPLC method suitable for the study. These included methanol–water, 75:25 (%, v/v); acetonitrile–water, 75:25; methanol–water, 50:50; methanol–water, 95:5; and acetonitrile–phosphate buffer (pH 3.5-6.5), 80:20. The suitability of the mobile phase was decided on the basis of the sensitivity of the assay, suitability for stability studies, time required for the analysis, ease of preparation, and use of readily available cost-effective solvents.

Standard curve

The stock solution (500 µg/ml) was prepared by dissolving an appropriate amount of solid substance of SRT in methanol. The calibration curve was made using five standard solutions of different concentrations (10, 20, 50, 100, and 200 µg/ml). The standard solutions were prepared by diluting an appropriate volume of stock solution with methanol. Each solution was analyzed in triplicate. The peak area values were plotted against the corresponding analyte concentrations to obtain the linear calibration.

Validation of method

Precision

Precision was considered at two levels, i.e., repeatability and intermediate precision. Repeatability of sample application was determined as intra-day variation, whereas intermediate precision was determined by carrying out inter-day variation at three different concentration (20, 60, and 120 µg/ml) levels in triplicates.

Accuracy, as recovery

Accuracy was determined by standard addition method. The preanalyzed samples of SRT (20 µg/ml)

were spiked with the extra 0, 50, 100, and 150% of the standard SRT and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate. The % recovery of samples, relative standard deviation (% RSD), and standard error of mean (SEM) were calculated at each concentration level.

Robustness

To evaluate HPLC method robustness, a few parameters were deliberately varied. The parameters included variation in percentage of water and methanol in the mobile phase, flow rate, and column temperature. Each factor selected to examine was charged at three levels. One factor at a time was changed to estimate the effect. Thus, replicate injections (n = 6) of mixed standard solution at three concentration levels were performed under small changes of four chromatographic parameters (factors).

Limit of detection (LOD) and quantification (LOQ)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

To estimate the limits of detection (LOD) and quantification (LOQ), blank methanol was applied (n = 6) and the standard deviation (σ) of the analytical response was determined. The LOD and LOQ values were calculated from the calibration curves as $k\sigma/b$, where k = 3.3 for LOD and 10 for LOQ, σ is the standard deviation of the intercept, and b is the slope of the calibration curve.

Determination of stability

The forced degradation of SRT was carried out under the condition of acid, neutral, alkaline, and oxidative hydrolysis. Appropriate amount of SRT was dissolved in methanol to prepare SRT stock solution concentration of 400 µg/ml. One milliliter of SRT stock solution was transferred into each of four glass vials. One milliliter of HCl (1 N) was added into first vial, 1 ml of NaOH (10 N) into the second vial, 1 ml of water into third vial, and finally 1 ml of the solution of 3% H_2O_2 into the fourth vial. All vials were tightly closed and maintained at constant temperature (90°C) in a heating block with simultaneous stirring. After the periods of 30, 60, 180, and 360 min, 20 µl of each sample was analyzed employing HPLC. The blanks consisting of 1 ml of methanol and 1 ml of degradation medium were injected on to the column before every single analysis. In order to determine relative rate of hydrolytic decomposition of the drug, the logarithm of remaining concentration of SRT was plotted versus time. The rate constants were calculated from the slope of the kinetic curves.

RESULTS AND DISCUSSION

Optimization of mobile phase

The HPLC procedure was optimized with a view to developing a method for stability-indicating assay in stressed samples. No internal standard was used because no extraction or separation step was involved. Of the several solvents and solvent mixtures investigated, methanol–water 75:25 (%, v/v) was found to furnish sharp, well-defined peaks with very good symmetry (1.25) and low t_R (7.05 ± 0.12 min) [Figure 2]. With acetonitrile–water 75:25 as the mobile phase, t_R was 9.5 min and peak shape and sensitivity were poor. Acetonitrile was also more expensive than methanol. Methanol–water 95:05 and methanol–water 50:50 did not furnish sharp, well-defined peaks, and other mobile phases tried either resulted in much lower sensitivity or did not give well-defined peaks in a short time, and so were not considered. The final decision on mobile phase composition and flow rate was made on the basis of peak shape (peak area, asymmetry, tailing factor), baseline drift, time required for analysis, and cost of solvent, and methanol–water 75:25 (%, v/v) was selected as the optimum mobile phase. Under these

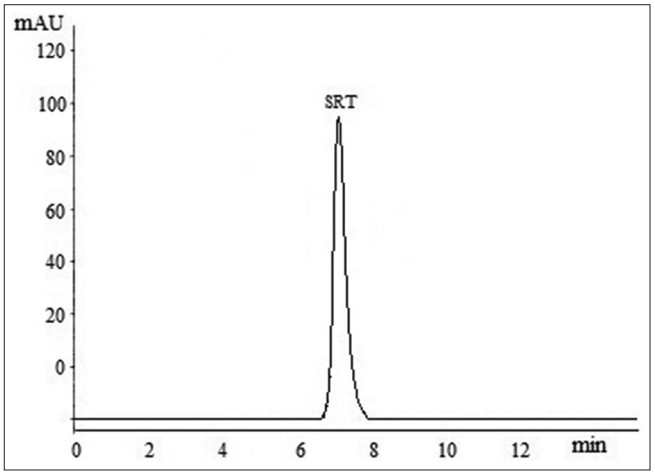

Figure 2: Chromatogram of standard SRT (200 µg/ml); Rt: 7.05 ± 0.12, mobile phase methanol–water (70:30, v/v) at 273 nm

conditions, the retention time and asymmetry factor were 7.05 min and 1.25, respectively.

Method validation

The validation of the procedures was examined via linearity, precision, accuracy as recovery, robustness, and by evaluation of the LOD and LOQ.

Linearity

The linearity of this method was proved using linear correlation of the peak area values and appropriate concentrations of SRT in a range of 10–200 µg/ml. The correlation coefficient of this dependence was calculated to be 0.998 [Table 1].

Precision

Precision was considered at two levels, i.e., repeatability and intermediate precision. Repeatability of sample application was determined as intra-day variation, whereas intermediate precision was determined by carrying out inter-day variation at three different concentration levels in triplicates. Results from determination of repeatability and intermediate precision, expressed as RSD (%), are listed in Table 2. The low values of RSD indicate the repeatability of the method.

Recovery

The recovery of the method, determined by spiking a previously analyzed test solution with additional drug standard solution, was 99.25–101.86%. The values of recovery (%), RSD (%), and SEM listed in Table 3 indicate the method is accurate.

Robustness

To evaluate the method robustness, a few parameters were deliberately varied. Results presented in Table 4 indicate that the selected factors remained unaffected by small variations of these parameters. Insignificant differences in peak areas and less variability in retention time were observed. The standard deviation of peak areas was calculated for each parameter and %RSD was found to be less than 2%. The low values of %RSD indicated robustness of the method.

LOD and LOQ

The LOD and LOQ values were calculated from the calibration curves as kSD/b, where k = 3.3 for LOD and 10 for LOQ, SD is the standard deviation of the intercept, and b is the slope of the calibration curve. The lowest LOD for HPLC and also the LOQ were found to be 28 ng/ml and 85.5 ng/ml, respectively.

Stability study

HPLC study of SRT hydrolytic decomposition suggested the following degradation behavior. After acid hydrolysis employing HPLC, two degradation products were detected at the retention times of 1.75 and 2.0, respectively [Figure 3]. It was observed that the area values of both peaks were growing in time and this observation was accompanied with decrease in concentration of SRT. The stability of SRT was also studied using water as a medium for degradation. Although both degradation products were detected on chromatogram, the ratio between the areas of peaks

Table 1: Results of least square regression analysis

Parameters	HPLC
Linearity range	10-200 µg/ml
Detection limits	28 (ng/ml)
Quantitative limits	85.5 (ng/ml)
Regression equation (Y)	Y=42262x+37254
SD on slope (S_b)	42262±402.40
SD on intercept (S_a)	37254±2503.40
SE of slope (S_e)	262.64
Correlation coefficient (r)	0.998

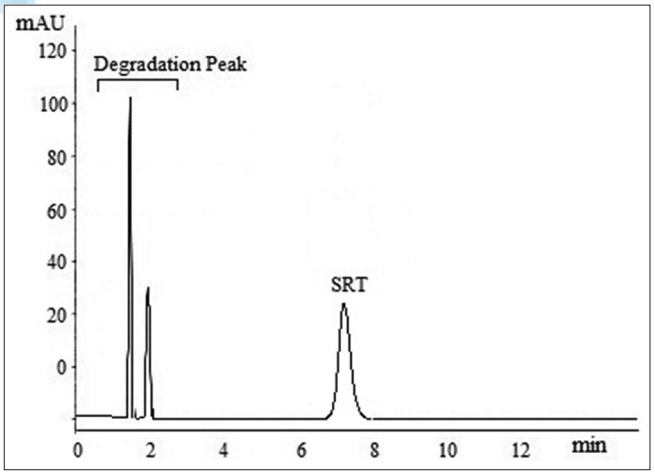

Figure 3: Chromatogram of SRT decomposition at 360 min in acid hydrolysis condition

Table 2: Intra-and inter-day precision (*n*=3)

Theoretical concentration	Intra-day precision			Inter-day precision		
HPLC (µg/ml)	Mean area±SD	SEM	% RSD	Mean area±SD (*n*=3)	SEM	% RSD
20	1,172,436±8512.52	4914.84	0.72	1,192,642±7841.66	4527.51	0.65
60	3,094,278±9948.94	5744.19	0.32	3,088,163±9715.74	5609.54	0.31
120	6,182,362±15,824.34	9136.45	0.25	6,251,683±16385.28	9460.32	0.26

Table 3: Accuracy as recovery (*n*=3)

Excess drug added to analyte (%)	Theoretical content (µg/ml)	Conc. found (µg/ml)±SD	% Recovery	% RSD	SEM
0	20	19.85±0.144	99.25	0.72	0.083
50	30	30.56±0.094	101.86	0.30	0.054
100	40	39.88±0.184	99.70	0.46	0.106
150	50	50.26±0.254	100.52	0.50	0.146

Table 4: Robustness (*n*=6)

Factor	Level	Retention time (Rt)	Capacity factor (K)	Tailing factor (T)	Mean±SD (min)
Percentage of water in the mobile phase (v/v)	−2	7.021	2.31	1.14	7.024±0.007
	0	7.032	2.28	1.16	2.27±0.035
	+2	7.019	2.24	1.13	1.14±0.015
Percentage of methanol in the mobile phase (v/v)	−2	7.018	2.30	1.12	7.021±0.004
	0	7.026	2.28	1.16	2.28±0.020
	+2	7.021	2.26	1.14	1.14±0.020
Flow rate (ml/min)	−0.25	7.038	2.24	1.14	7.054±0.014
	0	7.058	2.26	1.16	2.26±0.030
	+0.25	7.065	2.30	1.12	1.14±0.020
Temperature	−2	7.043	2.32	1.15	7.042±0.005
	0	7.036	2.26	1.13	2.28±0.030
	+2	7.047	2.28	1.16	1.14±0.015

was different [Figure 4] in comparison with previous experiment (acid hydrolysis).

In contrast to acid hydrolysis, alkaline conditions led to decomposition of SRT into three main degradation products. Their presence was detected at the retention times of 2.0 min, 2.5 min, and 2.6 min, respectively [Figure 5]. As it is evident from Figure 5, the peak area values of all main degradation products were growing in time. Besides the peaks of these degradation products, other small peaks were also found in the exposed samples, but none of them had the area value greater than 1% of concentration of SRT. Furthermore, the amount of these degradation products did not grow markedly during the time of the experiment.

The degradation of SRT in the solution of 3% H_2O_2 resulted in the formation of two major peaks [Figure 6]. The retention times indicated the agreement of oxidative degradation products with acid ones, but however, the decline of concentration of SRT was much higher than concentration fall in acid hydrolysis. As it is evident, acid, neutral, and oxidative hydrolysis led to the formation of the same degradation products.

Degradation behavior

The susceptibility of SRT to hydrolytic decomposition was determined as a fall of concentration of drug during the time of the experiment. The kinetic slopes are shown in Figure 7. The straight-line behavior

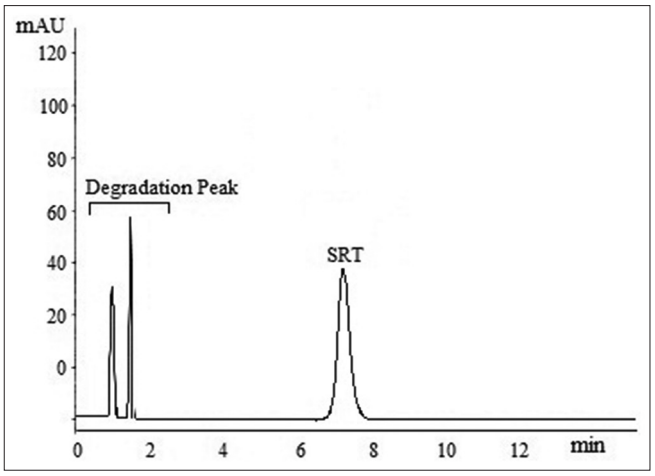

Figure 4: Chromatogram of SRT decomposition at 360 min in neutral hydrolysis condition

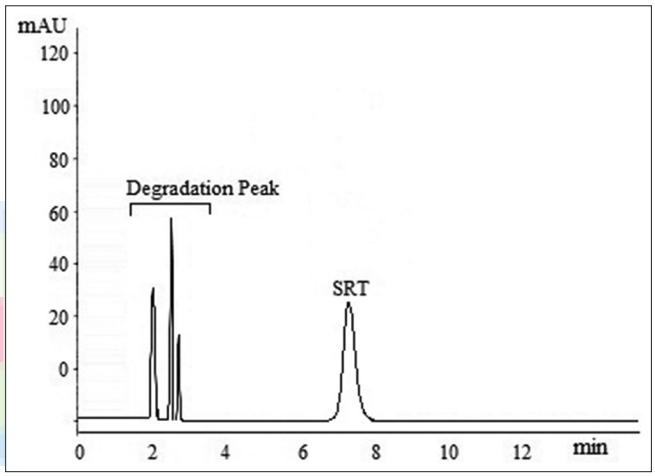

Figure 5: Chromatogram of SRT decomposition at 360 min in alkali hydrolysis condition

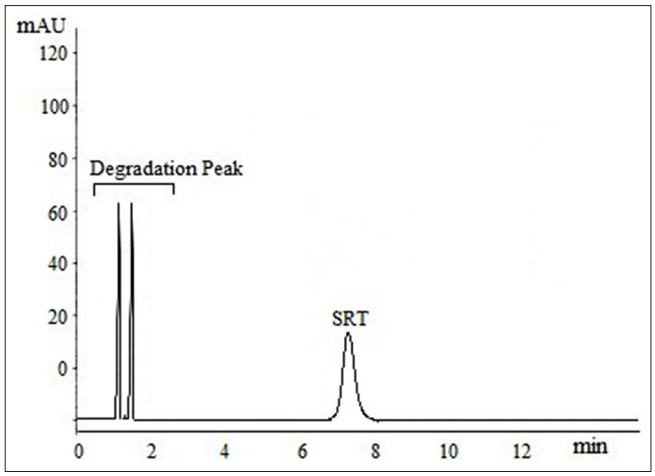

Figure 6: Chromatogram of SRT decomposition at 360 min in oxidative hydrolysis condition

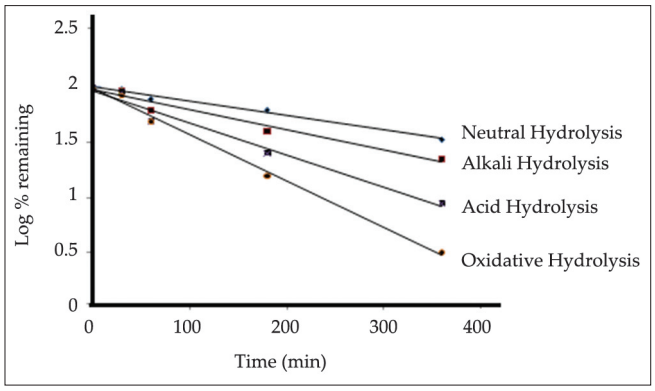

Figure 7: Kinetic curves of SRT decomposition

was obtained for neutral, alkaline, and oxidative conditions with correlation coefficients $R = 0.999$, $R = 0.998$ and $R = 0.996$, respectively. This fact implies that the hydrolytic degradation followed pseudo-first-order kinetic behaviour. The correlation coefficient for acid hydrolysis was calculated to be $R = 0.942$. The rate constants were determined from the slope of kinetic curves and their values are: $4.85 \times 10^{-2/min}$ (acid condition), $3.40 \times 10^{-2/min}$ (neutral condition), $4.32 \times 10^{-2/min}$ (alkaline condition), and $8.35 \times 10^{-2/min}$ (oxidative condition). The values of rate constants determined that the susceptibility of SRT to hydrolytic decomposition increased in the following manner: Neutral condition < alkaline condition < acidic condition < oxidative condition.

CONCLUSION

HPLC methods were developed and validated for the estimation of SRT in the presence of its degradation products. LOD and LOQ reported by this method are comparable to the reported one in previous literatures. The most striking feature of the developed method is its simplicity, accuracy, and rapidity. The behavior of SRT under the hydrolytic stress conditions in acid, neutral, alkaline, and oxidative media was studied. The information presented herein could be very useful for quality monitoring of bulk substance as well as the pharmaceutical preparation.

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