An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

DEVELOPMENT AND VALIDATION OF U V SPECTROPHOTOMETRIC METHOD AND RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF OLANZAPINE AND SERTRALINE HYDROCHLORIDE IN BULK AND SYNTHETIC MIXTURE

Nidhi S. Ghetiya[1], Dilip G. Maheshwari*[2]

Department of Quality Assurance and Pharm Regulatory Affairs,
L.J. Institute of Pharmacy, Ahmedabad, Gujarat, India.

Corresponding Author: dgmaheshwari@gmail.com

ARTICLE INFO

Key words:

Olanzapine,
Sertraline Hydrochloride,
First order derivative,
Synthetic mixture, Method validation

ABSTRACT

The present article describes simple, sensitive, accurate, precise and cost effective UV Spectrophotometric and RP-HPLC method for the simultaneous estimation Olanzapine and Sertraline Hydrochloride in Synthetic mixture. The first order derivative absorption at 271.368nm (zero crossing point for Olanzapine) was used for Sertraline Hydrochloride and 291.681nm (zero crossing point for Sertraline Hydrochloride) was used for Olanzapine. The linearity was obtained in the concentration range of 1-6 µg/ml for Olanzapine and 10-60 µg/mlµg/ml for Sertraline Hydrochloride with correlation coefficient (R^2) 0.996 and 0.997 respectively. The mean % recoveries were found to be in the range of 98-99.4% and 98.6 - 99% for Olanzapine and Sertraline Hydrochloride respectively. and in RP-HPLC using Peerless C18 (250×4.6 mm, 5 µm) column in Isocratic mode, with Mobile Phase Acetonitrile : Phosphate buffer, pH=3.00 (70:30 % v/v) (pH 3.1 adjusted with Orthophospheric acid). The Flow Rate was 1.0 ml/min and effluents were monitored at 226 nm. The Retention Time of were found to be Olanzapine and Sertraline Hydrochloride 2.230 min and 4.487 min respectively. The Linearity for Olanzapine and Sertraline Hydrochloride were found to be 1-6 µg/ml µg/ml 10-60 µg/ml respectively. The Recoveries of Olanzapine and Sertraline Hydrochloride were found to be 98– 99.42% and 98.66- 99.80 % respectively. The suitability of these methods for the quantitative determination of Olanzapine and Sertraline Hydrochloride was proved by validation. The proposed method has been validated as per ICH guideline and successfully applied to the simultaneous estimation of Olanzapine and Sertraline Hydrochloride in synthetic mixture.

INTRODUCTION:

Olanzapine has a higher affinity for 5-HT2A serotonin receptors than D2 Dopamine receptor and is chemically designated 2- Methyl – 4 - (4- methyl- 1-piperazinyl) -10H- thieno [2, 3-b] [1, 5] benzodiazepine. Which is a common property of all atypical antipsychotics, aside from the Benzamide antipsychotics such as Amisulpride. Olanzapine also had the highest affinity of any second-generation antipsychotic. **Sertraline hydrochloride** belongs to a class of antidepressant agents known as selective serotonin-reuptake inhibitors (SSRIs) is chemically designated (1S, 4S)- 4-(3, 4-dichlorophenyl)-N-methyl-1, 2, 3, 4-tetrahydronaphthalen-1-amine hydrochloride. As with other antidepressant

agents, several weeks of therapy may be required before a clinical effect is seen. SSRIs are potent inhibitors of neuronal serotonin reuptake. They have little to no effect on norepinephrine or Dopamine reuptake and do not antagonize α- or β-adrenergic, Dopamine D2 or histamine H1 receptors. During acute use, SSRIs block serotonin reuptake and increase serotonin stimulation of somatodendritic 5-HT1A and terminal auto receptors. Chronic use leads to desensitization of somatodendritic 5-HT1A and terminal auto receptors. Combination of Olanzapine and Sertraline Hydrochloride are useful in reduce the blood pressure. Combination of Olanzapine and Sertraline hydrochloride are effective in ameliorating symptoms of depression, anxiety and aggression, reducing sensitivity in interpersonal relationships and alleviating obsessive symptoms, pessimistic behaviors and somatization disorders in patients with personality disorders on methadone maintenance therapy. The purpose of the present work was to develop and validate first order derivative spectrophotometric method for simultaneous estimation of Olanzapine and Sertraline Hydrochloride in synthetic mixture.

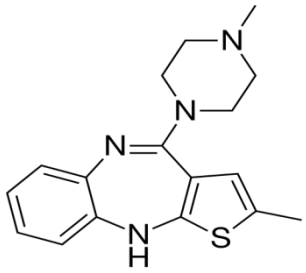


Fig. 1 Structure of Olanzapine

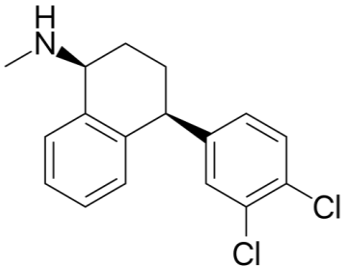


Fig 2: Structure of Sertraline

MATERIALS AND METHOD:

Spectrophotometric measurements were performed on Shimadzu UV –visible double beam spectrophotometer (Model-1800). All weighing were done on electronic analytical balance (Wensar Dab220).

Chemicals and Reagents

The bulk drug, Olanzapine was obtained from Chemdyes Corporation, Rajkot and Sertraline Hydrochloride was obtaining from Chemdyes Corporation, Rajkot. Fixed dose of synthetic mixture of Olanzapine 1 mg and Sertraline Hydrochloride 10 mg were prepared in laboratory scale as pilot batch. Analytical grade methanol was procured from Merck Fine chemicals (Mumbai).

Selection of a Solvent: Methanol was selected as solvent for studying spectral characteristic of drug.

Preparation of Standard Stock Solution

Accurately weighed 10 mg of Olanzapine and 10 mg of Sertraline hydrochloride standard were transferred to separate 100 ml volumetric flask and dissolved in 100 ml methanol. The flasks were shaken and volume was made up to the mark with methanol to give solution containing 100 µg/ml Olanzapine and 100 µg/ml Sertraline Hydrochloride.

Preparation of Working Standard Solution of Olanzapine and Sertraline Hydrochloride From above solution of Olanzapine pipetted out 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 ml of the stock solution was further diluted to 10 ml volumetric flasks individually with methanol to get concentrations 1, 2, 3, 4, 5, 6 µg/ml. From above solution of Sertraline Hydrochloride pipette out 1, 2, 3, 4, 5, 6,ml of the stock solution were further diluted to 10 ml volumetric flasks individually with methanol to get concentrations 10, 20, 30, 40, 50, 60 µg/ml.

Selection of Analytical Wavelength

Standard 1-6 µg/ml solutions of Olanzapine and 10-60 µg/ml solutions of Sertraline Hydrochloride were prepared in methanol by appropriate dilution and spectrum was recorded between 200-400 nm. All zero order spectrum (D0) were converted to first derivative spectrum (D1) using delta lambda 2.0 and scaling factor 10. The

overlain first derivative spectrums of Olanzapine and Sertraline Hydrochloride at different concentration were recorded. The zero crossing point (ZCP) of Olanzapine and Sertraline hydrochloride found to be 271.386 nm and 291.681nm.

Experimental work:

Instruments and apparatus: HPLC (Shimadzu), **Model:** LC-20 AD, **Column:** Peerless C18 (250 ×4.6 mm, 5µm), **Detector:** UV detector (SPD-20A) **Software:** Spinchrome, Hamilton syringe, Analytical weighing balance (Wensar DAB-220) Sonicator (Equitron), Digital pH Meter (Systronic) High vacuum pump (Parag engineering) Volumetric Flasks-10,50,100 ml (Borosilicate).

RP-HPLC Method:

Selection detection wavelength: The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. At 226 both drug give good peak height and shape. So, 226 nm was selected for simultaneous estimation of Olanzapine and Sertraline Hydrochloride in RP-HPLC method.

Mobile Phase Selection: The composition and flow rate of mobile phase were changed to optimize the separation condition using combined solution. After number of trial experiments, it was established that the mobile phase Acetonitrile: Phosphate buffer (pH 3.00 adjusted with 10% Ortho Phosphoric Acid) (70:30 %v/v) shows good peak shape and resolution.

Selection of Chromatographic Condition

- **Model:** LC-20 AD
- **Stationary phase:** C18
- **Mobile Phase:** Acetonitrile: Phosphate buffer (pH 3.00 adjusted with 10% Ortho Phosphoric Acid) (70:30 %v/v)
- **Detector:** UV detector (SPD-20A)
- **Flow rate:** 1 ml/min
- **Injection volume:** 20 µl
- **Temperature:** 30±2° C
- **Detection wavelength:** 226 nm

Preparation of 10% Ortho Phosphoric Acid: Take 1.33 ml of Ortho Phosphoric Acid in 10 ml of Volumetric Flask and make up the volume up to the mark with Water.

Preparation of Mobile phase

Preparation of Buffer (10mM KH_2PO_4) : Accurately weighed quantity of 1.36g of Potassium Di-Hydrogen Phosphate (KH_2PO_4) was transferred in 1000 ml water, dissolved in HPLC grade water and sonicated for 10 min and diluted with HPLC grade water. It was filtered through 0.45µm membrane filter. Buffer pH adjusted 3.00 using 10% Orthophospheric acid.

Preparation of Mobile phase: Mixtures of Acetonitrile and phosphate buffer in ratio of (70:30 % v/v) was mixed properly & adjust the pH 3.00 with 10% Orthophospheric acid.

Preparation of Standard Stock Solution

Preparation of standard stock solution of Olanzapine (100µg/ml): Accurately weigh 10 mg of Olanzapine was transferred into a 100 ml volumetric flask and diluted with ACN.

Preparation of standard stock solution of Sertraline Hydrochloride (100µg/ml): Accurately weigh 10 mg of Sertraline Hydrochloride was transferred into a 100 ml volumetric flask and diluted with ACN.

Preparation of working standard Solution:

Aliquots of stock solution of Olanzapine (100µg/ml) 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 ml and Sertraline Hydrochloride (100µg/ml) 1, 2, 3, 4, 5, and 6 ml were pipette out in same five different 10 ml volumetric flasks and further diluted with mobile phase to obtain the concentration of 1,2, 3 ,4 ,5, 6 µg/ml for Olanzapine and 10, 20, 30, 40, 50, 60 µg/ml for Sertraline Hydrochloride. 20 µl of each solution were injected into HPLC system and analyzed. Calibration curve was obtained by plotting respective peak area Vs Concentration in µg/ml and regression equation was obtained.

Preparation of Synthetic Mixture of Olanzapine and Sertraline Hydrochloride [11]: The Synthetic Mixture of Olanzapine and Sertraline Hydrochloride was prepared in ratio of 5: 50 Common excipients, Microcrystalline Cellulose, Lactose, Magnesium Stearate, Talc along with the drug Olanzapine 5 mg and Sertraline Hydrochloride 50 mg. Accurately weighed equivalently weight of Olanzapine (5 mg) and Sertraline Hydrochloride (50 mg) which transferred in 100 ml volumetric flask and make up half mark with Methanol. This solution was Sonicated till the drug dissolves and was made up to mark with Methanol. Then this solution was filtered through Whatmann filter paper. So, obtained concentration of Olanzapine is 50 µg/ml and Sertraline Hydrochloride is 500 µg/ml.

Preparation of Working Sample Solution:

Accurately 0.4 ml of the above solutions was pipette out into 10 ml volumetric flask and the volume was adjusted up to the mark with Methanol to make final concentration Olanzapine was 2 µg/ml and Sertraline Hydrochloride 20 µg/ml respectively.

Method Validation:

Method validation was performed following ICH guidelines. The proposed method has been extensively validated in terms of linearity, accuracy and precision, limit of detection and limit of quantification.

Linearity (Calibration curve)

Appropriate volume of aliquot from Olanzapine and Sertraline Hydrochloride standard stock solution was transferred to 10 ml volumetric flask. The volume was made up to the mark with methanol to give solution containing 1-6 µg/ml Olanzapine and 10-60 µg/ml Sertraline Hydrochloride. All D1spectrums were recorded using above spectrophotometric condition. D1 absorbance at 271.386 nm and 291.681 nm were recorded for Olanzapine and Sertraline Hydrochloride, respectively (n=6). Calibration curve were constructed by plotting average absorbance versus concentrations for both drugs. Straight line equations were obtained from these calibration curves and in RP-HPLC solution containing 1-6 µg/ml and 10 - 60 µg/ml Olanzapine and Sertraline Hydrochloride respectively(n=6). Calibration curve were constructed by plotting average area Vs concentration for both drugs.

Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the pre quantified sample preparation at three different concentration levels 50 %, 100 % and 150 %, taking in to consideration percentage purity of added drug sample. The amounts of Olanzapine and Sertraline Hydrochloride were estimated by applying obtained values to the respective regression line equations. Each concentration was analyzed 3 times and average recovery was measured.

Precision:

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the method was verified as repeatability, intra-day, inter- day and reproducibility. The repeatability was evaluated by assaying 6 times of sample solution of 2 µg/ml Olanzapine and 20 µg/ml Sertraline Hydrochloride prepared for assay determination without changing the parameter. The intra-day and inter-day precision study of Olanzapine and Sertraline Hydrochloride was carried out by estimating different concentration of Olanzapine (1, 2, 3 µg/ml) and Sertraline Hydrochloride (10, 20, 30 µg/ml), 3 times on same day and on 3 different day (first, second and third). The reproducibility was carried out by estimating Olanzapine and Sertraline Hydrochloride on changing of instrument and analyst. (Standardization of methodology) and the results are reported in terms of % RSD.

S.no	Parameters	Olanzapine	Sertraline Hydrochloride
1	Wavelength (nm)	271.368 nm	291.681 nm
2	Beer's Law Limit (µg/ml)	1-6	10-60
3	Regression Equation (y=mx+ c)	y = -0.0344x – 0.0122	y = 0.0046x - 0.0183
4	Correlation Coefficient (R^2)	0.9961	0.9975
5	Intraday Precision(% RSD, n=3)	0.50-0.64	0.82- 1.07
6	Interday (% RSD, n=3)	0.61-0.95	0.95-1.47
7	Repeatability (% RSD, n=3)	0.83	0.71
8	LOD (µg/ml)	0.059	0.272
9	LOQ (µg/ml)	0.180	0.826
10	% Recovery Study (n=3)	98 – 99.4%	98.6 – 99.8%

Table-1: Regression analysis data and summary of validation parameters for the proposed method of UV method. (First-Derivative UV Spectrophotometric)

Drug	% Level of Recovery	Test Amt. (µg/ml)	Amount of Drug Spiked (µg/ml)	Total Std Amt. (µg/ml)	Total Amt. Recovered (µg/ml)	% Recovery ±S.D(n=3)
Olanzapine	50	2	1	3	2.95	98.47 ± 0.1509
	100	2	2	4	3.95	98.75 ± 0.2419
	150	2	3	5	4.98	99.60 ± 0.269
Sertraline Hydrochloride	50	20	10	30	29.62	98.73 ± 0.110
	100	20	20	40	39.84	99.60 ± 0.149
	150	20	30	50	49.86	99.72 ± 0.209

Table 2: Recovery data of proposed method

S. no	System Suitability Parameter	Olanzapine	Sertraline Hydrochloride
1	Retention time(min)	2.230	4.487
2	Theoretical Plates	3163	4956
3	Tailing Factor	1.895	1.940
4	Resolution	10.914	

Table 3: System suitability parameters for Olanzapine and Sertraline Hydrochloride

S.no	Parameter	Olanzapine	Sertraline Hydrochloride
1	Beer's Law Limit (µg/ml)	1 – 6	10 – 60
2	Regression equation y=mx+c)	y= 22073x + 43210	y= 12766x – 99863
3	Correlation Coefficient (r^2)	0.996	0.995
4	Repeatability (%RSD, n=6)	0.786	0.802
5	Intraday Precision(%RSD,n=3)	0.50 – 0.75	0.59 – 0.97
6	Interday Precision(%RSD,n=3)	0.57 – 0.83	0.66 – 1.03
7	Accuracy (%Recovery, n=3)	98 – 99.4%	98.6 – 99.8%
8	LOD (µg/ml)	0.112	0.132
9	LOQ (µg/ml)	0.341	0.401
10	%Assay	98.2 %	99.7%

Table 4: Regression analysis data and summary of validation parameters for the proposed method of RP-HPLC method.

Name of Drug	% Level of Recovery	Test Amt. (µg/ml)	Amount of Drug Spiked (µg/ml)	Total Std Amt. (µg/ml)	Total Amt. Recovered (µg/ml)	% Recovery ±S.D(n=3)
Olanzapine	50	2	1	3	2.94	98 ±0.1509
	100	2	2	4	3.95	98.75 ± 0.2419
	150	2	3	5	4.98	99.60 ± 0.2696
	50	20	10	30	29.62	98.73 ± 0.1105
Sertraline Hydrochloride	100	20	20	40	39.84	99.60 ± 0.1493
	150	20	30	50	49.86	99.72 ± 0.2098

Table 5: Accuracy study data

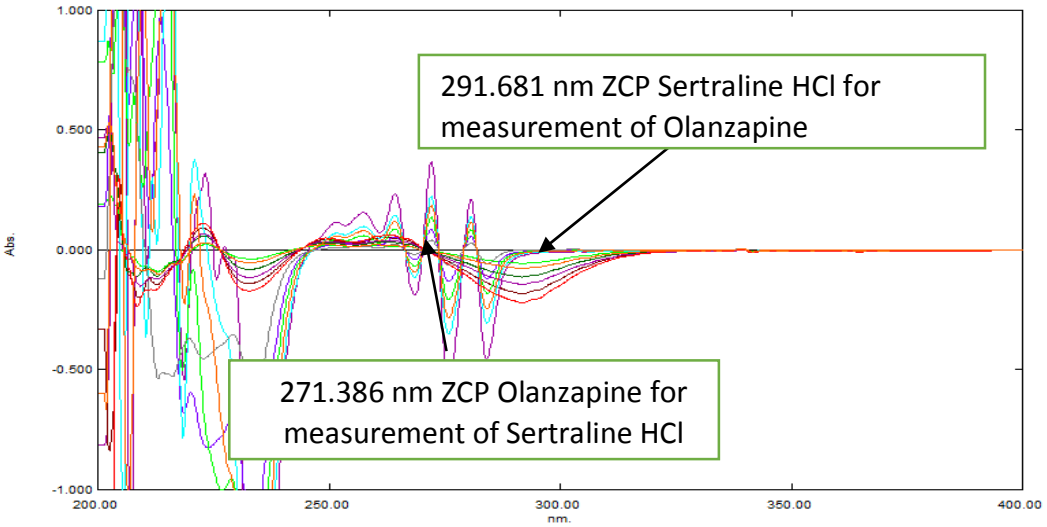
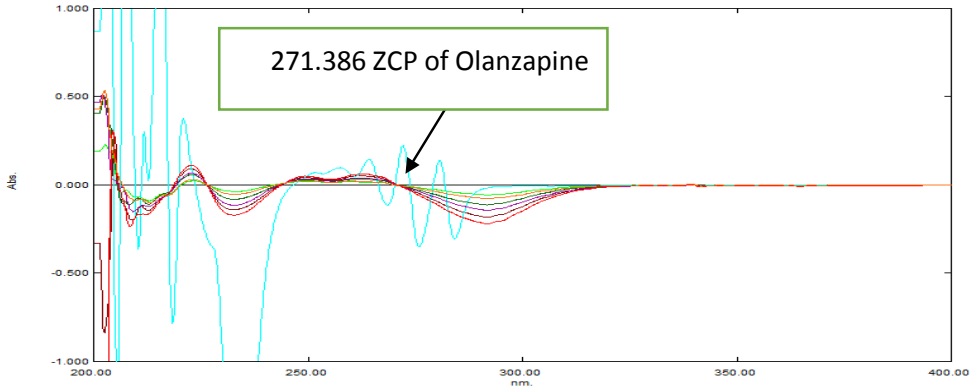


Fig. 3: Overlain D1 spectrum of Olanzapine (1-6 µg/ml) and Sertraline HCl (10-60 µg/ml) in Methanol

Fig. 4: D1 spectrum of Olanzapine (1-6 µg/ml) in Methanol.



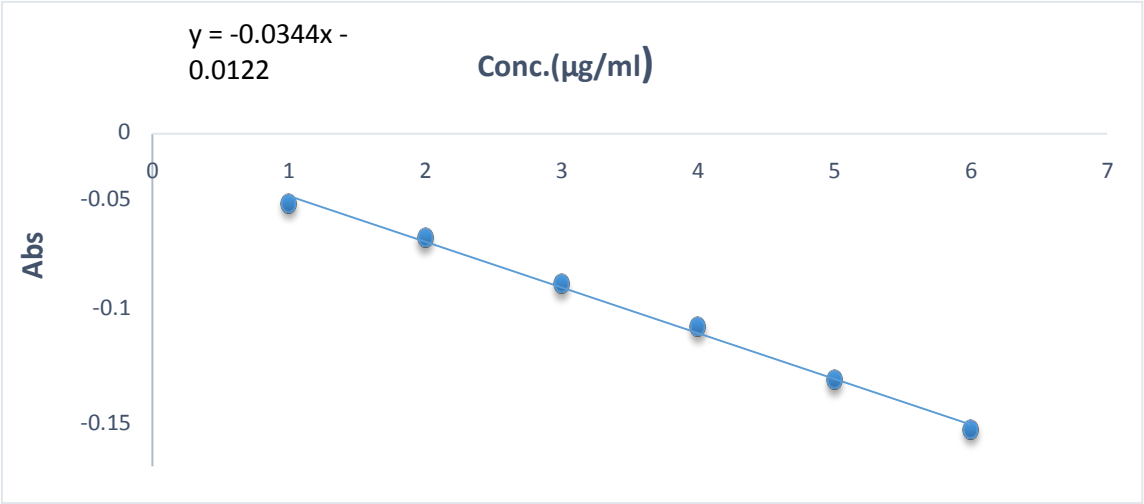


Fig. 5: Calibration curve of Olanzapine At 291.681nm in methanol

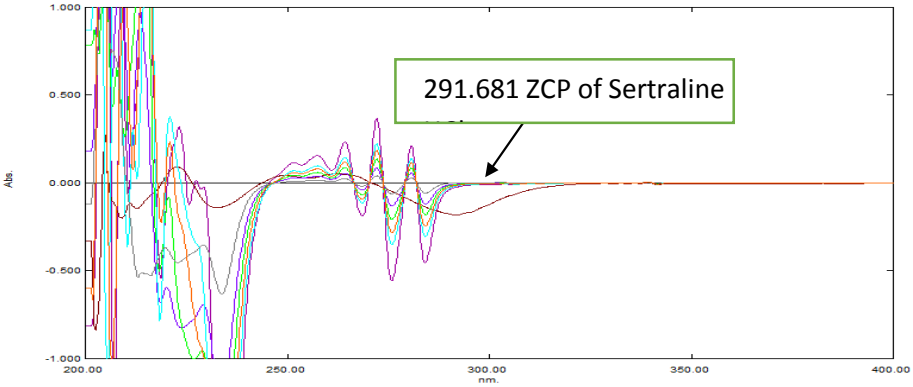


Fig.6: Overlain D1 spectrum of Sertraline HCl (10-60 µg/ml) in Methanol.

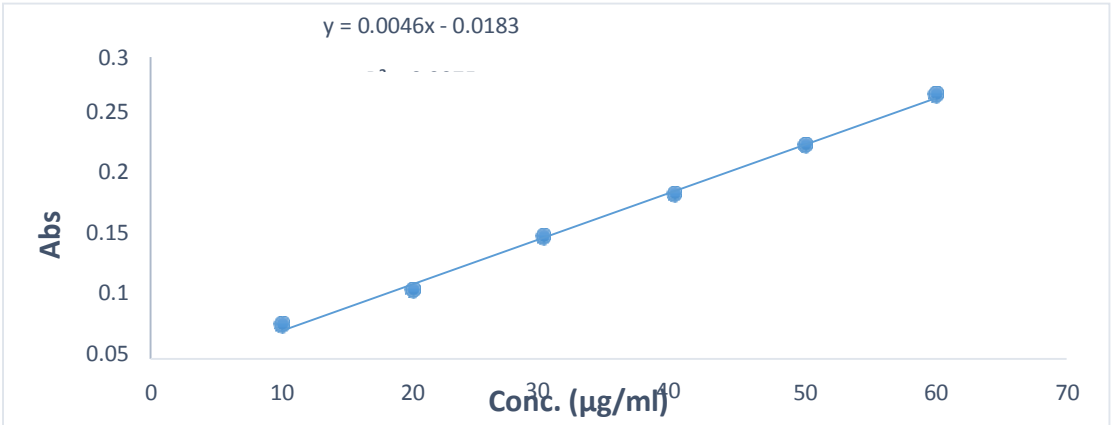


Fig. 7: Calibration curve of Sertraline HCl at 271.386 nm in Methanol.

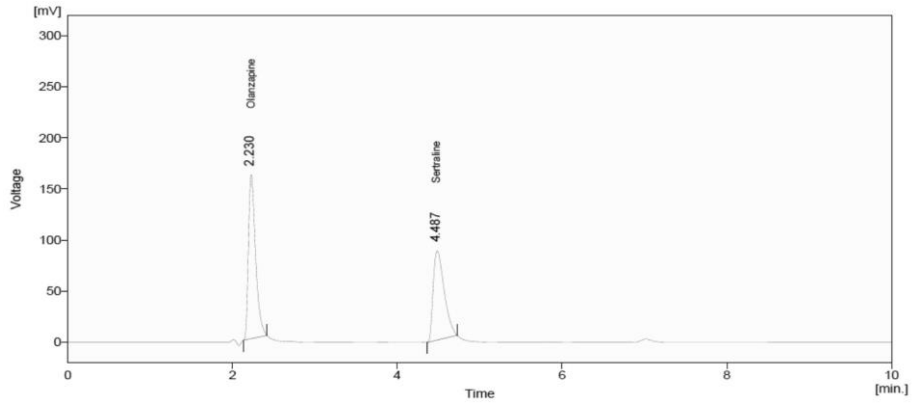


Fig. 8: HPLC chromatograph of Olanzapine (2µg/ml) and Sertraline Hydrochloride (20µg/ml) in ACN: Buffer (pH 3.00 adjust with ortho phosphoric acid) (70:30%v/v)

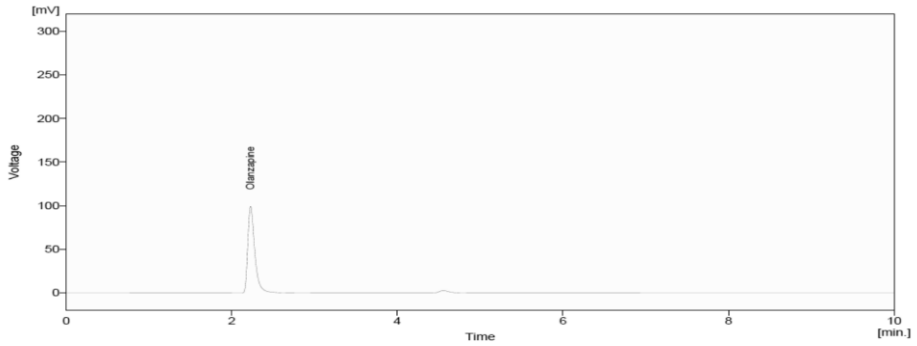


Fig. 9: HPLC chromatogram of Olanzapine (2µg/ml)

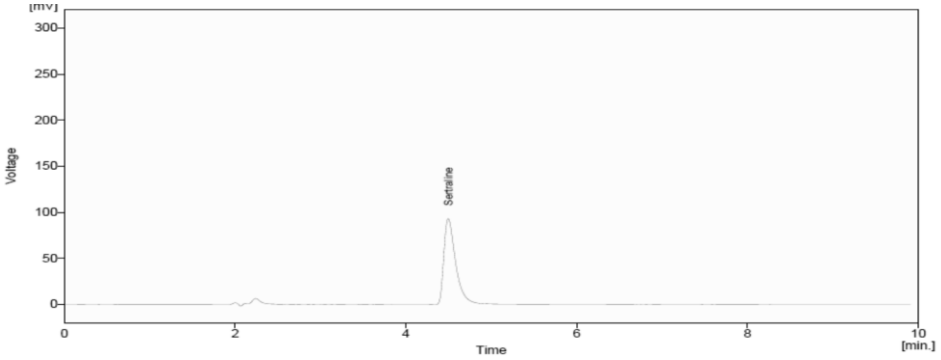


Fig.10: HPLC chromatogram of Sertraline Hydrochloride (20 µg/ml)

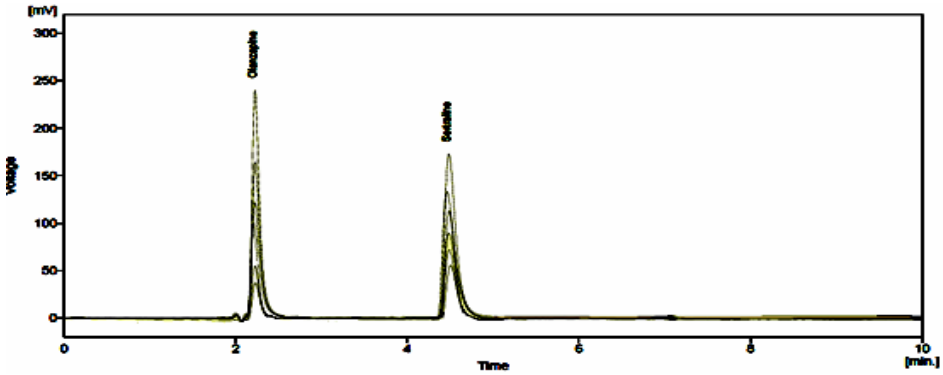


Fig. 11: Overlain chromatogram of Olanzapine and Sertraline Hydrochloride

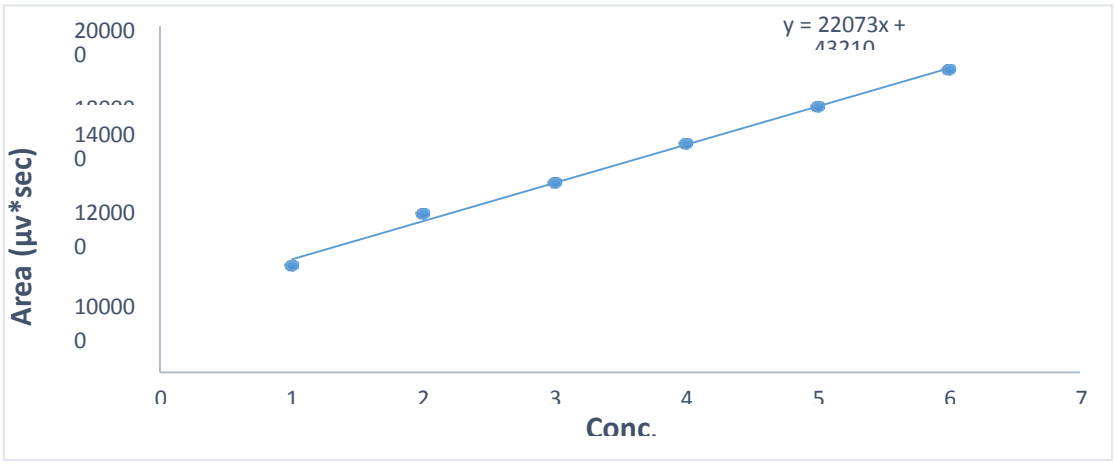


Fig 12: Calibration curve of Olanzapine ((1-6 µg/ml)

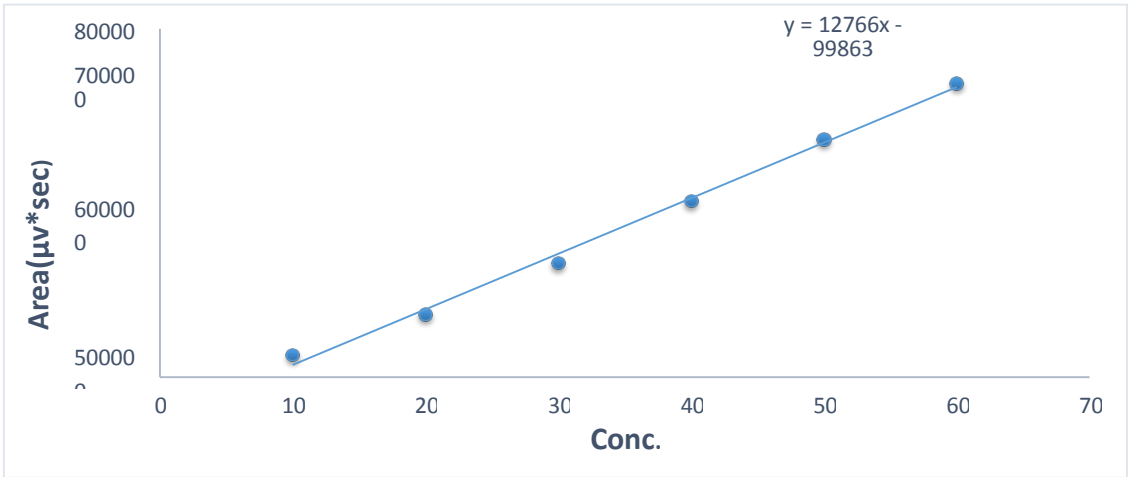


Fig 13: Calibration curve of Sertraline HCl ((10-60 µg/ml)

Limit of Detection (LOD) and Limit of Quantification (LOQ)

ICH guideline describes several approaches to determine the detection and quantification limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the 3.3 × (SD/Slope) and 10 × (SD/Slope) criteria, respectively; where SD is the standard deviation of y-intercept of regression line and S is the slop of the calibration curve.

RESULT AND DISCUSSION

U.V Spectrophotometric method

Reliable first order derivative spectrophotometric method was developed for simultaneous estimation of Olanzapine and Sertraline Hydrochloride in synthetic mixture by UV Spectrophotometry. Beers law was obeyed in concentration range of 1-6 µg/ml for Olanzapine and 10-60 µg/ml for Sertraline Hydrochloride at 291.681nm and 271.386 nm wavelengths, respectively. The correlation coefficient Olanzapine and Sertraline Hydrochloride was found to be R2= 0.9961 and 0.9975.

The mean % recoveries were found to be in the range of 98%-99.4 % and 98.6%-99%, respectively. The LOD and LOQ were 0.074µg/ml and 0.225 µg/ml of Olanzapine and 0.272 µg/ml and 0.826µg/ml of Sertraline Hydrochloride, respectively. The proposed method was precise, accurate and. reproducible and acceptable recovery of the analyses, which can be applied for the analysis of Olanzapine and Sertraline Hydrochloride in synthetic mixture.

RP-HPLC Method:

A Reverse Phase Column proposed as a suitable method for the determination of Olanzapine and Sertraline Hydrochloride in synthetic mixture. The Chromatographic condition was optimized by changing the Mobile Phase Composition. Different ratios were experimented to optimize the Mobile Phase. Finally, Acetonitrile: Phosphate buffer, pH=3.00 (70:30 % v/v) (pH 3.00 adjusted with Orthophospheric acid). The Flow Rate was 1.0 ml/min and effluents were monitored at 226 nm.

The Retention Time of Olanzapine and Sertraline Hydrochloride were found to be 2.230 min and 4.487 min respectively. Mobile Phase which shows good resolution of Olanzapine and Sertraline Hydrochloride peak. The wavelength of detection selected was 226 nm, as the drug shows optimized absorbance at this wavelength .The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate. It can thereby easily adopt for routine quality control analysis. Hence the proposed method can be successfully applied in estimation of Olanzapine and Sertraline Hydrochloride in synthetic mixture.

CONCLUSION:

The results of present study indicate that the proposed UV spectroscopic and RP-HPLC method is simple, rapid, and precise and accurate. Statistical analysis proves that the method is repeatable and selective for the analysis of Olanzapine and Sertraline Hydrochloride in Synthetic mixture. It can therefore be concluded that the developed analytical method is precise & accurate and can be used for routine Analysis of both the drug in combination.

Acknowledgement:

We are heartly thankful to Dr. K. Pundarikakshudu, Director of L.J Institute of Pharmacy, Ahmedabad for providing us all facilities and the valuable Guidance during our Research work.

REFERENCES:

1. D.A. Skoog, J.A.Hollar, and T.A.Nieman, Principle of Instrumental Analysis; 5th Edn; Thomson Asia Pte Limited, pp 300-328,725-744.
2. Drug bank, "Olanzapine (DB00334) Drug Profile", September2013, http://www.drugbank.ca/drugs/DB00334 (Last Assessed 12/9/2016)
3. Kishanta kumar pradhan*, sandhya kumari and rojalini samanta Development and validation of a stability indicating uv spectroscopic method for olanzapine in bulk and pharmaceutical dosage forms, Int J Pharm Pharm Sci, Vol 6, Issue 4, 67-72.
4. R.Chatwal, and S.Anand Instrumental method of Chemical Analysis; 5th Edn; Himalaya Publishing House, New Delhi, 2002, pp 2.149-2.184, 2.624-2.639
5. Mojhgan Jariani, Mandana Saaki, Hedayat Nazari & Mehdi Birjandi, "the effect of olanzapine and sertraline on personality disorder in patients with methadone maintenance therapy" Psychiatric Danubina, 2010; Vol. 22, No. 4, pp. 544–547
6. H.H.Willard, L.L. Merritt, et.al Instrumental Method of Analysis; 7th Edn; CBS Publisher & Distributors, New Delhi, pp 177- 178, 580,640.
7. ICH, Q2 (R1) Validation of Analysis Procedure: Text and Methodology International Conference on Harmonization, *IFPMA*, Geneva, Switzerland, 2005.
8. Indian Pharmacopeia; Government of India Ministry of Health and Family Welfare Published by Indian Pharmacopeia Commission, Ghaziabad, 2014, Vol-III, pp 2370- 2371, 2371-2372, (Vol-II 527).
9. The Olanzapine Tablets Revision Bulletin Supersedes the Currently official Olanzapine Tablets monograph. The Revision Bulletin will be incorporated in the Second supplement to USP 39-NF 34.
10. Indian Pharmacopeia; Government of India Ministry of Health and Family Welfare Published by Indian Pharmacopeia Commission, Ghaziabad, 2014, *vol*-III, pp 2722-2724, 2720-2722, (Vol-II ,579)
11. International Foundation for Research and Education on Depression http://www.ifred.org/types-of-depression.
12. Symptoms of Depression http://www.webmd.com/depression/guide/detecting-depression, Pubchem, national chemistry database https://pubchem.ncbi.nlm.nih.gov/compound/olanzapine.
13. Drug bank, "Olanzapine (DB00334) Drug Profile", September2013, http://www.drugbank.ca/drugs/DB00334 (Last Assessed 12/9/2016)