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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ALPRAZOLAM AND SERTRALINE HYDROCHLORIDE BY HPLC

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ABSTRACT

A simple, rapid, sensitive RP-HPLC method for the simultaneous determination of Alprazolam and Sertraline Hydrochloride in pharmaceutical dosage forms was developed the analyte were resolved using ACN:0.05M Phosphate Buffer (55:45) Ph 7.2 with TEA (Triethanolamine) at a flow rate of 1.5 ml/min, on HPLC auto sampler system containing UV- visible detector with Empower software and C18 JNJ Analytical (4.6×25×5µ) Detector SPD-20AT was used for the estimation the detection wavelength was taken as 264 nm. Linearity for detector response was observed in the concentration range of 5-15 mcg/ml and for Sertraline Hcl 250-750 mcg/ml. Correlation coefficient (r) for calibration curve Sertraline Hydrochloride and Alprazolam was found to be 0.999 and 0.998 respectively. Retention times for Alprazolam and Sertraline Hydrochloride were found to be 3.7 min and 4.8 min respectively. Percentage recovery for Alprazolam and Sertraline Hydrochloride was 98.70 – 99.60 %, and 99.84 -99.93 % respectively. The percent RSD for the analyzed tablet and recovery studied was less than 2. The results of recovery studies were found to be linear in the range 50% to 150% of test concentration. Results of the analysis were validated statistically and by recovery studies. The developed method was found to be precise, selective and rapid for the simultaneous determination of Alprazolam and Sertraline Hydrochloride in bulk and in pharmaceutical dosage form.

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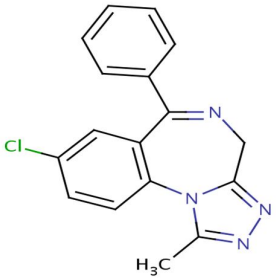
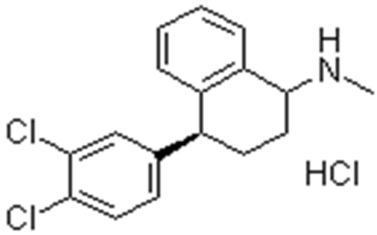
INTRODUCTION

Alprazolam: [1-8] Chemically 8-chloro-1-methyl-6-phenyl-4*H*-s-triazolo [4,3-α] [1,4] benzodiazepine $C_{17}H_{13}ClN_4$ 308.765 gm/mole white to off white crystalline powder Soluble in methanol or ethanol, Sparingly soluble in acetone and chloroform, slightly soluble in ethyl acetate, No appreciable solubility in water Partition Coefficient (Log P) Dissociation



Constant (Pka) belonging to Anti-anxiety Agents, Hypnotics and Sedatives, Benzodiazepines, GABA Modulators.

Sertraline Hydrochloride: [9-13] Chemically (1S-cis)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-N-methyl1-naphthalenamine hydrochloride Molecular Weight, 342.7 gm/mole belonging to Antidepressants, Selective Serotonin Reuptake Inhibitors (SSRIs), Antidepressive Agents, Serotonin Uptake Inhibitors white crystalline powder Solubility Slightly soluble in water and isopropyl alcohol and sparingly soluble in ethanol. Soluble in methanol Partition Coefficient (Log P) is 5.1Dissociation Constant (Pka) is 9.48 It belogs to Antidepressants, Selective Serotonin Reuptake Inhibitors (SSRIs), Antidepressive Agents, Serotonin Uptake Inhibitors category.



Literature survey reveals that several methods were reported for estimation of like HPLC [14-17], RPHPLC [18,19]. Derivative spectrophotmetry [20, 21],

EXPERIMENTAL WORK

INSTRUMENTATION:- HPLC Analytical Technologies Limited Detector SPD-20AT was used The column was C18 JNJ Analytical (4.6×25×5µ).The pH Meter, pH Tutor, Cyber Scan, Balance Sartorious & Mettler Toledo UV-Visible Spectrophotometer, Varian Single beam were other equipments used during the research work.

PREPARATION OF STANDARD SOLUTION

1. PREPARATION OF ALPRAZOLAM STANDARD SOLUTION:

Accurately weigh 25 mg of Alprazolam and transferred to 25 ml volumetric flask dilute with methanol up to 25 ml from this solution take 1.0 ml and transferred to 10 ml volumetric flask and dilute with methanol up to 10ml. from this solution take 1.0 ml and transferred to 10 ml volumetric flask and dilute with mobile phase up to 10 ml. (10 ug/ml)

2. PREPARATION OF SERTRALINE HYDROCHLORIDE STANDARD SOLUTION:

Accurately weigh 50 mg of Sertraline Hydrochloride and transferred to 10 ml volumetric flask dilute with methanol up to 10 ml from this solution take 1.0 ml and transferred to 10 ml volumetric flask and dilute with mobile phase up to 10 ml. (500 ug/ml)

3. PREPARATION OF STANDARD STOCK SOLUTION OF ALPRAZOLAM

Accurately weighed 25 mg Alprazolam was transferred to 25 ml volumetric flask and dissolved in methanol and take 1 ml from it and dilute with methanol up to 10 ml. This stock solution having strength 0.1 mg/ml (100 g/ml).

4. PREPARATION OF WORKING STANDARD SOLUTION OF ALPRAZOLAM

10 g•mL-1 of Alprazolam working standard solution was prepared by diluting 1.0 ml of stock solution to 10 ml with mobile phase.

5. PREPARATION OF STANDARD STOCK SOLUTION OF SERTRALINE HYDROCHLORIDE

Accurately weighed 50 mg Sertraline Hydrochloride was transferred into 10 ml volumetric flask and dissolved in mobile phase and diluted up to the mark with methanol to give a stock solution having strength 5.0 mg•mL-1 (5000 g•mL-1).

6. PREPARATION OF WORKING STANDARD SOLUTION OF SERTRALINE HYDROCHLORIDE

500 g•mL-1 of Sertraline Hydrochloride working standard solution was prepared by diluting 1 ml of stock solution to 10 ml with mobile phase.

7. PREPARATION OF BINARY MIXTURES OF ALPRAZOLAM AND SERTRALINE HYDROCHLORIDE

Accurately took 1.0 ml from Alprazolam stock standard and 1.0 ml from Sertraline HCL stock standard were transferred to 10 ml volumetric flask. It was dissolved sufficient methanol and diluted up to mark with mobile phase to give concentration of 10 g•mL-1 of Alprazolam and 500 g•mL-1 of Sertraline Hydrochloride. The solution was diluted further with mobile phase to get the concentration range of 5, 7.5, 10, 12.5, 15 g•mL-1 of Alprazolam and 250, 375, 500, 625, 750 g•mL-1 of Sertraline Hydrochloride.

RESULTS AND DISCUSSION

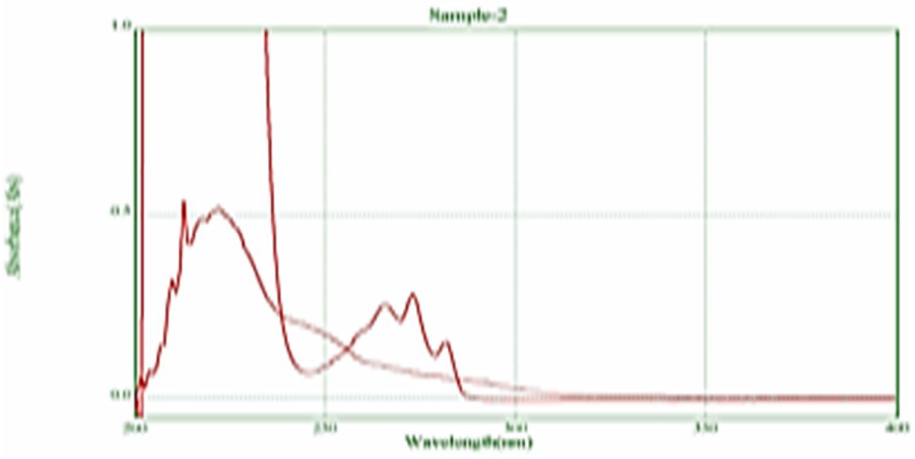
RP-HPLC METHOD DEVELOPMENT AND OPTIMIZATION:

SELECTION OF WAVELENGTH: The standard solution of Alprazolam (10 µg/ml) and Sertraline HCL (500 µg/ml) in methanol was scanned over the range of 200 nm to 400 nm wavelengths.

Table 1 Obtained λ_{max} of Alprazolam and SertralineHCL

Drug	Obtained λ_{max}
Alprazolam	230 nm
Sertraline HCL	272 nm

Figure 1.0:UV- spectra of Alprazolam and Sertraline HCL



RESULT: The isoabsorptive point was get at 254 nm.

SELECTION OF MOBILE PHASE:

The mobile phase was selected on the basis of best separation, peak purity index, peak Coreshell, theoretical plate etc. So, numbers of trial were taken. After numbers of trial Acetonitrile: 0.05M Phosphate Buffer (55:45) was selected.

CHROMATOGRAPY:

Optimization of the mobile phase was done on the trial and error base. The mobile phase Acetonitrile: 0.05M Phosphate Buffer (pH-7.2 with TEA (Triethanolamine) (55:45 v/v) was selected because it was found to resolve the peaks with retention time (RT) 3.7 min and 4.8 min for Alprazolam and Sertraline Hydrochloride respectively and the same is shown as chromatogram in figure

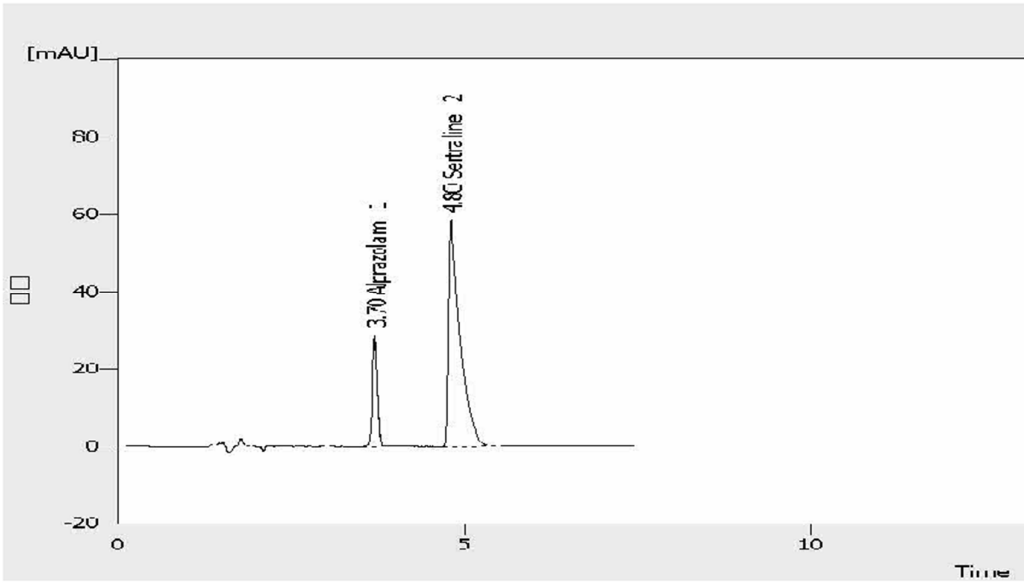


Figure 2.0: Chromatogram of standard at optimized condition

Table 2: Chromatographic Condition

1	Column	C18 JNJ Analytical (4.6×25×5µ)
2	Detector	SPD-20AT
3	Mobile phase	ACN:0.05M Phosphate Buffer (55:45) Ph 7.2 with TEA(Triethanolamine)
4	Flow rate	1.5 ml/min
5	Temperature	Ambient (room temperature)
6	Wavelength	264 nm
7	Injection Volume	20 l

VALIDATION OF RP-HPLC METHOD: LINEARITY AND RANGE

Linearity is expressed in terms of correlation co-efficient of linear regression analysis. The linearity response was determined by analyzing 5 independent levels of calibration curve. Aliquots of combined standard solution of Alprazolam and Sertraline HCL were taken to prepare in the range of 5.0-15 g/ml and 250-750 g/ml respectively. The graph of area obtained verses respective concentration was plotted. The mean area was calculated. Correlation co-efficient for calibration curve of Alprazolam and Sertraline HCL was found to be 0.999 and 0.998 respectively.

The regression line equation for Alprazolam is as follows: y = 994.3x + 123.4

The regression line equation for Sertraline Hydrochloride is as follows: y = 92.68x + 1046

Where, y= Peak area

x= Concentration of Alprazolam or Sertraline HCL (µg/ml)

Table 3: Linearity data for Alprazolam and Sertraline Hydrochloride

Alprazolam			**Sertraline Hydrochloride**		
Concentration (µg/ml)	**Area Mean ± SD**	**% RSD**	**Concentration (µg/ml)**	**Area Mean ± SD**	**% RSD**
5.0	5010.15±6.31	0.13	250	24103.8±5.80	0.23
7.5	7787.60±3.87	0.50	375	35984.32±8.00	0.22
10.0	9962.43±4.29	0.54	500	46230.99±7.20	0.15
12.5	12585.21±5.07	0.42	625	60205.75±7.35	0.12
15.0	15022.78±4.11	0.27	750	69848.48±56.62	0.80

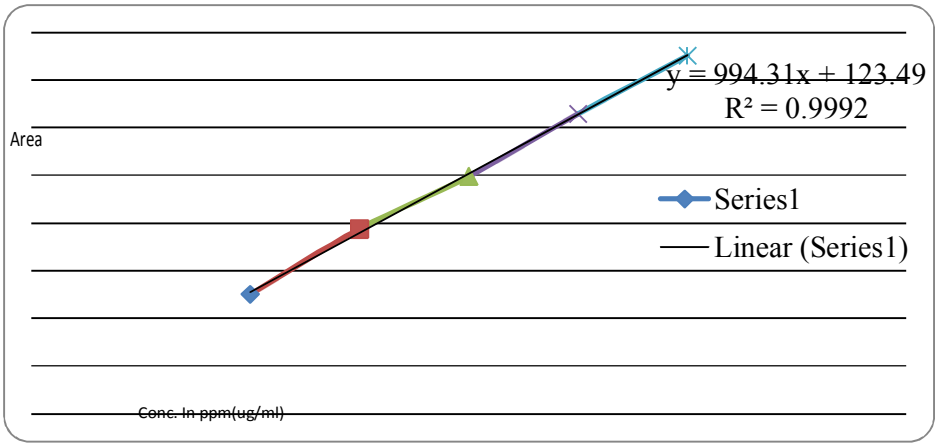


Figure 3.0: Calibration curve of Alprazolam

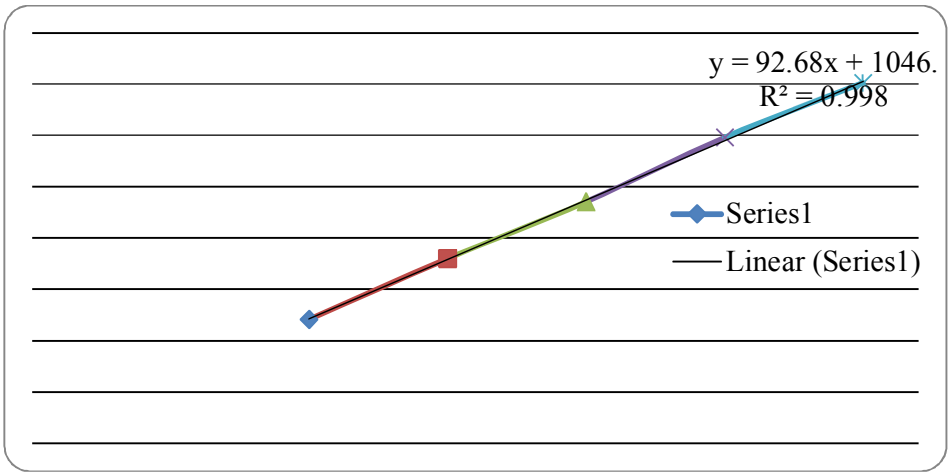


Figure 4.0: Calibration Curve of Sertraline Hydrochloride

Table 4: Linear regression data for calibration curves

Parameters (Units)	Alprazolam	Sertraline HCL
Linearity range (µg/ml)	5-15	250-750
Correlation Co-efficient r^2	0.999	0.998
Slope	994.3	92.68

Result: It can be seen that plot is linear over the concentration range 5-15 µg/ml of Alprazolam and 250-750µg/ml of Sertraline HCL with a correlation coefficient 0.999 for Alprazolam and 0.998 for Sertraline HCL.

ACCURACY:

Accuracy of the method was confirmed by recovery study from marketed formulation at three levels (50%, 100% and 150%) of standard addition. The results are shown in table 6.6 Percentage recovery for Alprazolam was 98.70 – 99.60 %, while 99.84 – 99.93 % for Sertraline Hydrochloride.

Table 5: Accuracy data for Alprazolam and Sertraline Hydrochloride

Drug	Accuracy level	Amt. taken (µg/ml)	Amt. added (µg/ml)	Total Amt. found (µg/ml)	Recovered Conc.(µg/ml)	Mean % Recovery±SD
Alprazolam	50%	10	5	14.89	4.98	99.60±48.86
	100%	10	10	19.69	9.87	98.70±60.30
	150%	10	15	24.94	14.88	99.20±62.75
Sertraline HCL	50%	500	250	749.56	249.62	99.84±291.56
	100%	500	500	999.83	499.56	99.91±32.107
	150%	500	750	1249.78	749.45	99.93±533.08

RESULT: % recovery, mean% recovery and % RSD were calculated at each level and recorded in Table 6.6. The % recovery was within limit (98.0 – 102.0 %) so the method was accurate.

PRECISION

INTRADAY PRECISION:

Combined standard solution having Alprazolam (5, 10, 15µg/ml) and Sertraline HCL (250, 500, 750 µg/ml) were analyzed three times on the same day. The data for intraday precision for Alprazolam and Sertraline Hydrochloride is shown in table 6.7. The % RSD for Intraday precision was found to be 0.22-0.94 for Alprazolam and 0.14-1.04 for Sertraline HCL.

Table 6: Intraday precision data for estimation of Alprazolam and Sertraline HCL

Alprazolam			Sertraline Hydrochloride		
Concentration(µg/ml)	Area Mean ± SD	% RSD	Concentration (µg/ml)	Area Mean ± SD	% RSD
5	10038.83±22.34	0.22	250	46019.63±481.42	1.04
10	10051.62±7.80	0.75	500	45592.73 ± 99.45	0.22
15	10054.20±9.51	0.94	750	45644.35 ± 6.75	0.14

INTERDAY PRECISION:

Table 7: Interday precision data for estimation of Alprazolam & Sertraline HCL

Alprazolam			Sertraline Hydrochloride		
Concentration (µg/ml)	Area Mean ± SD	% RSD	Concentration (µg/ml)	Area Mean ± SD	% RSD
5	9876.972±18.40	0.19	250	45183.95±14.03	0.31
10	9864.327±9.99	0.10	500	45180.89±4.44	0.98
15	9882.993±6.42	0.65	750	45183.16±8.10	0.18

Combined standard solution of Alprazolam (5, 10, 15 µg/ml) and Sertraline HCL (250, 500, 750 µg/ml) were analyzed on three different days. The data for intraday precision for Alprazolam and Sertraline HCL is shown in table 6.8. The % RSD for Interday precision was found to be 0.10 – 0.65 for Alprazolam and 0.18-0.98 for SE

Result: the % RSD is 0.31 for Alprazolam and 0.49 for Sertraline HCL which indicate that the method was precise.

LIMIT OF DETECTION AND LIMIT OF QUANTITATION (LOD & LOQ)

Calibration curves of Alprazolam (5-15 g/ml) and Sertraline HCL (250-750 g/ml) were repeated five times and the standard deviation (SD) of the intercepts was calculated. LOD andLOQ were calculated as follows: -

LOD=3.3
*σ/S

LOQ=10*
σ /S

Where, σ = Standard deviation of intercepts

S = mean slope of calibration curve

Table 8: LOD and LOQ data of Alprazolam and Sertraline Hydrochloride

Parameter	Alprazolam (µg/ml)	Sertraline HCL (µg/ml)
Mean slope	994.3	92.68
SD of intercept	123.4	1046
LOD (µg/ml)	0.41	37.24
LOQ (µg/ml)	1.24	112.9

ROBUSTNESS

The robustness was studied by analyzing the samples of Alprazolam and Sertraline HCL by deliberate variation in the method parameters. The changes in the response of Alprazolam and Sertraline HCL were noted and compared with the original one. The robustness of the method was established by making deliberate minor variations in the following method parameters:

Table 9: Robustness data of Alprazolam and Sertraline Hydrochloride

Drug Name	Parameter	Variation	Area	Theoretical plate	Tailing factor	Mean Area ± SD	% RSD
Alprazolam	Flow rate	+0.2 ml	11598.35	11553	0.984	11596.85±2.13	0.18
		– 0.2ml	7907.85	5977	0.935	7909.38±2.16	0.27
	P^H	+ 0.2	10053.00	10921	0.983	10054.23±1.74	0.17
		– 0.2	10118.32	10921	1.000	10121.86±5.00	0.49
Sertraline HCL	Flow rate	+0.2 ml	53209.51	4039	1.200	53210.82±1.85	0.34
		– 0.2ml	36004.32	4398	1.450	36007.58±4.61	0.12
	P^H	+ 0.2	46656.03	4112	1.456	46658.72±3.80	0.81
		– 0.2	46538.28	4141	1.315	46542.58±6.07	0.13

RESULT: The low % RSD value (< 2%) reveal that the proposed method was robust for this variation as shown in the Table 6.10.

SOLUTION STABILITY:

Standard and sample solution stability was evaluated at room temperature for 24 hrs. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution was stable up to 24 hrs at room temperature.

Solution stability data for standard and sample preparation showed in table 6.11 and 6.12

Table 10: Solution stability data for standard preparation

Time (hrs)	Area		% Assay	
	Alprazolam	Sertraline HCL	Alprazolam	Sertraline HCL
Initial	10157.736	46551.508	100	100
6	10142.368	46591.745	99.65	99.85
12	10162.482	46530.067	99.50	99.48
18	10150.486	46535.289	99.40	99.20
24	10162.256	46554.322	99.11	99.07

Table 11: Solution stability data for sample preparation

Time (hrs)	Area		% Difference	
	Alprazolam	Sertraline HCL	Alprazolam	Sertraline HCL
Initial	9916.198	45864.146	100	100
6	9921.271	45872.201	99.85	99.94
12	9928.738	45875.987	99.68	99.76
18	9916.376	45871.000	99.62	99.66
24	9930.059	45865.170	99.54	99.44

RESULT: Solution stability period for standard and sample preparation was determined and recorded in Table 6.11 and 6.12.

APPLICATION OF DEVELOPED METHOD TO PHARMACEUTICAL FORMULATION:

The proposed validated method was successfully applied to determination of Alprazolam and Sertraline HCL tablet dosage form.

PROCEDURE

Twenty tablets were weighted, crushed and finely powdered taken. Powder equivalent to 25 mg of Alprazolam and 50 mg of Sertraline Hydrochloride was weighed and transferred into 250 ml of volumetric flask. 30 ml of methanol was added and sonicated for 15 min. The solution was diluted up to mark with methanol, filtered using Whatman filter paper no.42 and first few drops of filtrate were discarded. (100 g/ml of Alprazolam and 5000 g/ml of Sertraline Hydrochloride). Aliquot of 1.0 ml of this solution was transferred into 10 ml volumetric flask and diluted up to mark with mobile phase to obtained strength of 10 g/ml of Alprazolam and 500 g/ml of Sertraline Hydrochloride

Table 12: Analysis of Marketed formulation

Tablet	Name of Drug	mg/5 ml	Amount found (mg)	% Assay
ALPRAX-PLUS	Alprazolam	0.5 mg	0.509 mg	101.86%
	Sertraline HCL	25 mg	25.142 mg	100.57%

Result of System Suitability & Validation parameters

Table 13: Result of System Suitability & Validation parameters

Parameters		Data obtained	
		Alprazolam	Sertraline HCL
Theoretical plate		10921	4112
Asymmetry factor		0.983	1.45
Retention time (min)		3.7	4.8
Linearity	Linearity Range	5-15 µg/ml	250-750 µg/ml
	Correlation Coefficient	0.999	0.998
Precision (%RSD)	Intraday	0.64%	0.47%
	Interday	0.31%	0.49%
Accuracy (% Recovery)		98.70-99.60 %	99.84 -99.93 %
LOD		0.41 µg/ml	37.24 µg/ml
LOQ		1.24 µg/ml	112.9 µg/ml
Robustness		Robust	Robust
% Assay		101.86%	100.57%

DISCUSSION:

Data reveals that the developed RP-HPLC method having LOD 0.41 g/ml for Alprazolam and 37.24 g/ml for Sertraline Hydrochloride, LOQ 1.24 g/ml for Alprazolam and 112.9 g/ml for Sertraline Hydrochloride. Linearity was observed in the range of 5-15 g/ml for Alprazolam and 250-750 g/ml for Sertraline Hydrochloride. Regression coefficient was 0.999 and 0.998 respectively for Alprazolam and Sertraline Hydrochloride. Precision was less than 2 for both the drugs. Percentage recovery was obtained in the limit of 98.70-99.60 % for Alprazolam and 99.84-99.93% for Sertraline Hydrochloride. Data showed in Table 6.14 that developed RP HPLC method had Retention time 3.7 min for Alprazolam and 4.8 min for Sertraline Hydrochloride. % Assay of marketed formulation was found to be 101.86% for Alprazolam and 100.57% for Sertraline Hydrochloride

CONCLUSION:

Developed RP-HPLC method was validated for its linearity, accuracy, precision and robustness. The developed method was found to be linear, accurate, precise and robust.

Recovery study suggests that the developed RP-HPLC method was used for estimation of Alprazolam and Sertraline HCL in tablet dosage form.

The developed RP-HPLC method was specific for determination of Alprazolam and Sertraline HCL in tablet dosage form without interference from excipients.

The developed RP-HPLC method was used for the estimation of Alprazolam and Sertraline HCL in tablet dosage form.

The method developed here could be useful for routine quality monitoring of Alprazolam and Sertraline HCL in tablet dosage form.

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