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Research Article

DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC AND RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ARIPIPRAZOLE AND CLOZAPINE IN SYNTHETIC MIXTURE

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ABSTRACT

The present Article portrays simple, sensitive, accurate, precise and cost effective First order derivative Spectrophotometric method and RP-HPLC method for the simultaneous estimation of Aripiprazole and Clozapine in Synthetic Mixture. In The first order derivative method absorption at 227.79 nm (zero crossing point for Clozapine) was used for Aripiprazole and 310.425 nm (zero crossing point for Aripiprazole) was used for Clozapine. The linearity was taken in the concentration range of 1- 5 µg/ml for Aripiprazole and 10-50 µg/ml for Clozapine with correlation coefficient (R2) 0.996 and 0.999, respectively. For The RP-HPLC method linearity was taken in the concentration range of 1- 5 µg/ml for Aripiprazole and 10-50 µg/ml for Clozapine with correlation coefficient (R2) 0.998 and 0.998, respectively. Proposed technique has been validated as per ICH guideline and successfully applied to the simultaneous estimation of Aripiprazole and Clozapine in their Synthetic Mixture. The results of analysis have been validated statistically and by recovery studies.

INTRODUCTION

Aripiprazole and Clozapine is an Atypical Antipsychotic drug. Chemically Aripiprazole is 7-{4-[4-(2, 3-dichlorophenyl) piperazin-1-yl] butoxyl}-1,2,3,4-tetrahydroquinolin-2-one. It is primarily used in the schizophrenia and bipolar disorder. Although it is used as an add-on treatment in major depressive disorder, tic disorders, and irritability associated with autism.

Aripiprazole have partial agonistic activity at D2 receptor, also have partial agonist activity at 5-HT1A receptor, and have antagonist activity at 5-HT2A receptor. Clozapine is chemically 8-chloro-11-(4-methylpiperazin-1-yl)-5H-dibenzo [b,e] [1,4]diazepine. It works by changing the activities of chemicals in the cerebrum.

It is utilized as a part of extreme schizophrenia, or to lessen the danger of self-destructive conduct in individuals with schizophrenia or comparative issue. It is also used in Parkinson's disease. Combination of Aripiprazole and Clozapine was studied under clinical trial phase and was proved that the synergistic effect was observed by improving psychotic symptoms and reducing side effects such as agranulocytosis, sedation, weight gain, sialorrhoea, and enuresis as compare to Clozapine monotherapy.

Also combination therapy leads to dose reduction of Clozapine. Although combination of Aripiprazole and Clozapine leads to improve in positive and negative symptoms. Also significant improvement in mean BPRS score (brief psychiatric rating scale) in combination therapy. There is higher metabolic risk in Clozapine monotherapy due to its strong blockade of 5HT2C and Histamine H1 receptors and stimulation of hypothalamic AMPK (adenosine monophosphate activated protein kinase), an enzyme that reverses the effect of leptin.

Unlike Clozapine, Aripiprazole has no histaminergic activity, and is a 5HT2C agonist. Moreover, it has some agonist activity at 5HT1A receptors, believed to lower blood glucose levels. Therefore, there is a mechanistic reasoning behind augmenting Clozapine with Aripiprazole- the effect of Aripiprazole on 5HT2C and 5HT1A receptors may in fact protect against the diabetes, weight gain and dyslipidaemia induced by Clozapine.

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MATERIAL AND METHODS

Method A

Instruments

UV Visible Spectrophotometer: A Shimadzu UV-visible double beam spectrophotometer model 1800 (Japan) with spectral width 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell.

Spectra were automatically obtained by UV probe system software (UV probe version 2.31) Digital analytical weighing balance: Wenser DAB-220
Sonicator: Equitron

Method B: RP-HPLC method

Chromatographic condition

- **Column:** Peerless C-18 (250×4.6 mm, 5 µm)
- Mobile phase: Phosphate buffer: ACN (pH 3.6 adjusts with 10% ortho phosphoric acid) (50:50 %v/v)
- Flow rate: 1ml/min
- Detection Wavelength: 219nm
- Run time: 10min
- Detector: UV detector
- Injection volume : 20µl

Chemicals and Materials

- Aripiprazole (Torrent Pharmaceuticals, Ahmedabad)
- Clozapine (Sun Pharmaceuticals, Vadodara)
- Methanol (Aventor Performance Material, India)

Synthetic mixture of Aripiprazole and Clozapine were prepared in the fixed dose of 15 mg Aripiprazole and 150 mg Clozapine respectively in laboratory scale as pilot batch.

Selection of a Solvent

Both The Drugs were soluble in Methanol. So, Methanol was selected as a solvent for estimation of both the Drugs.

Preparation of standard stock solution

Preparation of standard stock solution of Aripiprazole (1000µg\ml)

Weighed accurately 100 mg of Aripiprazole and was transferred into 100 ml volumetric flask, diluted to half and sonicated and made up to the mark with Methanol. (1000 µg/ml)

Preparation of working standard stock solution of Aripiprazole (100µg\ml)

Pipetted out 10 ml from the stock solution and transferred into 100 ml volumetric flask and diluted with Methanol to obtain 100µg/ml.

Preparation of standard stock solution of Clozapine (1000µg\ml)

Weighed accurately 100 mg of Aripiprazole and was transferred into 100 ml volumetric flask, diluted to half and sonicated and made up to the mark with Methanol. (1000 µg/ml)

Preparation of working standard stock solution of Clozapine (100µg\ml)

Pipetted out 10 ml from the stock solution and transferred into 100 ml volumetric flask and diluted with Methanol to obtain 100µg/ml.

The solutions were scanned in the range 200-400 nm and λmax found to be 256 nm for Aripiprazole and 294 nm for Clozapine which match standard λmax of Aripiprazole and Clozapine.

Procedure of selection of wavelength

0.2 ml working standard stock solution of Aripiprazole (100 µg/ml) and 2.0 ml working standard stock solution of Clozapine (100 µg/ml) was transferred into different 10 ml volumetric flask and dilute up to mark with Methanol to get 2 µg/ml of Aripiprazole and 20 µg/ml of Clozapine. Each solution was scanned in the range of 200-400 nm. Zero Order spectra were converted into First Order spectra. Aripiprazole shows ZCP (Zero Crossing Point) at 310.425 nm and Clozapine show ZCP at 227.799 nm. Hence, these wavelengths 227.799 and 310.425 were selected as analytical wavelengths.

Method Validation

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

Linearity (Calibration curve)

The linearity of Aripiprazole and Clozapine was found to be in the range of 1-5 µg/ml and 10-50 µg/ml, respectively. Linearity of both the drugs was checked in term of slope, intercept and correlation coefficient. All D1 spectrums were recorded using above spectrophotometric condition. D1 absorbance at 227.79 nm and 310.425 nm were recorded for Aripiprazole and Clozapine, respectively (n=6).Calibration curve were obtained by plotting average absorbance versus concentrations for both the drugs. Straight line equations were obtained from these calibration curves. The linear regression equation of Aripiprazole was y = -0.042x-0.024 (R2= 0.995) and Clozapine was y = -0.008x-0.004 (R2= 0.999).

Accuracy

Accuracy of the developed method was confirmed by doing recovery study 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 Aripiprazole and Clozapine were estimated by applying obtained values to the respective regression line equations. Each concentration was analyzed 3 times and average recoveries were 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 4µg/ml Aripiprazole and 10µg/ml Clozapine prepared for assay determination without changing the parameter. The intra-day and inter-day precision study of Aripiprazole and Clozapine was carried out by estimating different concentration of Aripiprazole (2, 4, 6µg/ml) and Clozapine (5, 10, 15µg/ml), 3 times on same day and on 3 different day (first, second and third).

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.

Chromatography

The composition and flow rate of mobile phase were changed to optimize the separation condition using combined solution. The pKa value for Aripiprazole and Clozapine is 7.46 and 3.70 respectively. After number of trial experiments, it was established that the mobile phase ACN: potassium dihydrogen Ortho phosphate buffer (pH 3.5 adjusts with Ortho phosphoric acid) (50:50) shows good peak shape and resolution.

System suitability parameters

The resolution, tailing factor and number of theoretical plates are shown in table. The values obtain confirmed the suitability of the system for the analysis of these drugs in combination.

RESULT AND DISCUSSION

A Simple, Precise and Accurate First Order Derivative Spectrophotometric Method have been developed for simultaneous estimation of Aripiprazole and Clozapine in Synthetic Mixture. Aripiprazole shows ZCP (Zero Crossing Point) at 310.425 nm and Clozapine show ZCP at 227.799 nm. At 227.799 (ZCP of Clozapine) Aripiprazole shows considerable absorbance while at 310.425 nm (ZCP of Aripiprazole) Clozapine shows considerable absorbance. Linearity Range of 1-5 µg/ml for Aripiprazole and 10-50 µg/ml for Clozapine with Correlation Coefficient of 0.996 and 0.999

Table 1 Linearity data of Aripiprazole at 227.79 nm

Aripiprazole		
Conc. (µg/ml)	**Mean Absorbance ± SD (n=6)**	**% RSD**
1	|-0.0965|±0.0015	1.554
2	|-0.2025|±0.0019	0.938
3	|-0.2745|±0.0021	0.765
4	|-0.3635|±0.0027	0.742
5	|-0.4405|±0.0030	0.681

Table 2 Linearity data of Clozapine at 310.425 nm

Clozapine		
Conc. (µg/ml)	**Mean Absorbance ± SD (n=6)**	**% RSD**
10	|-0.0428|±0.0007	1.635
20	|-0.0868|±0.0011	1.267
30	|-0.1245|±0.0013	1.044
40	|-0.1621|±0.0014	0.863
50	|-0.2008|±0.0017	0.846

for Aripiprazole and Clozapine respectively was obtained and the Precision data obtained with less than 2% RSD.

Table 3 Precision study of Aripiprazole at 227.79 nm

Intraday precision of Aripiprazole		
Conc. (µg/ml)	**Mean Absorbance ±SD (n=3)**	**% RSD**
1	|-0.0970| ± 0.0010	1.03
2	|-0.2016| ± 0.0020	0.99
3	|-0.2736| ±0.0025	0.91
Interday precision of Aripiprazole		
Conc. (µg/ml)	**Mean Absorbance ±SD (n=3)**	**% RSD**
1	|-0.0970| ± 0.0014	1.44
2	|-0.2026| ± 0.0025	1.23
3	|-0.2723| ± 0.0030	1.09
Repeatability of Aripiprazole		
Conc. (µg/ml)	**Mean Absorbance ± SD (n=6)**	**% RSD**
2	|-0.2025| ± 0.0019	0.93

Table 4 Precision study of Clozapine at 310.425 nm

Intraday precision of Clozapine		
Conc. (µg/ml)	**Mean Absorbance ±SD (n=3)**	**% RSD**
10	|-0.0406| ± 0.0005	1.23
20	|-0.087| ± 0.0010	1.14
30	|-0.1263| ± 0.0011	0.87
Interday precision of Clozapine		
Conc. (µg/ml)	**Mean Absorbance ±SD (n=3)**	**% RSD**
10	|-0.0407| ± 0.0006	1.47
20	|-0.0873| ± 0.0011	1.26
30	|-0.1266| ± 0.0015	1.18
Repeatability of Clozapine		
Conc. (µg/ml)	**Mean Absorbance ±SD (n=6)**	**% RSD**
20	|-0.0866| ± 0.0008	0.92

Accuracy was carried out by Recovery Studies and was obtained in the range of 99.42-99.81 for Aripiprazole and 99.28-99.44 for Clozapine. LOD and LOQ values were found to be 0.0589 and 0.178 µg/ml respectively for Aripiprazole and for Clozapine value were found to be 0.077 and 0.233 µg/ml respectively.

Table 5 LOD and LOQ data for Aripiprazole and Clozapine of first order derivative method

Parameter	**Aripiprazole**	**Clozapine**
LOD(µg/ml)	0.0589	0.077
LOQ(µg/ml)	0.178	0.233

Table 6 Recovery study

Name of Drug	**% Level of recovery**	**Amt Taken (µg/ml)**	**Amt Added (µg/ml)**	**Total Amt (µg/ml)**	**Amt Recovered (µg/ml)**	**% Mean Recove-ry ± S.D. (n=3)**
Aripiprazole	50	2	1	3	2.95	99.81± 0.3026
	100	2	2	4	3.94	99.42± 0.3704
	150	2	3	5	4.93	99.75± 0.3500
Clozapine	50	20	. 10	30	29.60	99.35± 0.3041
	100	20	20	40	39.40	99.44± 0.2857
	150	20	30	50	49.40	99.28± 0.2581

Table 7 Analysis of synthetic mixture

Name of Drug	**Amount taken (µg/ml)**	**Mean Amount found (µg/ml)**	**% Assay ± S.D.**	**% RSD**
Aripiprazole	2	1.965	99.55 ± 0.3288	0.3303
Clozapine	20	19.76	99.25 ± 0.1637	0.1649

A Simple, Precise and Accurate RP-HPLC Method have been developed for simultaneous estimation of Aripiprazole and Clozapine in Synthetic Mixture. Linearity Range of 1-5 µg/ml for Aripiprazole and 10-50 µg/ml for Clozapine with Correlation Coefficient of 0.998 and 0.998 for Aripiprazole and Clozapine respectively was obtained and the Precision data obtained with less than 2% RSD.

Table 8 Summary of validation parameters

Sr. No.	Parameters	Aripiprazole	Clozapine
1	Wavelength (nm)	227.79 nm	310.425 nm
2	Beer's Law Limit (µg/ml)	1-5	10-50
3	Regression equation (y = mx +c)	y = -0.084x-0.020	y = -0.003x-0.006
4	Correlation Coefficient (r^2)	0.996	0.999
5	Intraday Precision (%RSD, n=3)	0.91-1.03	0.87-1.23
6	Interday Precision (% RSD, n=3)	1.09-1.44	1.18-1.47
7	Repeatability (% RSD, n=6)	0.93	0.92
8	Accuracy (% Recovery, n=3)	99.42-99.81	99.28-99.44
9	LOD (µg/ml)	0.0589	0.077
10	LOQ (µg/ml)	0.178	0.233
11	%Assay	99.55	99.25

Table 9 System suitability parameter

Sr. No.	Parameters	Aripiprazole	Clozapine
1	Retention Time	6.947	4.720
2	Theoretical Plates	10892	6297
3	Tailing Factor	1.270	1.786
4	Area (µV.s)	254294	145207
5	Resolution	8.833	

Table 10 Calibration Data for Aripiprazole (1-5 µg/ml) and Clozapine (10-50 µg/ml)

Aripiprazole			Clozapine		
Conc. (µg/ml)	Mean Peak Area (µV.s) ± S.D. (n=6)	% RSD	Conc. (µg/ml)	Mean Peak Area (µV.s) ± S.D. (n=6)	% RSD
1	143367± 1165.31	0.8128	10	72747± 518.42	0.7126
2	254294± 1820.60	0.7159	20	145207± 929.37	0.6400
3	404665± 2706.23	0.6687	30	227506± 1327.01	0.5832
4	545805± 2886.55	0.5288	40	317165± 1640.18	0.5171
5	675942± 2932.96	0.4339	50	406793± 1827.73	0.4493

Table 11 Precision study of Aripiprazole

Aripiprazole		
Intraday precision		
Conc. (µg/ml)	**Mean Peak Area (µV.s) ± S.D. (n=3)**	**% RSD**
1	142507± 754.86	0.5297
2	252850± 1253.15	0.4956
3	402601± 1764.69	0.4383
Interday Precision		
Conc. (µg/ml)	**Mean Peak Area (µV.s) ± S.D. (n=3)**	**% RSD**
1	142574± 997.54	0.6996
2	253191± 1370.15	0.5414
3	403913± 1934.37	0.4789
Repeatability		
Conc. (µg/ml)	**Mean Peak Area (µV.s) ± S.D. (n=3)**	**% RSD**
2	254109± 1709.29	0.6726

Table 12 Precision study of Clozapine

Clozapine		
Intraday precision		
Conc. (µg/ml)	**Mean Peak Area (µV.s) ± S.D. (n=3)**	**% RSD**
10	72625± 363.86	0.5010
20	144727± 686.24	0.4741
30	227502± 966.98	0.4250
Interday Precision		
Conc. (µg/ml)	**Mean Peak Area (µV.s) ± S.D. (n=3)**	**% RSD**
10	72576± 498.50	0.6868
20	144540± 853.45	0.5904
30	227368± 1162.38	0.5112
Repeatability		
Conc. (µg/ml)	**Mean Peak Area (µV.s) ± S.D. (n=3)**	**% RSD**
20	145207± 929.37	0.6400

Table 13 Recovery Study Data

Name of Drug	% Level of recovery	Amt Taken (µg/ml)	Amt Added (µg/ml)	Total Amt (µg/ml)	Amt Recovered (µg/ml)	% Mean Recove-ry ± S.D. (n=3)
Aripiprazole	50	2	1	3	2.98	99.66± 0.2150
	100	2	2	4	3.97	99.70± 0.2335
	150	2	3	5	4.99	100.15± 0.2783
Clozapine	50	20	10	30	29.8	99.29± 0.2137
	100	20	20	40	39.9	99.69± 0.1743
	150	20	30	50	49.8	99.64± 0.3524

Table 14 LOD and LOQ Data

Parameter	Aripiprazole	Clozapine
LOD(µg/ml)	0.012	0.0046
LOQ(µg/ml)	0.037	0.015

Table 15 Analysis of synthetic mixture

Name of Drug	Amount taken (µg/ml)	Mean Amount found (µg/ml)	% Assay ± S.D.	% RSD
Aripiprazole	2	1.99	99.70 ± 0.2335	0.2342
Clozapine	20	19.76	100.11± 0.1955	0.1953

Table 16 Summary of validation parameters

Sr. No.	Parameters	Aripiprazole	Clozapine
1	Beer's Law Limit (µg/ml)	1-5	10-50
2	Regression equation (y = mx +c)	y = 13566x-2183	y = 8400x-18131
3	Correlation Coefficient (r^2)	0.998	0.998
4	Intraday Precision (%RSD, n=3)	0.4383-0.5297	0.4250-0.5010
5	Interday Precision (% RSD, n=3)	0.4789-0.6996	0.5112-0.6868
6	Repeatability (% RSD, n=6)	0.6726	0.6400
7	Accuracy (% Recovery, n=3)	99.66-100.15	99.29-99.69
8	LOD (µg/ml)	0.012	0.0046
9	LOQ (µg/ml)	0.037	0.015
10	%Assay	99.70	100.11

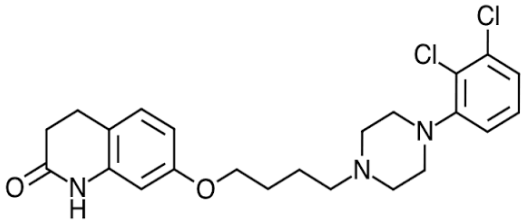

Figure 1 Structure of Aripiprazole

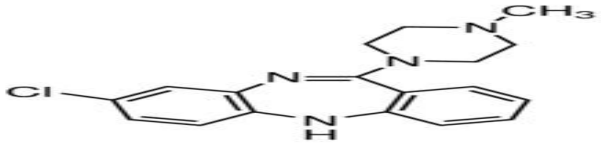

Figure 2 Structure of Clozapine (20µg/ml) in Methanol

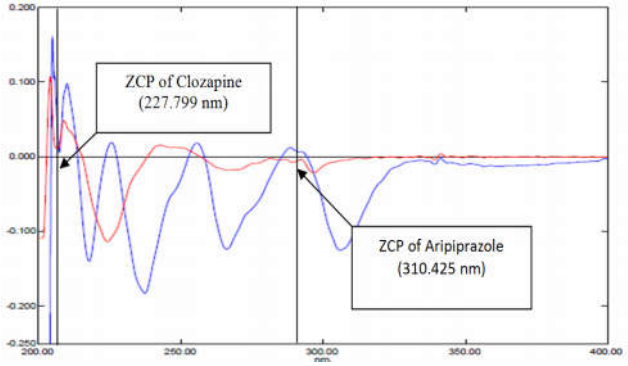

Figure 3 Overlain spectra of Aripiprazole (2µg/ml) and Clozapine (20 µg/ml) in methanol (First order)

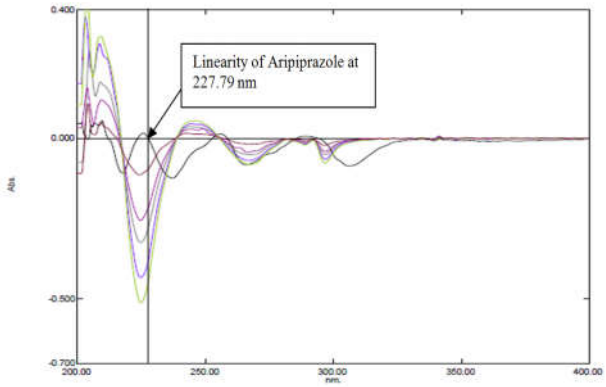

Figure 4 Linearity of 1st Derivative Spectra of Aripiprazole (227.79 nm)

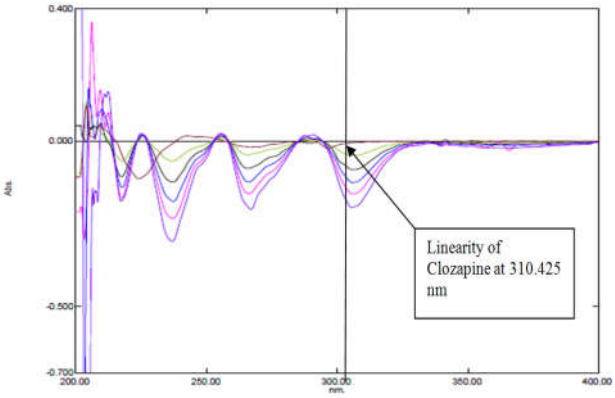

Figure 5 Linearity of 1st Derivative Spectra of Clozapine (310.425 nm)

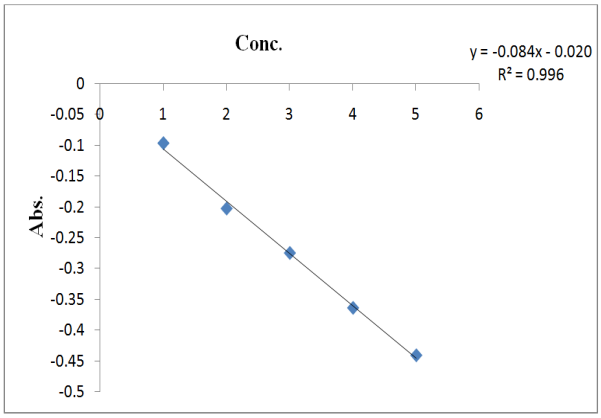

Figure 6 Calibration curve of Aripiprazole at 227.79 nm

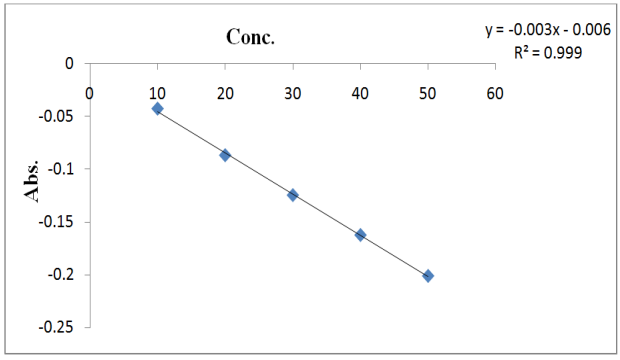

Figure 7 Calibration curve of Clozapine at 310.425 nm

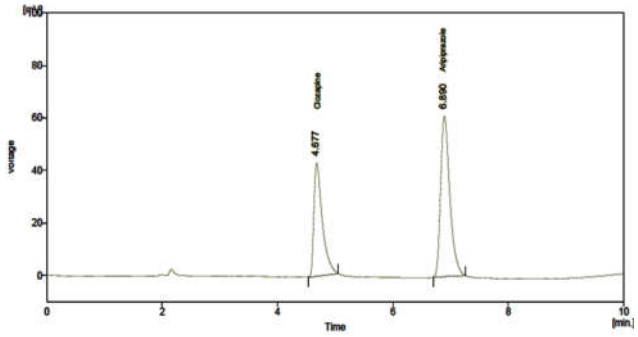

Figure 8 Chromatogram of Aripiprazole (2 µg/ml) and Clozapine (20 µg/ml) in ACN: potassium dihydrogen Ortho phosphate Buffer (pH 3.5) (50: 50)

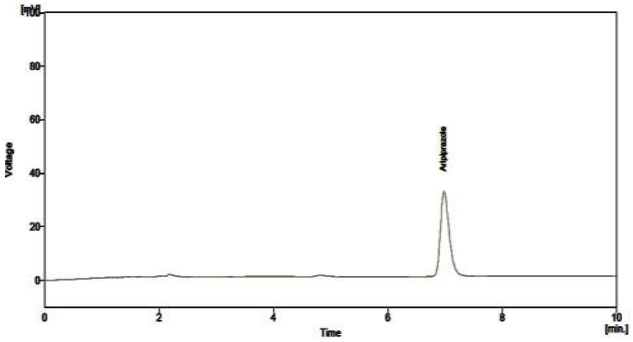

Figure 9 Chromatogram of Aripiprazole (2 µg/ml) in ACN: potassium dihydrogen Ortho phosphate Buffer (pH 3.5) (50: 50)

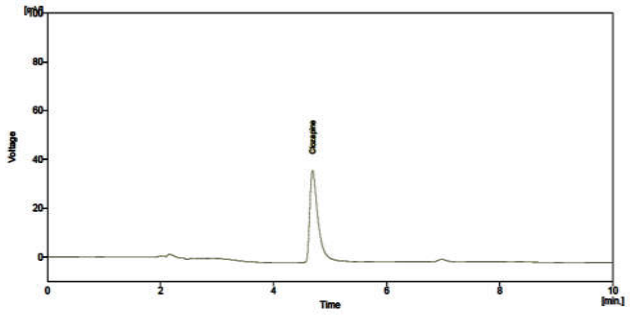

Figure 10 Chromatogram of Clozapine (20 µg/ml) in ACN: potassium dihydrogen Ortho phosphate Buffer (pH 3.5) (50:50)

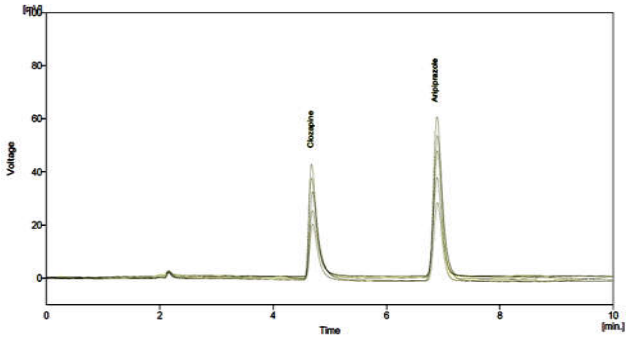

Figure 11 Overlay Chromatogram of Aripiprazole (1-5 µg/ml) and Clozapine (10-50 µg/ml)

Accuracy was carried out by Recovery Studies and was obtained in the range of 99.66-100.15 for Aripiprazole and 99.29-99.69 for Clozapine. LOD and LOQ values were found to be 0.012 and 0.037 µg/ml respectively for Aripiprazole and for Clozapine value were found to be 0.0046 and 0.015 µg/ml respectively.

The proposed method was precise, accurate and reproducible with acceptable recovery, which can be applied for the analysis of Aripiprazole and Clozapine in synthetic mixture.

CONCLUSION

The results of present study indicate that the proposed UV spectroscopic method is simple, precise and accurate. Statistical analysis proves that the method is repeatable and selective for the analysis of Aripiprazole and Clozapine in combination. It can therefore be concluded that the developed analytical method was precise & accurate and can be use for routine Analysis of both the drug in combination.

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