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Research Article

DEVELOPMENT AND VALIDATION OF REVERSE-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OF OLANZAPINE AND ARIPIPRAZOLE IN SYNTHETIC MIXTURES

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ABSTRACT

Objective: A simple, rapid, accurate, precise, specific, and sensitive reverse-phase high-performance liquid chromatography (RP-HPLC) method has been developed and validated for simultaneous estimation of olanzapine (OLZ) and aripiprazole (APR) in synthetic mixtures.

Methods: The stationary phase used for chromatographic separation was Phenomenex C_{18} column (250 mm × 4.6 mm i.d, particle size 5 µm) and mobile phase used for separation was methanol: Phosphate buffer (pH 3) taken in ratio of 75:25 %v/v. The flow rate was used 1.0 ml/min at room temperature and drugs detected at 240 nm with injection volume 20 µL.

Results: The retention time for OLZ and APR was found to be 4.231 and 6.523 min, respectively. The linearity was performed using a concentration range of 0.5–3.0 for both drugs. The correlation coefficient was found to be 0.999 for OLZ and APR. The % purity of both the drug was found to be 98–102%. The proposed RP-HPLC method has been validated, according to International council on harmonization Q2 (B) guidelines.

Conclusion: There was no interference of any diluents and excipients in the determination of drugs from synthetic mixture. Hence, the developed method can be used for routine quality control analysis.

Keywords: Olanzapine, Aripiprazole, Reverse-phase high-performance liquid chromatography, Validation.

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INTRODUCTION

Schizophrenia is a chronic and severe mental confusion affecting 20 million people worldwide. It is characterized by distortion in thinking, sensitivity, emotions, sense of self, and behavioral problems. Common experiences such as hallucinations and delusions and may affect educational and occupational performance. Stigma, discrimination, and violation of the human rights of people with schizophrenia are common [1,2]. Schizophrenia is treatable with medicines and psychosocial support is effective. The psychopharmacological management of therapy-resistant symptoms of schizophrenia is a big challenge for psychiatrists because this dilemma is generally approached with a realistic and polypharmaceutical medical treatment, for which evidence is lacking. There is some proof data that the combination of olanzapine (OLZ) and aripiprazole (APR) has advantages regarding symptoms control and side effects [3-5]. OLZ is a second-generation neuroleptic drug approved by the food and drug administration (USA) for the treatment of psychiatric patients suffering from schizophrenia to bipolar disorder. OLZ (Fig. 1), 2-methyl-4-(4-methylpiperazin-1-yl)-10*H*-thieno [2,3-b][1,5]benzodiazepine, is a psychotropic agent belonging to the synthetic derivative of thienobenzodiazepine [6]. APR (Fig. 2), (7-[4-[4-(2, 3-dichlorophenyl)-1-piperazinyl] butoxy]-3, 4-dihydrocarbostyril, is belonging to the chemical class of benzisoxazole derivatives and is indicated for the treatment of schizophrenia [7]. From a deep literature survey, various methods have been reported for the determination of OLZ and APR individually and in combination with other drugs [8-30]. However, the reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous determination of OLZ and APR in combination has not yet been reported. In the presented research work, RP-HPLC method was developed and validated according to the International council on harmonization (ICH) [31].

METHODS

Chemicals and reagents

OLZ and APR of pharmaceutical grade were kindly supplied as gift samples by Sun Pharmaceutical Advanced Research Center, Vadodara. Methanol, acetonitrile, and water used were of HPLC grade and purchased from Merck specialist Pvt. Ltd., India. Methanol, potassium dihydrogen orthophosphate, triethylamine, and orthophosphoric acid used were of AR grade.

Instrumentation

A HPLC system with LC solution data handling system (Shimadzu LC-20 10- Japan) was equipped with injector (Rheodyne, 20 µL), ultraviolet (UV) and PDA detector. The chromatographic analysis was performed using LC solution software on a Phenomenex C_{18} column (250 mm × 4.6 mm i.d., particle size 5 µm). Digital weighing balance (Shimadzu ATX 224, Japan), pH meter (Janki Impex Pvt. Ltd.), vacuum filtration assembly and ultrasonic bath cleaner were used during the study.

Standard stock solution preparation of OLZ (100 µg/mL) and APR (100 µg/mL)

Ten milligrams of OLZ and 10 mg of APR were weighed accurately and transferred to a 100 ml volumetric flask. This stock solution was prepared in methanol sonicated for 15 min; the volume was adjusted up to the mark with the same solvent. The solution was filtered through Whatman filter paper no 41. This stock solution contained 100 µg/ml of OLZ and ARP, both.

Preparation of synthetic mixture solution

For analysis of synthetic mixture, the powder of 10 mg OLZ and 10 mg APR was taken, dissolved in 100 ml volumetric flask and made up to 100 ml with methanol. The solution was sonicated for 15 min and

filtered through Whatman filter paper No 41. From a clear solution, further dilutions were made to get 10 µg/ml of OLZ and APR, both.

Selection of detection wavelength
For the selection of wavelength, spectra of both the drug were taken in the HPLC grade methanol, and then drug solutions were scanned in 200–400 nm region and the spectrum was recorded to get maximum absorbance of analyte in the mobile phase.

Selection of mobile phase
Mostly selection of mobile phase was based upon good resolution, less symmetric factor, and theoretical plates (≥2000). Therefore, the number of trial was taken for the selection of the mobile phase

Preparation of phosphate buffer solution (pH 3)
Dissolve 3.4 g of potassium dihydrogen orthophosphate and 2 ml of triethylamine in 800 ml of water adjust the pH 3 with orthophosphoric acid and add sufficient water to produce 1000 ml with distilled water.

Preparation of mobile phase
Mobile phase was prepared by mixing 75 ml of methanol with 25 ml of 25 mM phosphate buffer having pH 3 mixed well and sonicated. Then, the mobile phase is filtered with a 0.45 µm membrane filter.

Optimized chromatographic condition
The chromatographic condition for the estimation of both the drug is:
1. Stationary phase: Phenomenex C18 column (250 mm × 4.6 mm i.d., particle size 5 µm)
2. Column temperature: Room temperature
3. Mobile phase: Methanol:phosphate buffer (pH 3) (75:25%v/v)
4. Flow rate: 1.0 ml/min
5. Injection volume: 20 µl
6. Detection: UV detector SPD-20A at 240 nm
7. Run time: 10 min.

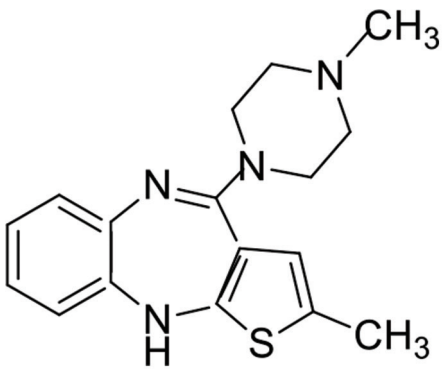

Fig. 1: Chemical structure of olanzapine

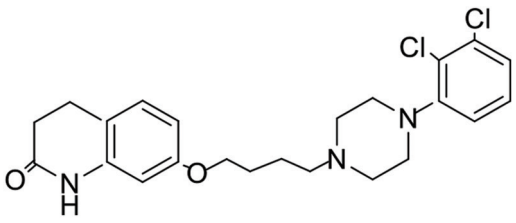

Fig. 2: Chemical structure of aripiprazole

Method validation
The developed method was validated according to ICH (Q2B) guideline for parameters such as accuracy, precision, specificity, system suitability, limit of detection (LOD), limit of quantification (LOQ), and robustness.

System suitability
The values for evaluation of system suitability of chromatographic procedure are relative standard deviation (RSD) <2%, tailing factor<1.5, and theoretical plates >2000. The retention time, resolution, theoretical plates, and tailing factor were evaluated for the system.

Linearity
Fresh aliquots were prepared from the stock solution of OLZ and APR (100 µg/ml) ranging from of 0.5 to 3.0 µg/ml for both OLZ and APR, and they were transferred into 10 ml volumetric flask and diluted up to 10 ml using methanol as a solution. The peak area of the solution was then measured at 240 nm. The calibration curve was constructed by plotting peak area versus concentration and the regression coefficient equation was calculated.

Precision

Repeatability

Six replicates of OLZ (2 µg/ml) and APR (2 µg/ml) were prepared and peak area was calculated at OLZ and APR, respectively, without altering the parameters of the proposed method. The result reported in terms of (%RSD).

Intermediate precision

It can be assessed by intraday and interday analysis. The intra-day study was performed by analyzing the three different concentrations of drug for three times in the same day. Inter-day precision was performed by analyzing three different concentrations of the drug for three different days. Different concentrations of the standard solution of OLZ and APR (1, 1.5, and 2 µg/ml) were measured for intraday and interday. The result was reported in terms of (%RSD).

Accuracy

The accuracy studies were carried out by spiking of the standard at three different concentrations, that is, 50%, 100%, and 150%. The recovery studies were carried out by adding a known amount of standard solution of three different levels. Accuracy was carried out by calculating recovery of OLZ and APR by standard addition method, from working sample solution of test 0.5 ml was taken for OLZ and APR and increasing aliquots of working standard solution (0.25 ml, 0.50 ml, and 0.75) ml from 100 µg/ml of OLZ and APR, were added and diluted up to 50 ml with methanol. These solutions were set in triplicate. Peak area of OLZ and APR was measured at a selected wavelength for OLZ and APR. The amount of OLZ and APR was calculated at each level % recoveries were computed.

Table 1: System suitability parameters of OLZ and APR

Parameters	Observed results±SD (n=6)		%RSD		Acceptance criteria
	OLZ	APR	OLZ	APR	
Retention time (Rt) (min)	4.23±0.04	6.572±0.02	0.10	0.39	%RSD <2
Peak area	2542.25±7.62	844.77±1.48	0.40	0.23	%RSD <2
Theoretical plates (N)	7328.66±26.25	4851.33±13.05	0.14	0.26	>2000
Tailing factor (N)	1.49±0.004	1.37±0.005	-	-	T ≤1.5
Resolution (Rs)	9.92±0.15		0.29		>2

OLZ: Olanzapine, APR: Aripiprazole, RSD: Relative standard deviation

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Typically, these might include impurities and degradants. A solution of placebo in mobile phase was prepared and injected. The chromatogram of placebo was compared with those acquired from OLZ and APR (2 µg/ml) standards, correlation terms of interference at retention time and peak area was evaluated to indicate the specificity of methanol.

LOD and LQC

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the three replicate determinations, y intercept was calculated and the standard deviation of the y intercept was computed. From these values, the parameters LOD and LOQ were determined on the basis of the response and slope of the regression equation.

LOD and LOQ were calculated by application of the following formula:

LOD = 3.3 σ/S

LOQ = 10 σ/S

Where, σ = standard deviation of response

S = slope of calibration curve

Robustness

Robustness was performed by deliberate changes in method parameters such as flow rate, detection wavelength on assay of the analyte of interest. Here, the mobile phase composition varied±2 nm, flow rate varied±1.0 ml, and pH varied±0.2.

RESULTS

System suitability

System suitability was checked by repeated preparations of 2 µg/ml of OLZ and APR, both. The typical values for evaluating system suitability of a chromatographic procedure are RSD <2%, tailing factor <1.5 and theoretical plates >2000. The retention time, peak area, theoretical plates, and tailing factor were evaluated for the system suitability data of OLZ and APR, the data are shown in Table 1 and Fig. 3.

Linearity

The linearity in chromatographic methods was good in the range of 0.5–3.0 µg/ml for OLZ and APR was shown in Figs. 4-6. The correlation coefficient value can be finding out by the regression equation. Correlation coefficient values for OLZ and APR are 0.999 and 0.999 for respectively.

Precision

Repeatability

The precision of the method was checked by repeated preparation (n=6) of 2 µg/ml of OLZ and APR, both. The %RSD was found to be <2% showing good repeatability. The values for OLZ and APR are shown in Table 2.

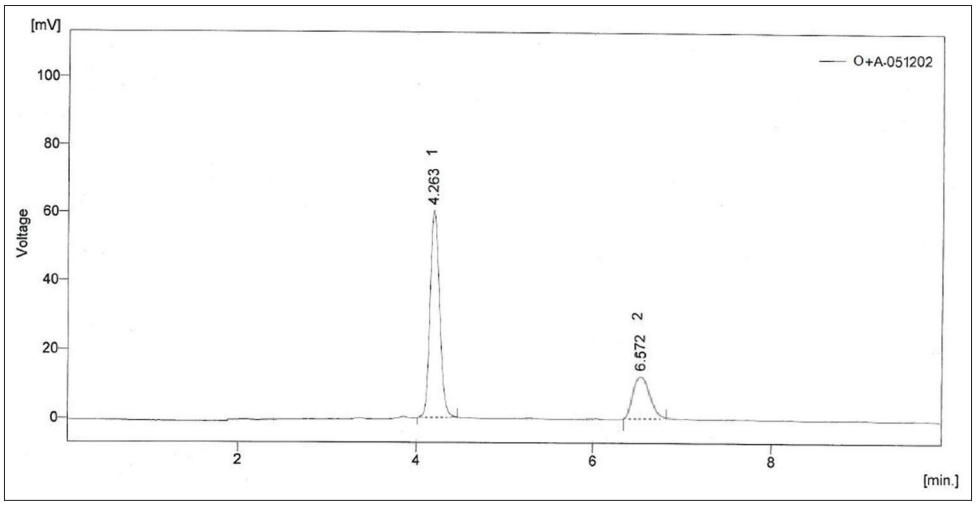

Fig. 3: Optimized chromatographic chromatogram of olanzapine (OLZ) (2 µg/ml) and aripiprazole (APR) 2 (2 µg/ml) (peak 1 OLZ and 2 APR)

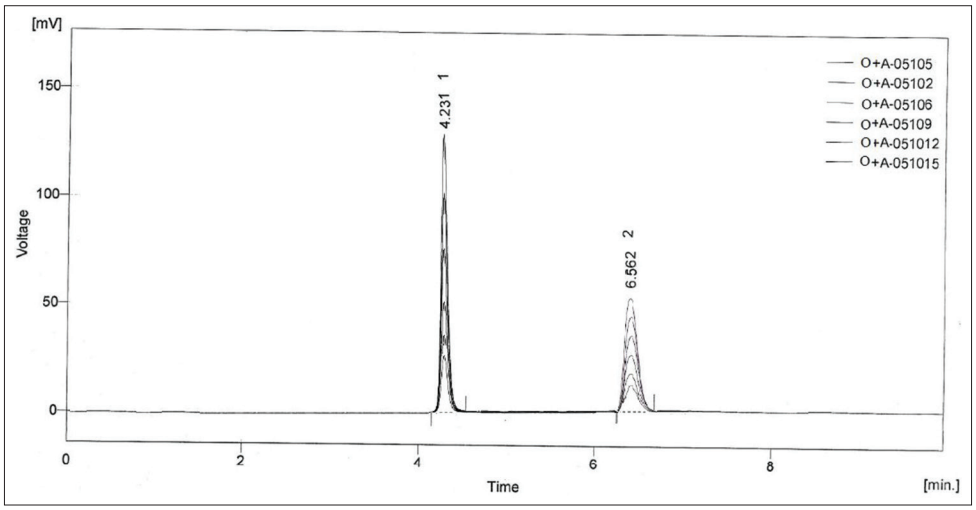

Fig. 4: Chromatogram of linearity (olanzapine [OLZ] and aripiprazole [APR]) using the optimized protocol (peak 1 OLZ and 2 APR)

Intermediate precision
The low %RSD value of intraday and interday was established to be (0.15–0.49 and 0.11–0.41) for OLZ and (0.34–0.70 and 0.28–0.42) APR, respectively, reveals that the intended method is precise (Table 3).

LOD and LOQ
LOD was found to be 0.021 µg/ml and 0.065 µg/ml for OLZ and APR and LOQ was found to be 0.13 µg/ml and 0.40 µg/ml for OLZ and APR, respectively.

Accuracy
The accuracy studies were carried out by spiking of the standard at three different concentrations, that is, 50%, 100%, and 150%. The %recovery study was found out by adding known quantity of standard. Each concentration was analyzed for 3 times and average recovery was calculated. %Recovery of OLZ and APR was found to be within acceptance criteria, that is, 98–102% (Table 4).

Specificity
Mobile phase (methanol) and common excipients such as starch, lactose, and magnesium stearate were dispersed in methanol, filtered, and injected. The chromatogram showed no interfering peaks at the retention time of the two drugs which indicates the specificity of method (Figs. 7-9).

Robustness
Robustness was performed by deliberate changes in method parameters such as flow rate, detection wavelength on assay of analyte of interest. Here, the mobile phase composition varied±2 nm, flow rate varied±1.0 ml, and pH varied±0.2. The robustness data for OLZ and APR are shown in Table 5. The result indicates that the chosen factors remained unchanged through minute variation in parameters and %RSD was found which is <2; therefore, it reveals that the proposed method is robust in the scenery.

Table 2: Repeatability data of APR and OLZ

Conc. (µg/ml)		Mean of peak area		SD (n=6)		% RSD	
OLZ	APR	OLZ	APR	OLZ	APR	OLZ	APR
2	2	2542.51	845.21	25.20	4.09	0.99	0.48
2	2	2530.12	845.23				
2	2	2545.36	847.56				
2	2	2515.23	848.21				
2	2	2525.63	845.26				
2	2	2587.23	836.84				

OLZ: Olanzapine, APR: Aripiprazole, RSD: Relative standard deviation

Applicability to synthetic mixtures
Applicability of the proposed RP-HPLC method was tested by analyzing the synthetic mixture (Table 6).

DISCUSSION

All the method validation parameters were well within the limits, as specified in the ICH Q2B guidelines. Moreover, the % RSD (less variation) showed good precision of developed method. The calculated LOQ and LOD concentrations confirmed that the methods were sufficiently sensitive. Various system suitability parameters are shown which show that the method is simple, accurate rapid, and precise. The method was

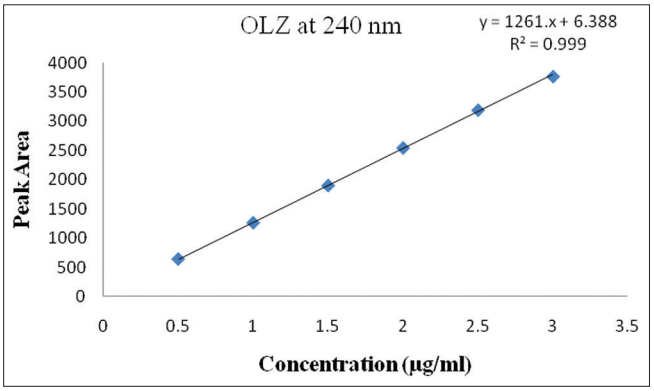

Fig. 5: Calibration curve of olanzapine (0.5–3.0 µg/ml)

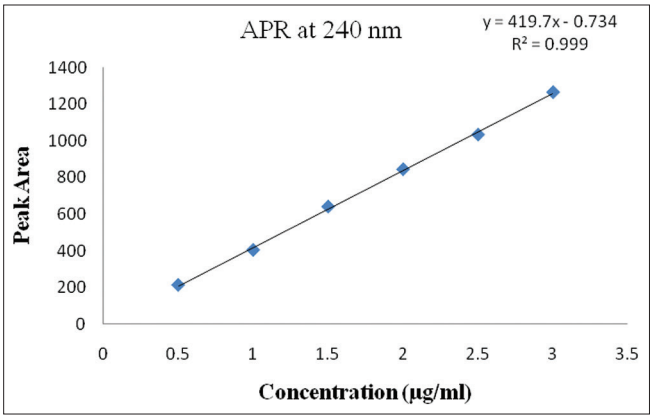

Fig. 6: Calibration curve of aripiprazole (0.5–3.0 µg/ml)

Table 3: Intraday and interday precision of OLZ and APR

Concentration (µg/ml)		Mean of peak area±SD (n=3)		%RSD		Mean of peak area±SD (n=3)		%RSD	
OLZ	APR	OLZ	APR	OLZ	APR	OLZ	APR	OLZ	APR
1	1	1257.73±2.67	402.92±2.65	0.21	0.65	1255.02±2.82	401.25±1.15	0.22	0.28
1.5	1.5	1895.31±3.02	640.53±2.20	0.15	0.34	1893.85±2.18	639.28±2.34	0.11	0.36
2	2	2527.07±12.39	843.98±5.91	0.49	0.70	2524.29±10.59	841.74±3.61	0.41	0.42

OLZ: Olanzapine, APR: Aripiprazole, RSD: Relative standard deviation

Table 4: Results of accuracy study of OLZ and APR

Drugs	Level (%)	Amount present (µg/ml)	Amount added (µg/ml)	Total amount of drug (µg/ml)	Amount found (µg/ml)	%Recovery±SD (n=3)	%RSD
OLZ	50	1	0.5	1.5	1.49	99.80±0.13	0.13
	100		1	2	2.01	100.66±0.05	0.05
	150		1.5	2.5	2.52	100.89±0.09	0.09
APR	50	1	0.5	1.5	1.52	101.82±0.19	0.19
	100		1	2	2.01	100.93±0.30	0.30
	150		1.5	2.5	2.46	98.74±0.51	0.52

OLZ: Olanzapine, APR: Aripiprazole, RSD: Relative standard deviation

Table 5: Robustness study of OLZ and APR

S. No.	Parameters	Variation	Mean Area		Retention time (min)		Tailing factor	
			OLZ	APR	OLZ	APR	OLZ	APR
1.	Flow rate (1±0.2 ml/min)	0.8	1252	402	3.68	5.00	1.52	1.25
		1.0	1305	417	3.72	5.23	1.49	1.34
		1.2	1310	428	3.72	5.23	1.32	1.38
2.	Mobile phase (75:25%v/v±2)	73:27	1238	384	3.83	5.07	1.40	1.31
		75:25	1282	413	3.75	5.10	1.40	1.64
		77:23	1365	423	3.71	5.18	1.43	1.71
3.	pH±0.2	2.8	1164	387	3.59	5.15	1.28	1.41
		3	1235	420	3.60	5.22	1.48	1.40
		3.2	1268	426	3.62	5.24	1.57	1.48

OLZ: Olanzapine, APR: Aripiprazole

Table 6: Assay result of synthetic mixtures

Synthetic mixture	Drug	Amount taken in synthetic mixture	Amount found (mg) n=3±SD	% Amount obtained±SD
	OLZ	10 mg	9.97±0.17	99.70±0.75
	APR	10 mg	9.93±0.85	99.36±1.02

OLZ: Olanzapine, APR: Aripiprazole

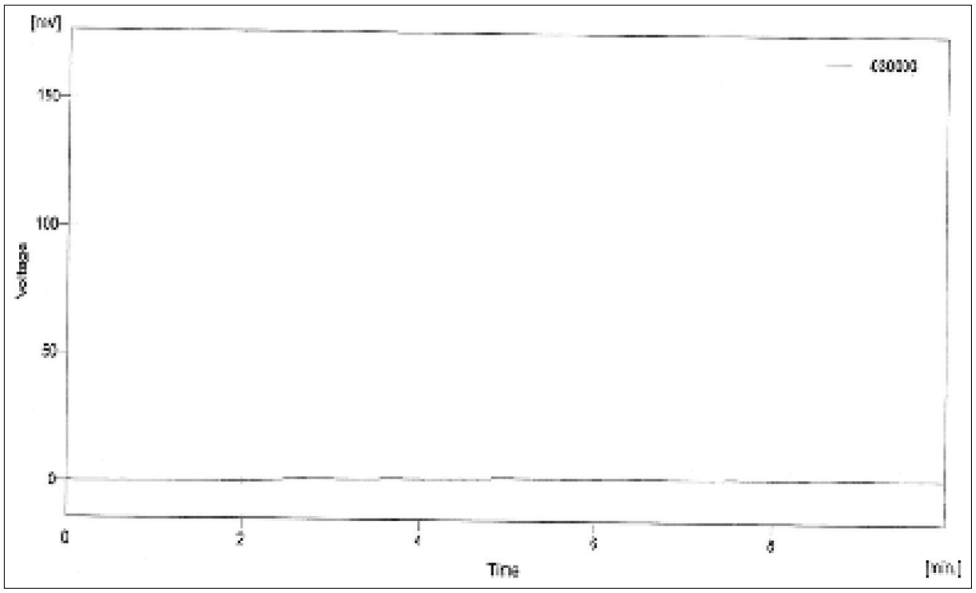

Fig. 7: Chromatogram of blank using optimized conditions

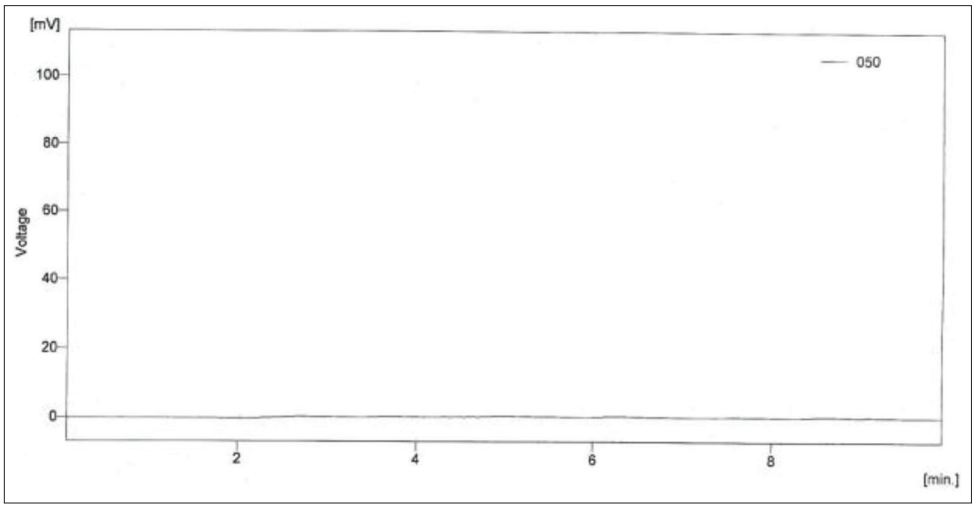

Fig. 8: Chromatogram of excipient using optimized conditions

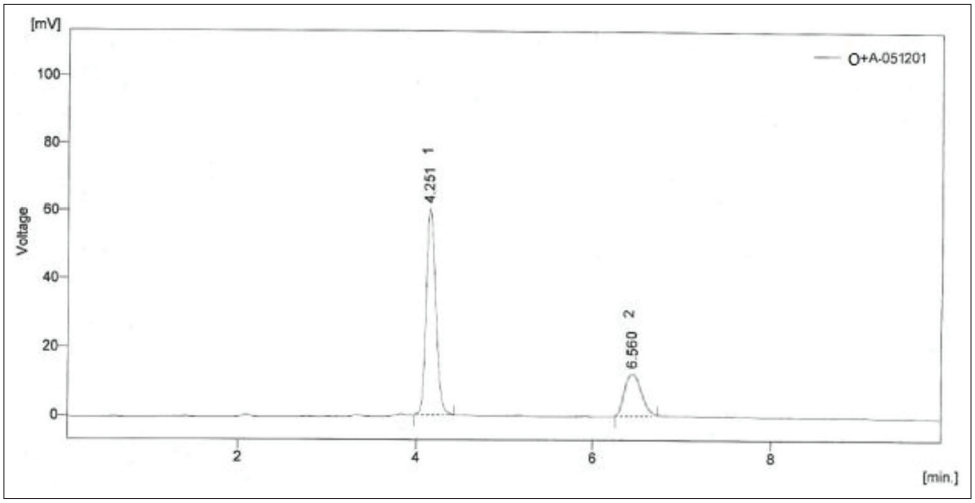

Fig. 9: Chromatogram of binary mixtures (olanzapine + aripiprazole)

suitably employed for assaying all the drugs in commercial marketed formulation. Hence, the developed can be used for further studies, and it helps researches to determine the drug content in formulations as well as in synthetic mixtures.

CONCLUSION

From the experimental result and parameters, it was concluded that this newly developed method for OLZ and APR was found to be simple, precise, accurate, and high resolution and shorter retention time makes the method more acceptable and cost-effective, and it can be effectively applied for routine analysis in research institution, quality control department, and approved testing laboratories.

AUTHORS' CONTRIBUTIONS

Ms. Megha has generated the research idea and interpreted the data and draft the manuscript, check plagiarism, and submitting the manuscript. Dr. Paresh Patel has suggested the research idea and participated in the design of the study. Dr. Dhara Patel has participated in the research idea and reviewed the manuscripts.

CONFLICTS OF INTEREST

The authors confirm that this article content has no conflicts of interest.

AUTHORS' FUNDING

None.

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