NEW RP-HPLC METHOD FOR DETERMINATION OF VORTIOXETINE AND ARIPIPRAZOLE IN SYNTHETIC MIXTURE

Suresh Kumar[a]*, Ancy Mathew[b] and Dhara Vashi[b]

(Received 26 July 2019) (Accepted 14 August 2020)

ABSTRACT

The present study was aimed to develop a novel, simple, rapid, accurate, stability-indicating Reversed-Phase High-Performance Liquid Chromatography method and its validation for the simultaneous estimation of vortioxetin and aripiprazole in synthetic mixture. RP-HPLC method was carried out by isocratic technique on a reversed phase Cosmosil C 18 (250 mm × 4.6 mm, 5 µm) column with acetonitrile: triethanolamine buffer (50:50 V/V, adjusted at pH 3.0) as mobile phase at a flow of 1.0 mL min^{-1}. The detection wavelength was 228 nm. The retention times for vortioxetine and aripiprazole were found to be 5.75 and 4.02 minutes respectively. The calibration curves were linear (r^2 >0.997) over the concentration range 2-20 µg mL^{-1} for vortioxetine and 2-32 µg mL^{-1} for aripiprazole. The values of Relative Standard Deviation (RSD) and percentage recovery were found to be satisfactory. The method was tested and validated as per ICH guidelines. Further, both the methods were compared statistically using student t-test and no significant difference found at 99 % level.

Keywords: Vortioxetine, aripiprazole, ICH guidelines, HPLC, method development, validation

INTRODUCTION

Vortioxetine is the first antidepressant which was released in the late 1950s. It was officially approved by the FDA on September 30, 2013. Vortioxetine represents another option for the treatment of MDD[1]. Aripiprazole is primarily used in the treatment of schizophrenia and bipolar disorder. It was approved by the FDA for schizophrenia on November 15, 2002[2]. The combination of vortioxetine and aripiprazole is mainly used for the treatment of Obsessive Compulsive Disorder. The combination of both drugs has been approved since 2017[3]. Obsessive-compulsive disorder (OCD) is a mental health condition characterized by distressing, intrusive, obsessive thoughts and repetitive, compulsive physical or mental acts[4]. An abnormality, or an imbalance in neurotransmitters, is thought to be involved in OCD. It is said that people with OCD are found to have different brain activity. Serotonin is the chemical in the brain that sends messages between brain cells and it is thought to be involved in regulating everything from anxiety, memory and sleep[5]. Due to the imbalance in the serotonin level, it may lead to OCD. The combination of vortioxetine and aripiprazole helps to maintain the serotonin level. Symptoms often appear in teens or young adults[6]. Stress can make symptoms worse. It has observed that 15 mg of vortioxetine and 10 mg of aripiprazole has been showing better results for OCD treatment. The combination of vortioxetine and aripiprazole is safe for the treatment of OCD. Vortioxetine hydrobromide monohydrate is used instead of vortioxetine[3]. The IUPAC name of vortioxetine hydrobromide monohydrate is 1{2[2, 4 dimethyl phenyl) sulfanyl] phenyl} piperazine hydrobromide monohydrate. Molecular formula is $C_{18}H_{25}BrN_2SO$[7].

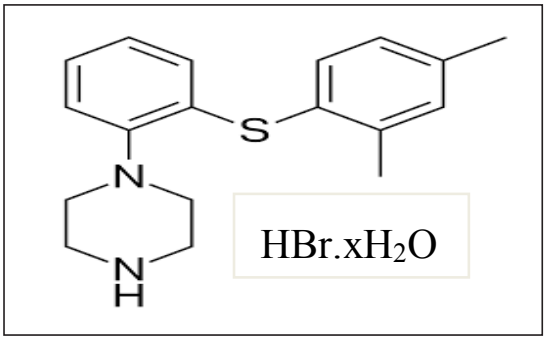

Fig. 1: Chemical structure of vortioxetine hydrobromide monohydrate[1]

[a] Department of Pharmaceutical Quality Assurance, B.Pharmacy College Rampura, Godhra- 388 713, Gujarat, India
[b] Department of Pharmaceutical Quality Assurance, Shree Dhanvantary Pharmacy College, Kim, Surat-394 110, Gujarat, India
*For Correspondence: E-mail: jdrsuresh@gmail.com

https://doi.org/10.53879/id.59.06.12034

The IUPAC name of aripiprazole is 7-[4-[4-(2,3-dichlorophenyl)piperazin-1yl]butoxy]-3,4-dihydro-1*H* quinolin-2-one. Molecular formula is $C_{23}H_{27}Cl_2N_3O_2$[8] .

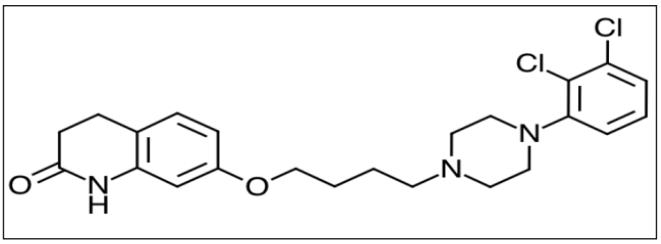

Fig. 2: Chemical structure of aripiprazole[2]

Vortioxetine act as an antidepressant and aripiprazole act as an antipsychotic, the combination of both drugs is used for the treatment of OCD. The combination of these two drugs provides synergistic treatments for OCD. The study was conducted in compliance with Good Clinical Practice. The combination of these drug shows effect in dosage ratio of 15 mg vortioxetine and 10 mg aripiprazole. Hence, vortioxetine in combination with aripiprazole can be useful for the treatment of OCD. There is no formulation available in this combination till date, as per our knowledge.

MATERIALS AND METHODS

Apparatus and instrumentation

- **HPLC:** Semi automatic liquid chromatography Model-SPD 10A-LC 10AT (Shimadzu, Japan), Pump-single pump systems using UV-VIS Detector with Winchrome software to acquire and process the data.
- **Column:** Cosmosil C_{18} (250 mm × 4.6 mm i.d., 5µm).
- **Semi micro analytical balance** (Sartorius CP225D, Germany)
- **Magnetic stirrer** (Remi)
- **pH meter** (313927, Eutech Instruments)
- **Ultrasonic cleaner** (D 120/1H, Trans-O- Sonic)
- **Nylon membrane filters** (0.22 µm, 47 mm D)

Chemicals

Vortioxetine hydrobromide monohydrate API was received as a gift sample from Torrent Pharmaceutical, Dahej. Aripiprazole API was received as a gift sample from Akhil Health Care, Vadodara. Required excipients were provided by Shree Dhanvantary Pharmacy College, Kim. Methanol and acetonitrile HPLC grade (Finar), HPLC grade water and triethanolamine AR grade (Astron), were used for development purpose.

METHOD DEVELOPMENT

Preparation for standard solution of vortioxetine hydrobromide monohydrate (VHM)

10 mg of drug VHM was taken in 100 mL volumetric flask and dissolved in a mixture of ACN and triethanolamine buffer (50:50 V/V) and adjusted at pH 3. Then, the volume was made upto the mark to make the stock solution with concentration 100 µg mL^{-1}.

Preparation for standard solution of aripiprazole (APZ)

10 mg of APZ was taken in 100 mL volumetric flask and dissolved in a mixture of ACN and triethanolamine buffer (50:50 V/V) and adjusted at pH 3 upto the mark to make the stock solution with concentration 100 µg mL^{-1}.

Preparation for standard solution of vortioxetine hydrobromide monohydrate and aripiprazole in combination

0.3 mL standard solution of VHM (100 µg mL^{-1}) and 0.2 mL standard solution of APZ (100 µg mL^{-1}) were taken in a common 10 mL volumetric flask and diluted up to the mark with acetonitrile: triethanolamine buffer (50:50 V/V).

Preparation of test (formulation) solution

All the ingredients mention in Table I were sifted and blended to achieve uniformity of mixing. A powder equivalent of about 200 mg was taken in 100 mL volumetric flask.

Table I: Composition of synthetic mixture

Sr. No.	Excipient name	Quantity (mg)
1.	Vortioxetine hydrobromide monohydrate	30
2.	Aripiprazole	20
3.	Mannitol	120
4.	Corn starch	10
5.	Methyl cellulose	10
6.	Sodium starch	4
7.	Magnesium stearate	6
	Total	200

Weighed amount of above was dissolved in 25 mL of acetonitrile: triethanolamine buffer (50:50 V/V) and sonicated for 15 min.

100 mL was diluted using solvent, shaken vigorously then the solution was filtered through Whatman filter paper No. 42 and further diluted to form the concentration 300 µg mL^{-1} for VHM and 200 µg mL^{-1} for APZ.

From the above solution, 1 mL was pipetted out in to a 10 mL volumetric flask and diluted up to the mark with acetonitrile: triethanolamine buffer (50:50 V/V) to obtain a concentration of VHM (30 µg mL^{-1}) and APZ (20 µg mL^{-1}).

From the above solution 1 mL was pipetted out in to a 10 mL volumetric flask and volume was made up to the mark with acetonitrile: triethanolamine buffer (50:50 V/V) to get a concentration of VHM (3 µg mL^{-1}) and APZ (2 µg mL^{-1}).

Table II: Chromatographic conditions

Parameter	Condition
Mobile phase	Acetonitrile: triethanolamine buffer (50:50 V/V)
pH	3.0 adjusted by orthophosphoric acid
Column	C18 , 250 mm X 4.6 mm, 5 µm
Wavelength	228nm
Injection volume	20 µL
Flow rate	1.0 mL min^{-1}
Run time	10 minutes

METHOD VALIDATION

Linearity and range

The linearity response was determined by analyzing 6 independent levels of concentrations in the range of 2-20 µg mL^{-1} for VHM and 2-32 µg mL^{-1} APZ.

Preparation of calibration curve for VHM

0.2, 0.6, 1.2, 1.4, 1.6, 1.8 and 2.0 µg mL^{-1} were injected from standard stock solution (100 µg mL^{-1}) to obtain seven concentrations ranging from 2-20 µg mL^{-1} for VHM. A graph was plotted between peak area versus concentration and treated by linear least square regression analysis.

Preparation of calibration curve for APZ

0.2, 0.4, 0.8, 1.2, 2.0, 3.0, and 3.2 µg mL^{-1} were injected from standard stock solution (100 µg mL^{-1}) to obtain seven concentrations ranging from 2-32 µg mL^{-1} for APZ. A graph was plotted between peak area versus concentration and treated by linear least square regression analysis.

Precision

Repeatability (n = 6)

Absorbance was measured using VHM (12 µg mL^{-1}) and APZ (8 µg mL^{-1}) without change in the parameters of the proposed method. This procedure was repeated six times in a day, having an interval of 30 min. Calculate % RSD value.

Intraday precision

Samples of the same batch with three standard solutions containing concentrations 6, 12 and 18 µg mL^{-1} for VHM and 4, 8, and 12 µg mL^{-1} for APZ were analyzed and three replicates (n=3) each on same day were run. % RSD value was calculated.

Interday precision

Samples of the same batch with three standard solutions containing concentrations 6, 12 and 18 µg mL^{-1} for VHM and 4, 8, and 12 µg mL^{-1} for APZ and three replicates (n=3) each on different days were run. % RSD value was then calculated.

Accuracy

Composition of synthetic mixture was made as per the Table II.

All ingredients were sifted and blended to achieve uniformity of mixing. Powder equivalent about 10 mg VHM in four different 100 mL volumetric flasks was taken.

1st flask was considered as a placebo and remaining flasks 2, 3 and 4 were spiked with 80, 100 and 120 % of standard drug (API). Same procedure was repeated for APZ .

In each volumetric flask, 25 mL of acetonitrile: triethanolamine buffer was added and sonicated for 15 min. The volume was made up with acetonitrile: triethanolamine buffer to 100 mL to a get a final concentration of 6, 11, 12, and 13 µg mL^{-1} for VHM and 4,7.2, 8 and 9 µg mL^{-1} for APZ, respectively (Table III).

The solutions were filtered through Whatman filter paper No. 42.

From above solutions, 3 mL was pipetted out in 10 mL volumetric flask and diluted up to the mark with acetonitrile and the amount recovered determined. % recovery was then calculated.

Table III: Spiking of formulation in accuracy test

Concentration of formulation (mg)		Concentration of API spiking (mg)		Total concentration (mg)	
VHM	APZ	VHM	APZ	VHM	APZ
6	4	-	-	6	4
6	4	4.8	3.2	11	7.2
6	4	6	4	12	8
6	4	7.8	4.8	13	9

LOD (Limit of detection) and LOQ (Limit of quantification)

The LOD and LOQ were estimated from the set of 6 calibration curves used to determine method linearity.

The LOD and LOQ may be calculated as

LOD=3.3*SD/Slope

LOQ=10*SD/Slope

where SD = SD of six intercepts of calibration curve

slope = the mean slope of the 6 calibration curves

Robustness

Robustness and ruggedness of the method were determined by subjecting the method to slight changes in the method condition, such as

- Pump flow rate
- Mobile phase pH, composition ratio
- Detected wavelength
- Analyst

Three replicates were made for the same concentration (15 µg mL^{-1} of VHM and 10 µg mL^{-1} of APZ) and % RSD was calculated.

Analysis of VHM and APZ in synthetic mixture

- Composition of synthetic mixture was made as per the Table II.
- All the listed ingredients were sifted and blended to have uniformity of mixing. Powder from synthetic mixture equivalent about 30 mg VHM and 20 mg APZ in 100 mL volumetric flask was taken.
- Contents were dissolved in 25 mL of acetonitrile: triethanolamine buffer (50:50 V/V) and sonicated for 15 min. It was diluted up to 100 mL with solvent and shaken vigorously, Solution was filtered through Whatman filter paper No. 42 and further diluted to produce concentration of solution of 300 µg mL^{-1} for VHM and 200 µg mL^{-1} for APZ.
- From the above solution, 1 mL was pipetted out in 10 mL volumetric flask and diluted up to the mark with acetonitrile: triethanolamine buffer (50:50 V/V) to get a concentration of VHM (30 µg mL^{-1}) and APZ (20 µg mL^{-1}).
- From the above solution, 1 mL was pipetted in 10 mL volumetric flask and diluted up to the mark with acetonitrile: triethanolamine buffer (50:50 V/V) to obtain a concentration of VHM (3 µg mL^{-1}) and APZ (2 µg mL^{-1}).

RESULTS AND DISCUSSION

Selection of detection wavelength

Detection wavelength was determined using HPLC. Both the drugs were detected at 228 nm.

Mobile phase selection

Various mobile phases with different ratios of different solvents and pH were used. The mixture of acetonitrile: triethanolamine buffer (50:50 V/V) provided optimum polarity for proper elution, separation and resolution of vortioxetine hydrobromide monohydrate and aripiprazole peaks. Under these conditions, the eluted peaks were well defined, resolved and free from tailing. Due to the non-polar nature of the stationary phase, more polar component aripiprazole will be eluted first because of its higher affinity towards the polar mobile phase and less polar component vortioxetine hydrobromide monohydrate will be eluted later due to its higher affinity towards non-polar stationary phase. Trials show chromatograms for various mobile phases tried for method development.

System suitability parameters

Table IV: Observed values for system suitability test (n=5)

Parameter	Observed Values		IP 2014 specification
	VHM	APZ	
Retention time (min)	5.98	4.19	-
Peak area	379133	36552	-
Theoretical plates	3242.834	2613.803	Not less than 2000
Resolution	4.779		>2

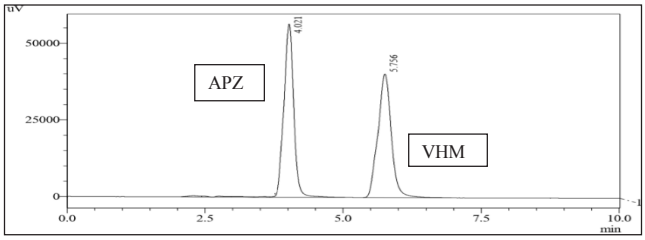

Fig. 3: Chromatogram of standard drug in ACN: triethanolamine buffer (50:50) as mobile phase

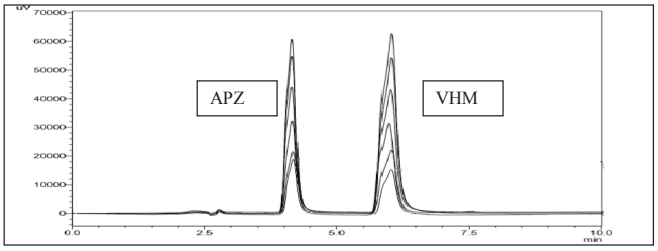

Fig. 4: Overlain chromatogram for six concentration of VHM (2-20 µg mL^{-1}) and APZ (2-32 µg mL^{-1})

Validation parameters

Linearity and range

Linearity was in the concentration range 2-20 µg mL^{-1} and 2-32 µg mL^{-1} for VHM and APZ, respectively. (Fig. 4, Fig. 5, Table V)

Table V: Calibration data for VHM and APZ (n=5)

Sr. No	Concentration (µg mL^{-1})		Peak Area ± SD VHM	%RSD	Peak Area ± SD APZ	%RSD
	VHM	APZ				
1.	3	2	255579± 260	0.1019	202362± 397	0.1965
2.	6	4	426220± 651	0.1529	302205± 873	0.2890
3.	9	6	624459± 1011	0.1619	449295± 457	0.1018
4.	12	8	841139± 938	0.1115	595764± 1659	0.2786
5.	15	10	1055636± 2265	0.2145	701600± 2000	0.2850
6.	18	12	1242832± 3458	0.2782	832446± 1550	0.1861

PRECISION

Intraday precision

The % R.S.D. was found to be 0.118-0.202 % for VHM and 0.138-0.168 % for APZ (Table VI).

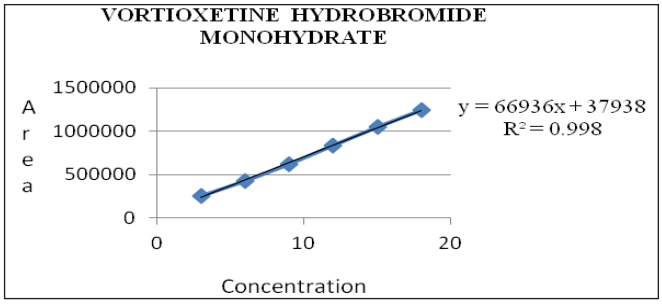

Fig. 5: Calibration curve for vortioxetine hydrobromide monohydrate

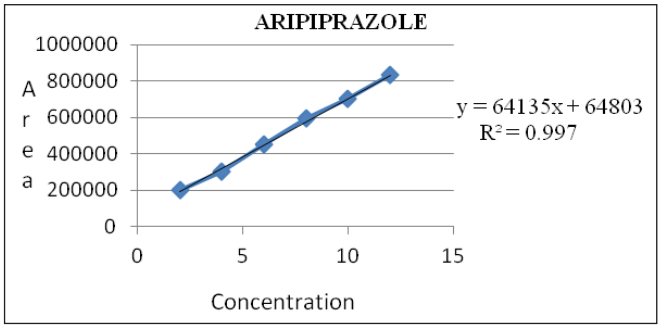

Fig. 6: Calibration curve for aripiprazole

Table VI : Intraday precision data for estimation of VHM and APZ (n=3)

Concentration (µg mL^{-1})		Peak Area± SD VHM	%RSD	Peak Area± SD APZ	%RSD
VHM	APZ				
6	4	441978± 893	0.2020	255610± 353	0.1381
12	8	865081± 1023	0.1183	536220± 902	0.1682
18	12	1316072± 2132	0.1620	826516± 1274	0.1541

Interday precision

The % R.S.D was found to be 0.205-0.353 % for VHM and 0.325-0.420 % for APZ (Tablel VII).

Table VII : Interday precision data for estimation of VHM and APZ (n=3)

Concentration (µg mL^{-1})		Peak Area± SD VHM	% RSD	Peak Area± SD APZ	% RSD
VHM	APZ				
6	4	426729 ± 995	0.2332	302252± 1210	0.4004
12	8	844160 ± 280	0.3531	595195± 1937	0.3255
18	12	1242501± 2549	0.2052	833540± 3504	0.4204

Table VIII : Repeatability precision data for estimation of VHM and APZ (n=3)

Conc. (µg mL^{-1})	No. of replicates						Peak area±SD	%RSD
	1	2	3	4	5	6		
15	1056398	1051195	1057495	1056195	1056895	1056398	1055763± 2286	0.216
10	700600	701601	701907	701902	700150	700400	701093±798	0.113

Table IX : Recovery data of VHM (n=3)

Target Conc. of VHM from synthetic mixture (µg mL^{-1})	Con. of standard VHM spiked (µg mL^{-1})	Total amount of VHM (µg mL^{-1})	Total amt. of VHM recover (µg mL^{-1})	% Recovery Mean±SD	% RSD
6 (Placebo)	-	6	6	99.437±0.464	0.4669
6	4.8	10.8	11	99.431±0.259	0.2611
6	6	6	12	100.0±0.120	0.120
6	7.2	13.2	13	99.360±0.268	0.270

Table X: Recovery data of APZ (n=3)

Target conc. of APZ from synthetic mixture (µg mL^{-1})	Con. of standard APZ spiked (µg mL^{-1})	Total amount of APZ (µg mL^{-1})	Total amt. of APZ recover (µg mL^{-1})	% Recovery Mean ± SD	% RSD
4 (Placebo)	-	4	4	100.237±0.285	0.284
4	3.2	7.2	7	101.133±0.176	0.174
4	4	8	8	99.363±0.224	0.225
4	4.8	8.8	9	100.102±0.481	0.480

Repeatability

%RSD was found to be 0.216 % for VHM and 0.113 % for APZ, respectively (Table VIII).

Accuracy

Accuracy of the method was determined by recovery study from synthetic mixture at three levels (80%, 100%, and 120%) of standard addition.

Percentage recoveries for VHM and APZ were found in the range of 99.36-100.00 % and 99.36-101.13 %, respectively (Table IX, X).

Limit of detection (LOD) and Limit of quantification (LOQ)

The LODs for VHM and APZ were confirmed to be 0.014 µg mL^{-1} and 0.010 µg mL^{-1}, respectively.

The LOQs for VHM and APZ was confirmed to be 0.043 µg mL^{-1} and 0.031 µg mL^{-1}, respectively (Table XI).

Robustness and Ruggedness

The % R.S.D was found to be 0.1-0.3 for VHM and 0.1-0.4 % for APZ (Table XII, XIII) .

Table XI: LOD and LOQ data of VHM and APZ

Conc. (µg mL^{-1}) VHM:APZ	VHM		APZ	
	Peak area ±SD	%RSD	Peak area ±SD	%RSD
3:2	208654± 292	0.1400	11753 ± 203	1.7303
LOD (µg mL^{-1})	0.014		0.010	
LOQ (µg mL^{-1})	0.043		0.031	

Table XII: Ruggedness and robustness data of VHM

Ruggedness & robustness data of vortioxetine hydrobromide monohydrate (15 µg mL^{-1})				
Standard mobile phase composition (ACN: triethanolamine buffer 50:50 V/V)				
Sr. No.	**Factor**	**Level**	**VHM Peak area±SD**	**%RSD**
1.	Change in analyst	Analyst-1	1055029 ± 3365	0.3190
		Analyst-2	1087225 ± 2330	0.2143
2.	Change in pH ± 0.2	3.2	1064873± 2411	0.2264
		2.8	1766814± 2464	0.1394
3.	Change in wavelength (228±2)	226 nm	1126265 ± 1647	0.1462
		230 nm	1095113 ± 2895	0.2643
4.	Change in flow rate	0.8 mL min^{-1}	1412805 ± 2668	0.1888
		1.2 mL min^{-1}	917560± 2098	0.2287
5.	Change in concentration	48:54	1026856± 1643	0.1600
		51:49	1096364± 2902	0.2647

Table XIII : Ruggedness and robustness data of APZ

Ruggedness & robustness data of aripiprazole (10 µg mL^{-1})				
Standard mobile phase composition (ACN: Triethanolamine buffer 50:50 V/V)				
Sr. No.	**Factor**	**Level**	**APZ Peak area±SD**	**%RSD**
1.	Change in analyst	Analyst-1	702267±2887	0.4111
		Analyst-2	793258 ± 2279	0.2873
2.	Change in pH ± 0.2	3.2	721224± 870	0.1206
		2.8	421012±570	0.1355
3.	Change in wavelength (228±2)	226nm	793847 ± 2453	0.3090
		230nm	532808 ± 1654	0.3106
4.	Change in flow rate	0.8 mL min^{-1}	826343 ± 1281	0.1550
		1.2 mL min^{-1}	536274 ± 710	0.1325
5.	Change in concentration	48:54	701432 ± 1072	0.1529
		51:49	796245 ± 1742	0.2187

Table XIV: Specificity data for VHM and APZ

Sr. No.	Concentration of mixture (µg mL^{-1})	Pure sample peak area	Synthetic mixture peak area	% Interference
1.	VHM (6 µg mL^{-1})	426732	424857	0.4393
2.	APZ (4 µg mL^{-1})	301646	305296	1.2100

Table XV: Analysis data of commercial formulation *(n=3)

Sr. No.	Formulation (Synthetic mixture)		% Assay VHM ± SD	%RSD	% Assay APZ ± SD	%RSD
	VHM	APZ				
1.	**18**	**12**	100.66±0.206	0.2047	100.17±0.055	0.0552

Specificity

Specificity was checked by observing the influence caused by excipients.

Table XIV of specificity data indicates that there was no interference of excipients, so the proposed method can be applied to analyze VHM and APZ in synthetic mixture. % Interference is less than 5%.

Analysis of VHM and APZ in synthetic mixture

Chromatogram of the test solution containing 18 µg mL^{-1} of VHM and 12 µg mL^{-1} of APZ was recorded and peak areas were noted for estimation of VHM and APZ, respectively (Table XV).

DISCUSSION

A new RP-HPLC method for simultaneous determination of VHM and APZ in synthetic mixture has been developed. The method was found to be simple, sensitive, precise, accurate and specific for quantification of VHM and APZ in synthetic mixture. Parameters comply with the criteria of the ICH guidelines for the method validation and are found to be suitable for routine quantitative analysis in synthetic mixture. Assay results were derived by proposed method which were in fair agreement with actual values.

REFERENCES

1. Drug Profile of vortioxetine hydrobromide monohydrate https://www.drugbank.ca/drugs/DB09068 accessed on Oct 2018.
2. Drug Profile of aripiprazole https://www.drugbank.ca/drugs/DB01238 accessed on Oct 2018.
3. Luigi O., Francesco N. and Gabriella R.: vortioxetine and aripiprazole Combination in treatment Resistant Obsessive Compulsive Disorder, **J. Clin. Psychopharmacol.**, 2017, 37(6), 1-2.
4. About OCD https://www.medicalnewstoday.com/article/178508 accessed on Oct 2018.
5. Causes of OCD https://www.ocduk.org/ocd accessed on Oct 2018.
6. About OCD https://www.webmd.com/mentalhealth/obssesive accessed on Oct 2018.
7. Pravallika K.E., Ravi P. and Abdulla H.A.: Development and validation of UV spectrophotometric methods for the estimation of vortioxetine hydrobromide in bulk and pharmaceutical dosage forms**. Res. J. Pharm. Tech.**, 2017, 10(11), 3928-3932 .
8. Raju G.V, Ganapathy S, Sankar D.G and Naidu P Y,: Stability Indicating RRLC Method for Determination of aripiprazole and Its Intermediates in Bulk and Pharmaceutical Formulation. **Asian J. Res. Chem.**, 2010, 3(2), 380-385.