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Development and Validation of RP-HPLC for Estimation of Brexpiprazole in Bulk Drugs

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Abstract

A simple, rapid, accurate, and sensitive method was developed for quantitative analysis of Brexpiprazole in bulk dosage form using reverse phase high-performance liquid chromatography with UV detection. The chromatography separation was achieved on Kromasil -C18, (250 mm × 4.6 mm i.d., 5 µm particle size), at column temperature 25°C ± 2, in the isocratic mobile phase mode using acetonitrile 5% ortho-phosphoric acid: water (60:40 v/v) at a flow rate of 1.0 mL/min. The determination was performed using waters HPLC with UV detector set at 216 nm. Samples were prepared with diluent methanol, and the volume injected was 20 µL. The retention time for brexpiprazole was 3.89 min. The analytical curve was linear (r^2=0.99994) over a wide concentration range (0.51–7.50 µg/mL). The presence of components of the bulk did not interfere in the results of the analysis. The method showed adequate precision with a relative standard deviation <2%.

Keywords: Brexpiprazole, Reverse-phase high-performance liquid chromatography, Validation

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Introduction

Brexpiprazole (Rexulti®, Otsuka Pharmaceutical Co, Ltd, Tokyo, Japan) is an atypical antipsychotic that was approved by the US Food and Drug Administration in July 2015 for treatment of schizophrenia and as an adjunctive therapy to antidepressant medications for the treatment of major depressive disorder.[1-3] Brexpiprazole is a new serotonin–dopamine receptor modulator that acts as a partial agonist at the 5-HT_{1A} and D_2 receptors with less intrinsic activity than aripiprazole and as an antagonist at the 5-HT_{2A} and alpha-1A receptors, all with subnanomolar potency.[5,6]

It is a white-to-off white freely soluble powder in methanol and practically insoluble in water is chemically designated as 7-{4-[4-(1-benzothiophen-4-yl) piperazin-1-yl] Butoxy}-1,2-dihydroquinolin-2- one with molecular formula C25H27N3O2S, and molecular weight 433.57 [Tables 6-8].[2,8-14]

While the precise mechanism of action of brexpiprazole in treating schizophrenia is not fully understood, the pharmacology of brexpiprazole is believed to be mediated by a combination of high binding affinity and functional activities at multiple monoaminergic receptors.

Materials and Methods

Brexpiprazole is obtained from Alkem Laboratories Ltd. and reagents, namely, methanol high-performance liquid chromatography (HPLC grade), ethanol (Analytical grade), acetonitrile (HPLC grade), orthophosphoric acid (OPA) (HPLC grade), dimethyl formamide (DMF) (Analytical grade), dimethylsulfoxide (DMSO) (Analytical grade), and hydrocloric acid (Analytical grade) was obtained from Merk, whereas water (HPLC grade) was obtained from Siddhi Lab.

Instruments like (1) double-beam UV-visible spectrophotometer with model no. UV 550, Make-Jasco, (2) HPLC binary gradient system with model no. 1260 Infinity II, Make- Agilent, Pump- DEAX02386, Detector-DEACX16446, Column-Water spherisorb 100 mm * 4.6 mm, C18, 5 µ, Software-Openlab EZ Chrome, (3) Azcet high precision balance with Model- CY 224C, Maximum-220 g, Minimum-0.001 g, (4) digital pH meter Make- LabMan, (5) bio-technic ultra sonicator with capacity of 13.5 L, and (6) filters with membranes of Nylon 0.45 µm and PVDF 0.45 µm were used, whereas the glasswares and apparatus such as volumetric flask, funnel, UV-cuvettes, beakers, pipettes, glass rod, test tubes, test tube stands, GC vials, rubber bulb, and tissue papers were used.

Chromatographic separation was achieved on a Kromasil, C18, 250 mm*4.6 mm, 5µ. The mobile phase used for the separation of Brexpiprazole was Acetonitrile: 0.05%OPA in water in the ratio of 60:40. Flow rate was set at a 1ml/min at ambient temperature 30°C, an injection volume of 20 µL using UV-visible detector (UV3000-v) at wavelength of 216nm. The mobile phase was ultrasonicated for degassing and filtered through 0.45µm membrane nylon filter using vacuum pump before pumping into HPLC system [Table 9].

Brexpiprazole is evaluated for various preliminary parameters such as color, odor, and appearance and confirmed that they complied with official standards. The melting point for brexpiprazole is determined by an open capillary method and compared with standard literature values. The solubility was determined in solvents under consideration, that is, methanol, water, acetonitrile, ethanol, DMF, and DMSO and are shown in the result**.** Stock solution was prepared by weighing and dissolving 10 mg brexpiprazole with 50 mL of methanol and to this dilution was done with methanol (100 µg/mL) which was further diluted

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with 1–10 mL with methanol. Sonication was done to dissolve it completely.

The solution of brexpiprazole was scanned between 200 and 400 nm and the wavelength of maximum absorption was determined. Brexpiprazole showed maximum absorbance at 216 nm shown in results.

Aliquots of standard stock solution of brexpiprazole have diluted appropriately with diluents to get a concentration in the range of 0.8 µg/mL–8.0 µg/mL and absorbance of each of the resulting solutions was measured at 216 nm in 1.0 cm cell using solvent blank. Standard stock solution (100 µg/mL) was appropriately diluted to get 0.8 µg/mL, 2.0 µg/mL, 4.0 µg/mL, 6.0µg/mL, and 8.0 µg/mL for brexpiprazole. These solutions were analyzed at absorption maxima obtained in the UV spectrum and their Beer Lamberts plot was generated.

Fourier transformation infrared (FTIR) analysis was done [Figure 1 and Table 12].

Results And Discussion

Analysis of Brexpiprazole by HPLC Method was done as by Preparing

Standard stock solution

The standard solution was prepared by dissolving 10 mg of brexpiprazole into a 100 mL clean and dry volumetric flask, added about 70 mL of methanol to dissolve it completely, and made volume up to the mark with methanol (100 µg/mL).

Diluted OPA

Pipette out 5 mL of OPA and transferred it into a 50 mL volumetric flask and made volume up to the mark with water. Mixed well and sonicated for 5 min.

1.0% OPA in water

Pipette out 1 mL of OPA and transferred it into a 100 mL volumetric flask and made volume up to the mark with water. Mixed well and sonicated for 5 min.

10.00 mM phosphate buffer in water

Weighed about 1.36 g of potassium dihydrogen orthophosphate and dissolved in 1000 mL of water, adjusted its pH to 2.0 ± 0.05 by diluted OPA solution.

Optimization of HPLC Method was Done for Estimation of Brexpiprazole as per Following Trials

Observation: Brexpiprazole eluted but chromatography not acceptable.

Conclusion: Method rejected [Table 1 and Figure 2].

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Conclusion: Method rejected [Table 2 and Figure 3].

Observation: Brexpiprazole eluted at R.T. but chromatography not acceptable.

Conclusion: Method rejected [Table 3 and Figure 4].

Observation: Brexpiprazole eluted and good chromatography observed. R.T. will try to reduce.

Table 1: Trial 1

S. No.	*Parameter/condition*	*Description*
1.	Standard solution	Brexpiprazole 100 µg/mL
2.	Column	Kromasil C18
3.	Column dimension	(250 mm×4.6 mm i.d.) 5 µm
4.	Detector	U.V. Detector
5.	Wavelength	216 nm
6.	Flow rate	1.0 mL/min
7.	Mobile phase	Methanol: water (70:30)
8.	Injection volume	20 µL
9.	Run time	15 min

Table 2: Trial 2

S. No.	*Parameter/condition*	*Description*
1.	Standard solution	Brexpiprazole 100 µg/mL
2.	Column	Kromasil C18
3.	Column dimension	(250 mm×4.6 mm i.d.) 5 µm
4.	Detector	U.V. Detector
5.	Wavelength	216 nm
6.	Flow rate	1.0 mL/min
7.	Mobile phase	Methanol: Water (50:50)
8.	Injection volume	20 µL
9.	Run time	15 min

Table 3: Trial 3

S. No.	*Parameter/condition*	*Description*
1.	Standard solution	Brexpiprazole 100 µg/mL
2.	Column	Kromasil C18
3.	Column dimension	(250 mm×4.6 mm i.d.) 5 µm
4.	Detector	U.V. Detector
5.	Wavelength	216 nm
6.	Flow rate	1.0 mL/min
7.	Mobile phase	Acetonitrile: Water (20:80)
8.	Injection volume	20 µL
9.	Run time	7 min

Table 4: Trial 4

S. No.	*Parameter/condition*	*Description*
1.	Standard solution	Brexpiprazole 100 µg/mL
2.	Column	Kromasil C18
3.	Column dimension	(250 mm×4.6 mm i.d.) 5 µm
4.	Detector	U.V. Detector
5.	Wavelength	216 nm
6.	Flow rate	1.0 mL/min
7.	Mobile phase	Acetonitrile: 0.05% OPA in water (50:50)
8.	Injection volume	20 µL
9.	Run time	7 min

Table 5: Trial 5

S. No.	*Parameter/condition*	*Description*
1.	Standard solution	Brexpiprazole 100 µg/mL
2.	Column	Kromasil C18
3.	Column dimension	(250 mm×4.6 mm i.d.) 5 µm
4.	Detector	U.V. Detector
5.	Wavelength	216 nm
6.	Flow rate	1.0 mL/min
7.	Mobile phase	Acetonitrile: 0.05% OPA in water (60:40)
8.	Injection volume	20 µL
9.	Run time	7 min

Conclusion: Method accepted [Table 4 and Figure 5].

Observation: Brexpiprazole is eluted and good chromatography observed. The following chromatographic conditions were established by trial and error and were kept constant throughout the method.

Conclusion: Method accepted [Table 5 and Figure 6].

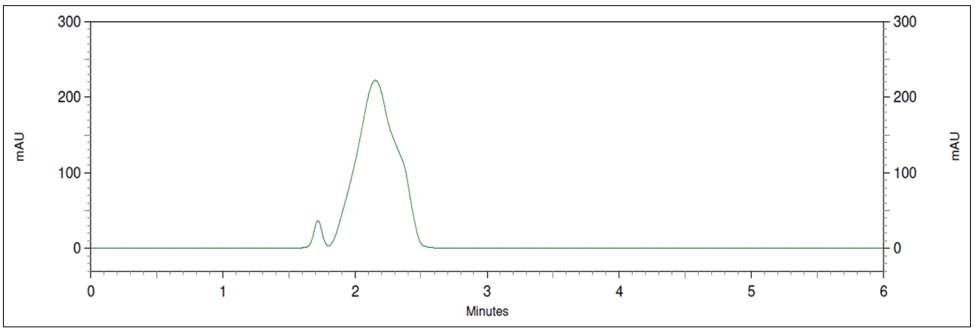


Figure 1: Typical chromatogram of Trial 1

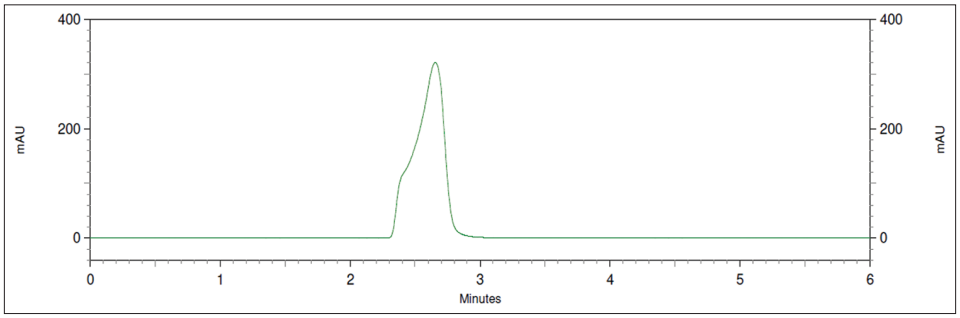


Figure 2: Typical chromatogram of Trial 2

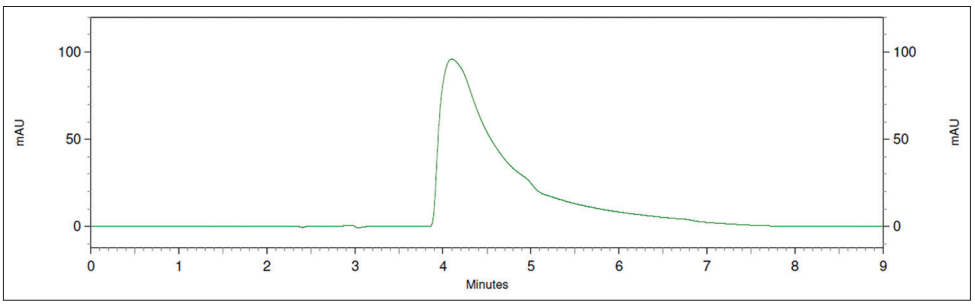


Figure 3: Typical chromatogram of Trial 3

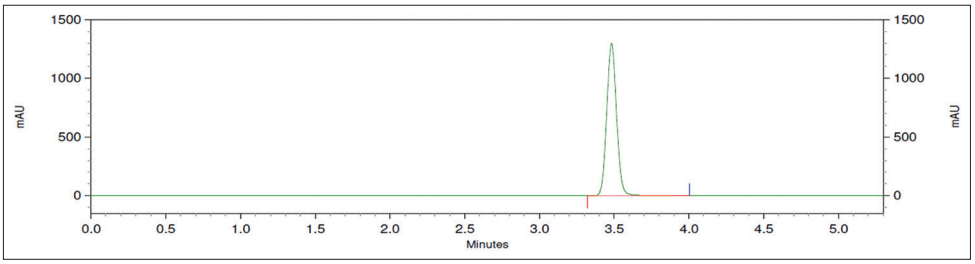


Figure 4: Typical chromatogram of Trial 4

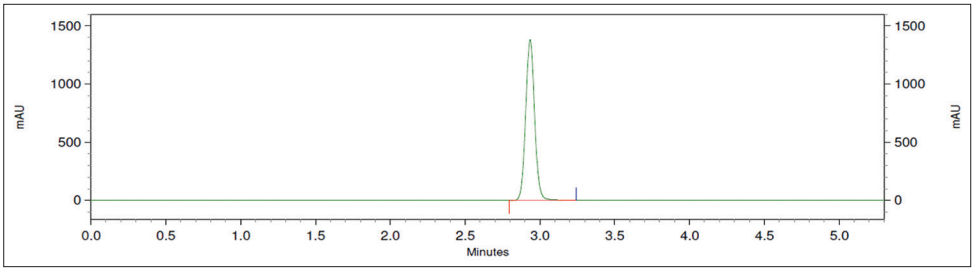


Figure 5: Typical chromatogram Trial 5

Determination of Wavelength Maxima and Beers Lamberts Law Study Using UV- Spectroscopy

Determination of wavelength maxima for brexpiprazole

Observation: The standard solution was scanned between 200 and 400 nm. The wavelength of maximum absorption was determined for drugs. Brexpiprazole showed maximum absorbance at 216 nm. It is shown in Figure 7. Therefore, 216 nm considered as an analytical wavelength for further determination.

Table 6: Color, odor, and appearance of drug

Parameters	*Observation*
Color	OffWhite
Odor	Odorless
Appearance	Amorphous powder

Table 7: Melting point (°C) of brexpiprazole

S. No.	*Observed MP (°BC)*	*Standard. M.P(°BC)*
1	271–273	272–274
2	273–275	
3	271–272	

Table 8: Solubility study of brexpiprazole

S. No.	*Name of solvent*	*Solubility*
1	Water	Insoluble
2	Methanol	Slightly soluble
3	Acetonitrile	Slightly soluble
4	Phosphate buffer	Soluble
5	0.1 N HCl	Insoluble
6	Ethanol	Soluble
7	DMF	Soluble
8	DMSO	Soluble

Table 9: Optimized chromatographic conditions

Parameter/condition	*Description*
Column name	Kromasil , C18 ,250 mm*4.6 mm, 5µ
Detector	UV3000M
Injection volume	20 µL
Wavelength	216 nm
Mobile phase	Acetonitrile: 0.05%OPA in water (60: 40% V/V)
Program	Isocratic
Flow rate	1.0 mL/min
Column oven temp	30°C
Run time	6 min
Buffer	0.5 mL Ortho Phosphoric Acid in 1000 ml water.(0.05%OPA)

Table 10 wavelength

λ max	216 nm

Table 11: Absorbance values for different concentration of brexpiprazole in methanol (λmax=216 nm)

X (Conc.µg/mL)	*Y (Absorbance)*
0.8	0.0858
2.0	0.2143
4.0	0.4294
6.0	0.6486
8.0	0.8611

Study of Beers-Lambert's law [Tables 10 and 11, Figure 8]

Aliquots of standard stock solution of Brexpiprazole have diluted appropriately with diluents to get a concentration in the range of 0.8 µg/mL -8.0 µg/mL and Absorbance of each of the resulting solutions was measured at 216 nm in 1.0 cm cell using solvent blank. Standard stock solution (100µg/mL) was appropriately diluted to get 0.8µg/mL, 2.0µg/mL, 4.0 µg/mL, 6.0µg/mL, 8.0µg/mL for Brexpiprazole. These solutions were analysed at absorption maxima obtained in the UV spectrum and their Beer Lamberts plot was generated.

Fourier transformation infrared attenuated total reflectance analysis [Table 12 and Figure 1]

a) Selection of solvent: Methanol was selected as the solvent for dissolving Brexpiprazole.
b) Preparation of standard stock solutions:

To prepare a stock solution, weigh accurately 10 mg Brexpiprazole and transferred into 100 mL volumetric flask, dissolve with 50 mL of methanol and sonicated to dissolve it completely further diluted up to the mark with methanol (100µg/mL). Further diluted 1 mL to 10 mL with methanol.

c) Selection of analytical wavelength

The standard solution was scanned between 200-400 nm. The wavelength of maximum absorption was determined for the drug. Brexpiprazole showed maximum absorbance at 216 nm shown in results.

Control Strategy

System suitability test (SST) [Table 13]

Acceptance criteria:

1. % relative standard deviation (RSD) of the five replicate injections is NMT 2.0%.

Table 12: ATR interpretation of brexpiprazole

S. No.	*Peak observed (cm^{-1})*	*Peak reported (cm^{-1})*	*Interpretation*
1.	3148	3500–3100	N-H- stretching for amide
2.	3067	3150–3050	C-H- (aromatic) stretching
3.	2940,2815	3000–2850	C-H- (aliphatic) stretching
4.	1650	1700–1640	-C=O- (amide) stretching
5.	1327	1300–1000	C-N stretch
6	1219,1140	1300–1000	-C-O- (ether) stretching
7.	1052	1050	C-S stretch

Table 13: Analytical data of System suitability test

S. No.	*Standard solution*	*Area*	*Asymmetry*	*Theoretical plates*
1	Standard_1	4546743	1.09	12671
2	Standard_2	4538416	1.09	12684
3	Standard_3	4554857	1.09	12666
4	Standard_4	4536986	1.09	12656
5	Standard_5	4548651	1.09	12676
Mean		4545131	1.09	12671
STD Dev		7433.30796		
% RSD		0.16		

Table 14: Analytical data of filter test

Sample	*Area*	*% Absolute difference*
Unfiltered	16256478	NA
0.45 µ PVDF filter	16178521	0.48
0.45 µ Nylon filter	16152546	0.64

2. Theoretical plates should be more than 2000.
3. Tailing factor should be NMT 2.

Data interpretation: It was observed from the data tabulated above; the method complies with system suitability parameters. Hence, it can be concluded that the system suitability parameter meets the requirement of method validation.

Chromatograms for SST [Figures 9-14].

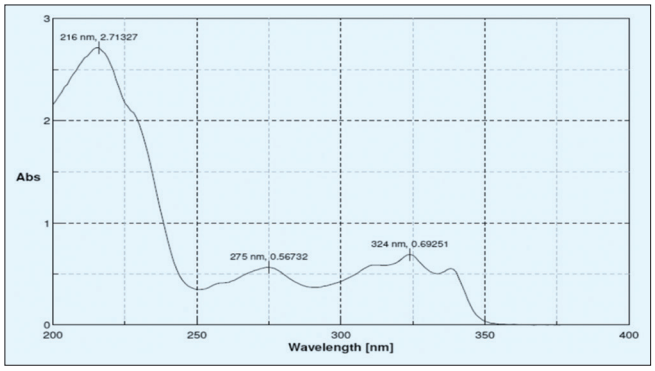


Figure 6: UV spectrum of in brexpiprazole methanol

Filter test [Table 14]

Acceptance Criteria: % Absolute difference NMT 2.0.

Conclusion: Both filters PVDF and Nylon pass the criteria for filter study; hence, both filters can be used.

Chromatograms of Filter Test [Figures 15-17].

Acceptance criteria: % Absolute difference of filtered samples NMT 2.0 w.r.t. Unfiltered sample.

Data interpretation: Both filters PVDF and Nylon pass the criteria for filter study; hence, both filters can be used.

Solution stability

Stability study was conducted for standard solution and test solution. Test solution stability was performed using test sample

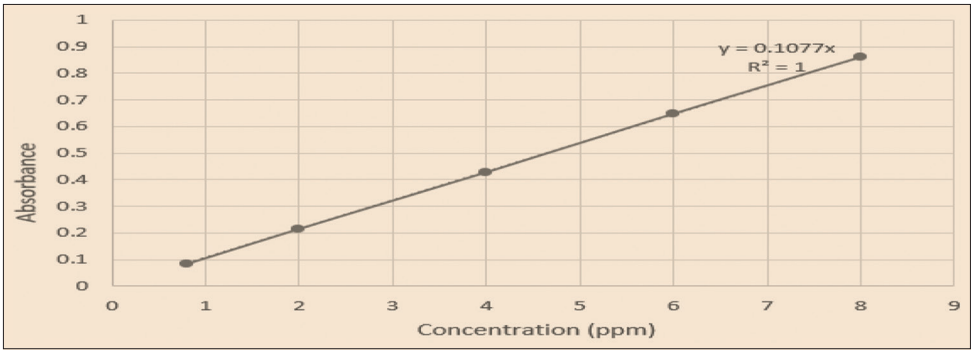


Figure 7: Beer-Lambert's plot for brexpiprazole in methanol

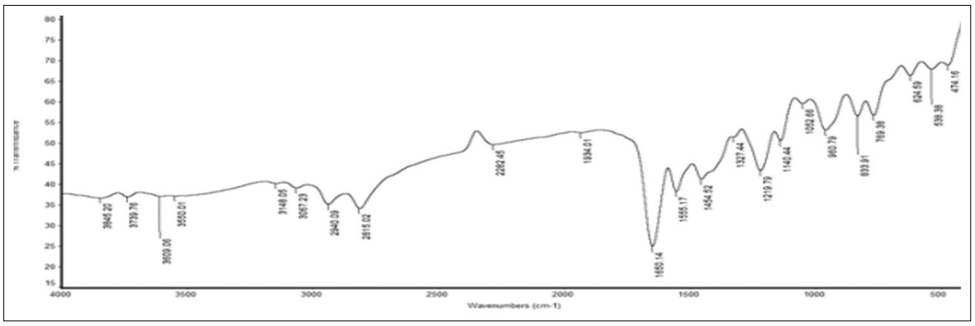


Figure 8: ATR spectra of brexpiprazole

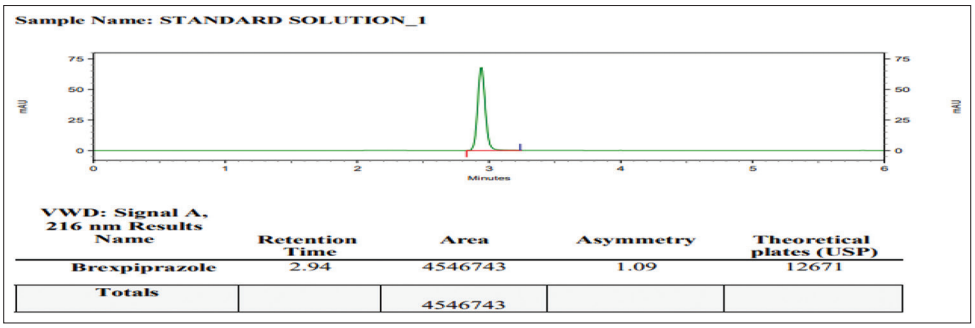


Figure 9: Chromatogram of Standard 1 for SST

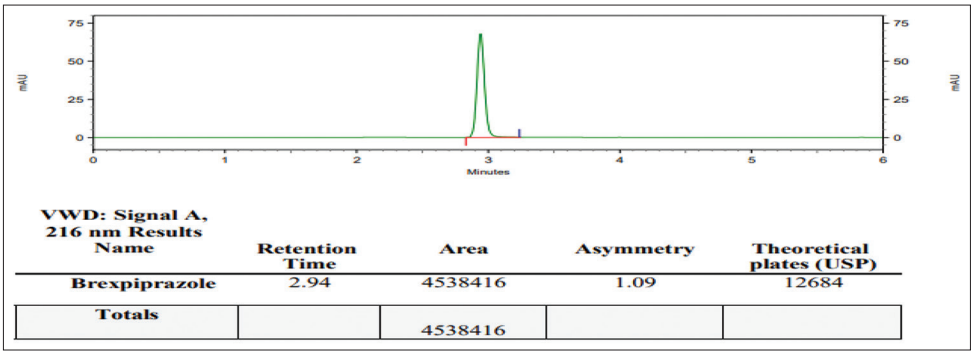


Figure 10: Chromatogram of Standard 2 for SST

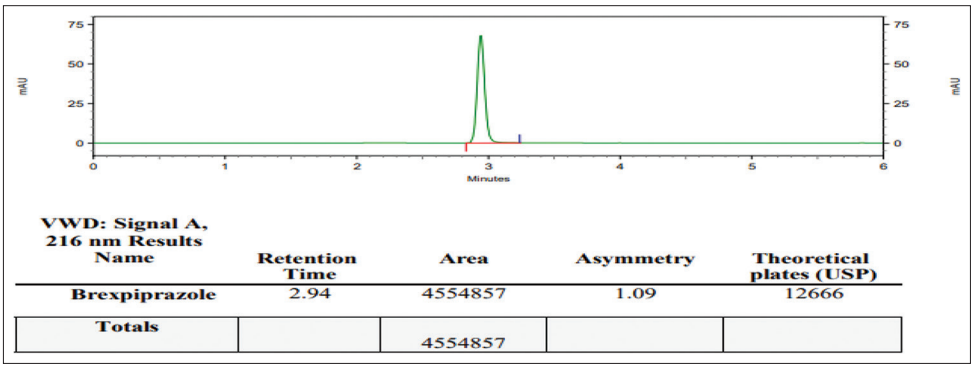


Figure 11: Chromatogram of Standard 3 for SST

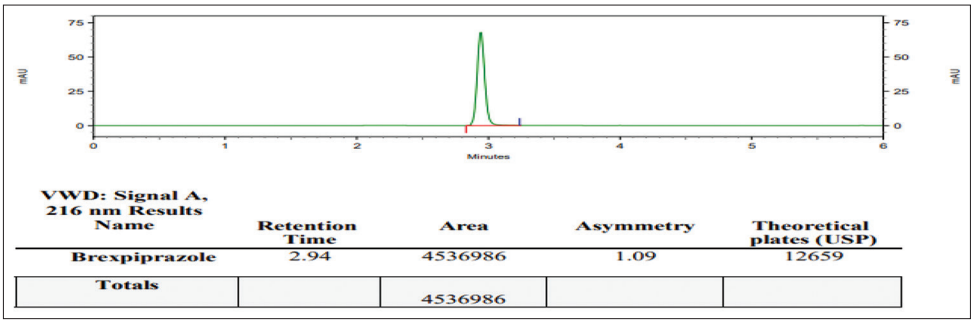


Figure 12: Chromatogram of Standard 4 for SST

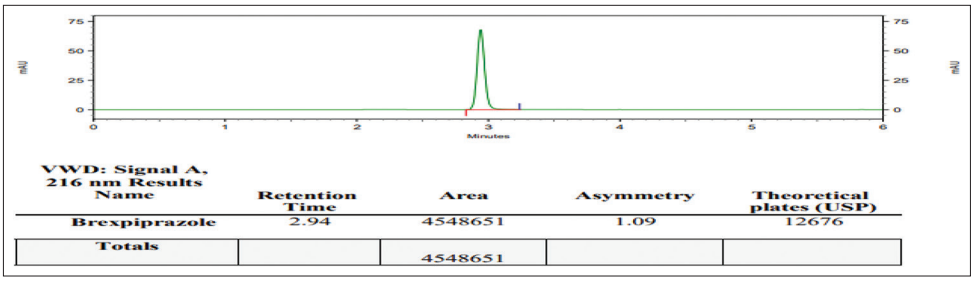


Figure 13: Chromatogram of Standard 5 for SST

Table 15: Analytical data of brexpiprazole for solution stability

Sample solution			*Standard solution*		
time point	*Area*	*% Absolute difference*	*time point*	*Area*	*% Absolute difference*
Initial	4467152	NA	Initial	4571699	NA
12 h	4453846	0.30	12 h	4559748	0.26
24 h	4434865	0.72	24 h	4539484	0.70

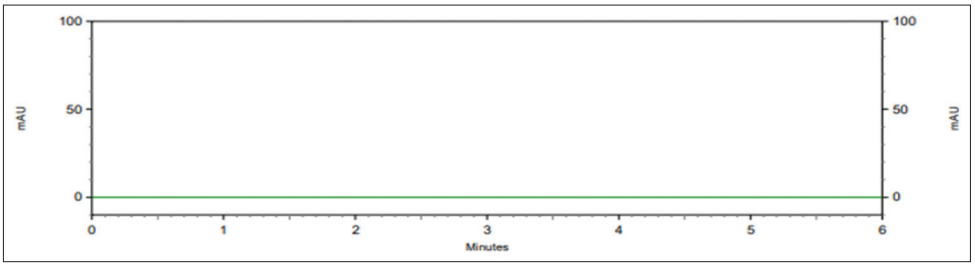
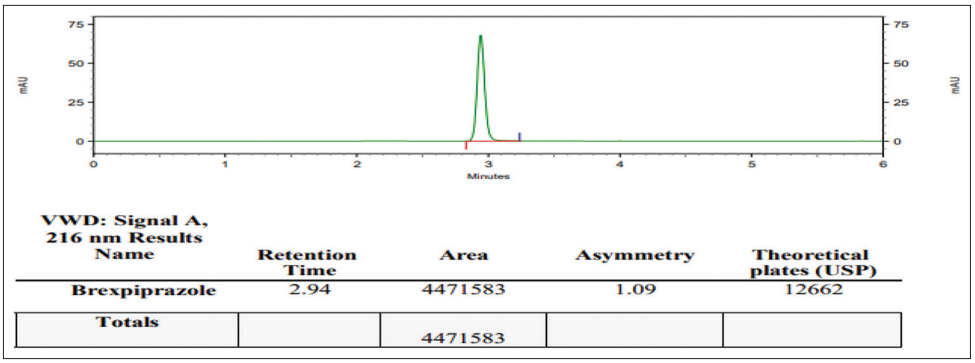


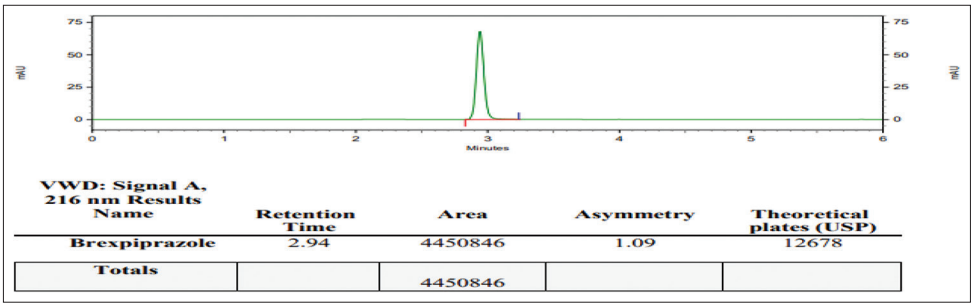
Figure 14: Chromatogram of blank mobile phase for SST



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4471583	1.09	12662
Totals		4471583		

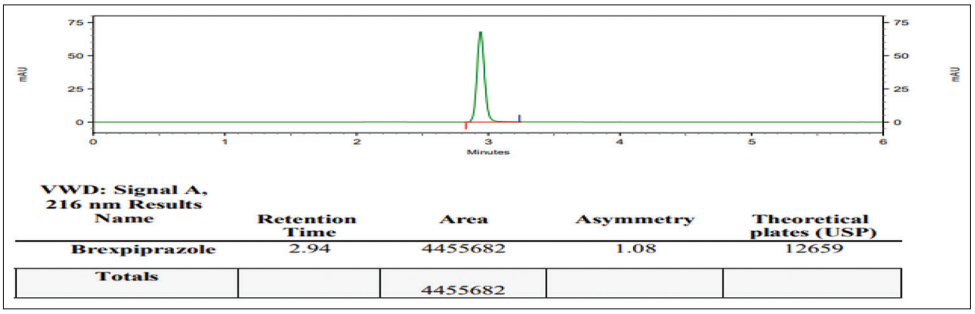
Figure 15: Typical chromatogram of unfiltered sample



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4450846	1.09	12678
Totals		4450846		

Figure 16: Typical chromatogram of sample filtered through 0.45 µ PVDF filter



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4455682	1.08	12659
Totals		4455682		

Figure 17: Typical chromatogram of sample filtered through 0: 45 µ Nylon filter

of 1 mg of Rexulti tablet. Stability study was performed at normal laboratory conditions. The solution was stored at normal illuminated laboratory conditions and analyzed after 12 h and 24 h [Table 15].

Acceptance Criteria: % Absolute difference NMT 2.0.

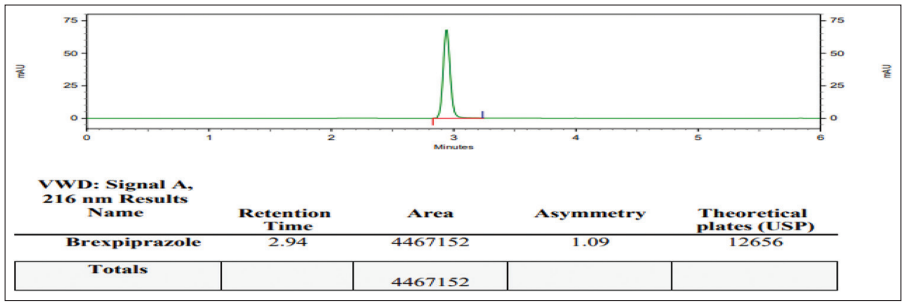
Conclusion: Both standard solution and sample solution were found stable for 24 h; hence, prepared solution can be used up to 24 h (User can check solution stability even after 24 h if he/she wants to inject solution after 24 h).

Chromatograms of solution stability: [Figures 18-23].

Specificity

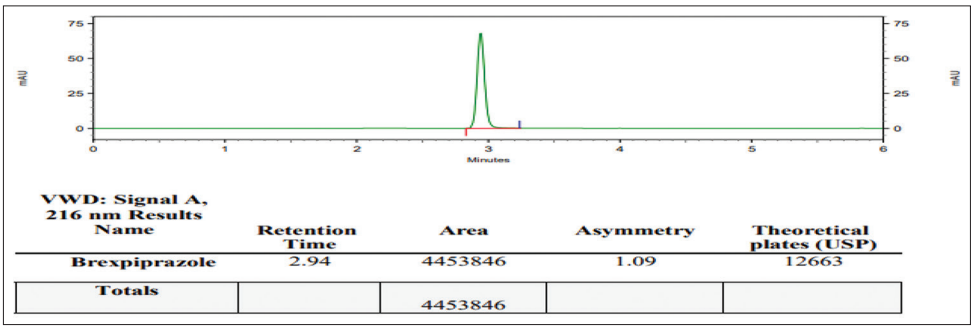
Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present.

Blank, standard solution, and test sample prepared and injected to check peak purity [Table 16].



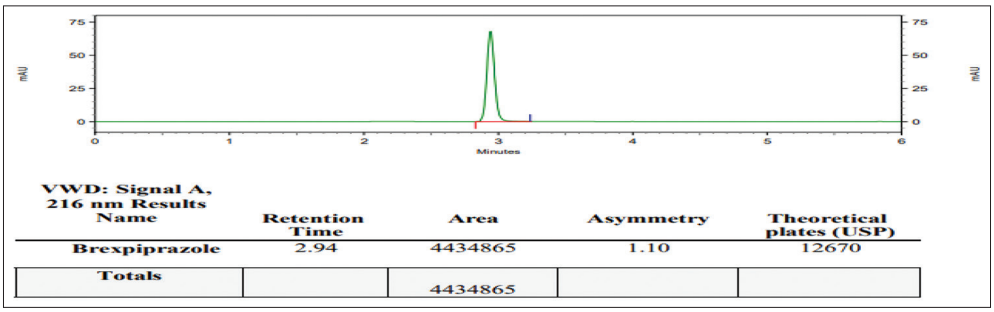
Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4467152	1.09	12656
Totals		4467152		

Figure 18: Typical chromatogram of test solution initial



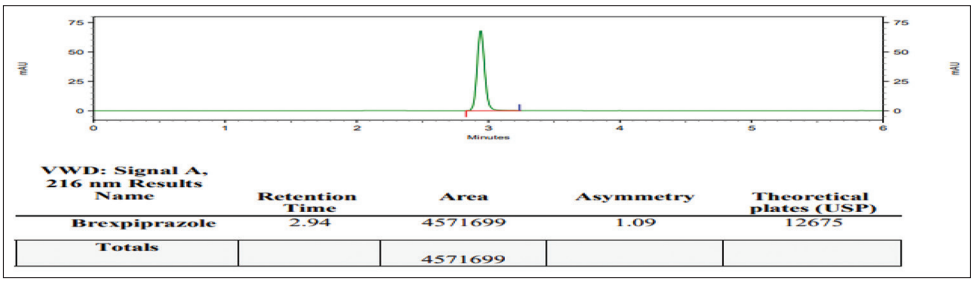
Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4453846	1.09	12663
Totals		4453846		

Figure 19: Typical chromatogram of test solution after 12 h



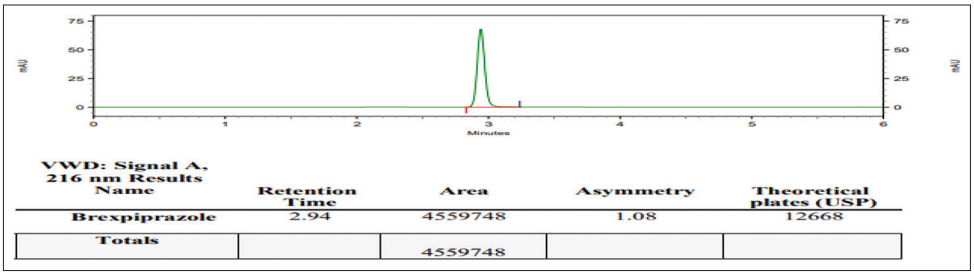
Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4434865	1.10	12670
Totals		4434865		

Figure 20: Typical chromatogram of test solution after 24 h



Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4571699	1.09	12675
Totals		4571699		

Figure 21: Typical chromatogram of standard solution initial



Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4559748	1.08	12668
Totals		4559748		

Figure 22: Typical chromatogram of standard solution after 12 h

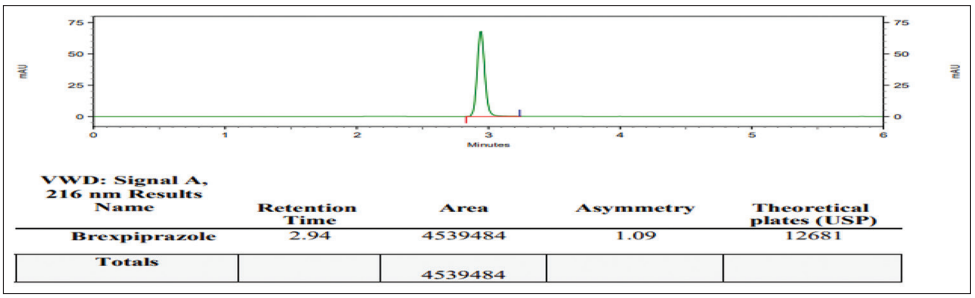


Figure 23: Typical chromatogram of standard solution after 24 h

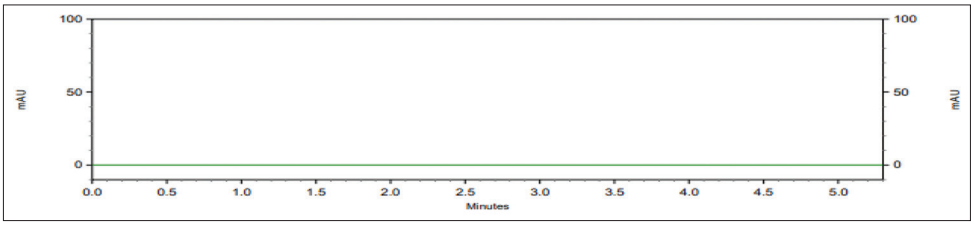


Figure 24: Typical chromatogram of blank solution

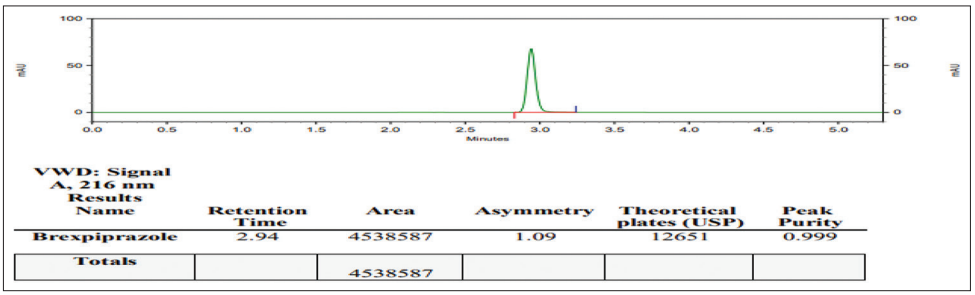


Figure 25: Typical chromatogram of standard solution

Table 16: Results of specificity

Description	*Observation*
Blank	No interference at R.T. of brexpiprazole in blank
Standard solution	Peak purity was 0.999
Sample solution	Peak purity was 0.998
Placebo	No interference at R.T. of brexpiprazole in placebo

Acceptance criteria: Blank and placebo solution: There should be no Interference at R.T. of Brexpiprazole.

Standard and sample solution: Peak purity: NLT 0.95.

Sample solution: Sample solution should exhibit at same R.T. as that of standard solution.

Conclusion: Blank and placebo solution is not having interference at R.T. of brexpiprazole. Peak purity for both standard as well as sample was within limits. Sample solution exhibits same R.T. as that of standard solution. Hence, developed chromatographic method passed the criteria for specificity.

Chromatograms for specificity: [Figures 24 and 25].

Acceptance Criteria: Blank and placebo solution: There should be no interference at R.T. of brexpiprazole standard and sample solution: Peak purity: NLT 0.95.

Test sample solution: Sample solution should exhibit at same R.T. as that of standard solution.

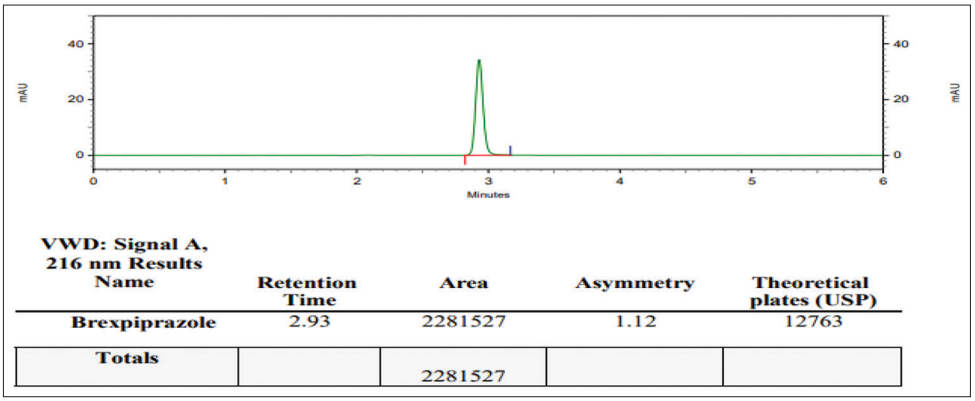
Data interpretation: Blank and placebo solution is not having interference at R.T. of brexpiprazole. Peak purity for both standard as well as sample was within limits. Sample solution exhibits same R.T. as that of standard solution. Hence, developed chromatographic method passed the criteria for specificity.

Table 17: Result and statistical data of accuracy

Level (%)	*Area*	*Added concentration*	*Recovered concentration*	*% Recovery*
50	2281527	2.55	2.53	99.22
	2235175	2.50	2.48	99.20
	2311405	2.55	2.57	100.78
100	4518476	5.05	5.02	99.41
	4602876	5.10	5.11	100.20
	4568571	5.05	5.08	100.59
150	6859143	7.55	7.62	100.93
	6789245	7.50	7.54	100.53
	6741486	7.55	7.49	99.21

Table 18: Analytical data intraday precision of brexpiprazole

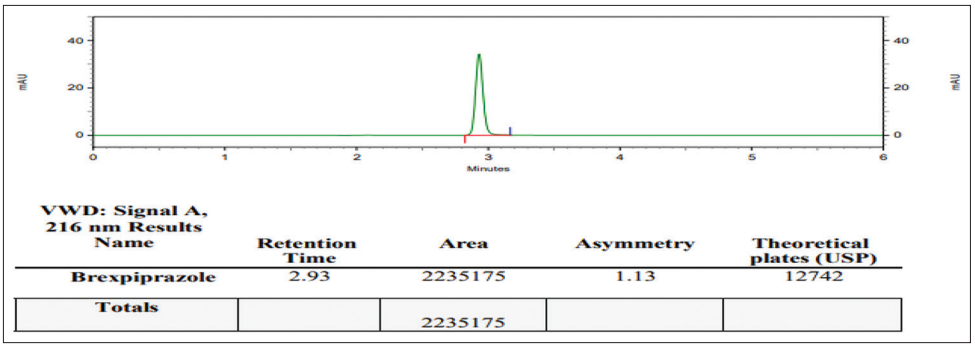
Sample	*Area*	*% Assay*
Sample 1	4498638	98.98
Sample 2	4527566	98.64
Sample 3	4625817	100.78
Sample 4	4605841	101.34
Sample 5	4667964	100.71
Sample 6	4598961	100.19
Mean		100.11
SD		1.073865
% RSD		1.073



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.93	2281527	1.12	12763
Totals		2281527		

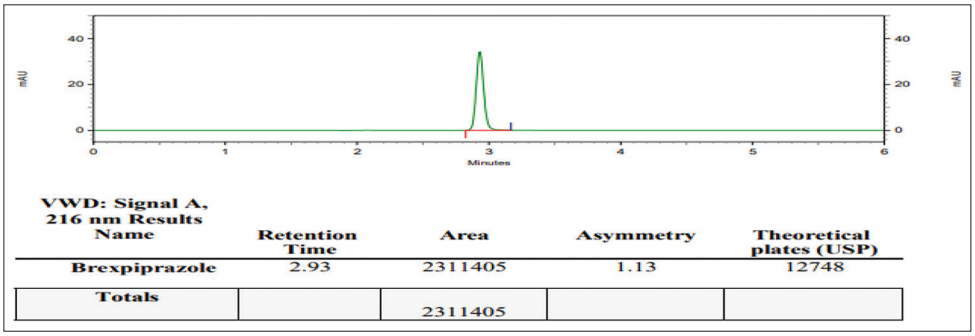
Figure 26: Chromatograms of accuracy for brexpiprazole at level of 50% -I



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.93	2235175	1.13	12742
Totals		2235175		

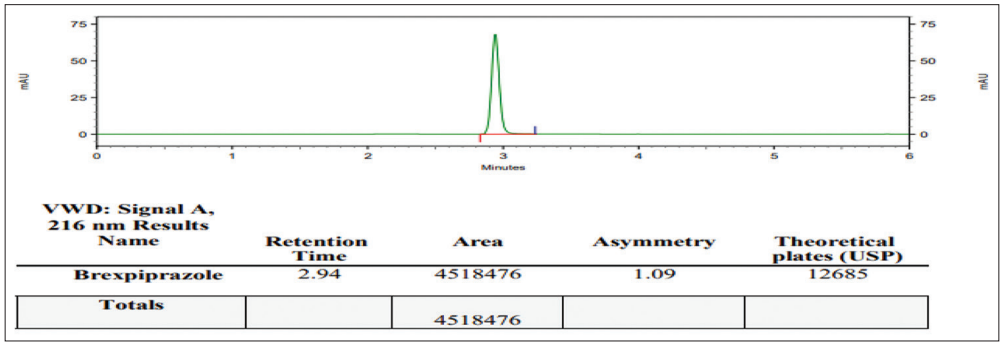
Figure 27: Chromatograms of accuracy for brexpiprazole at level of 50% -II



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.93	2311405	1.13	12748
Totals		2311405		

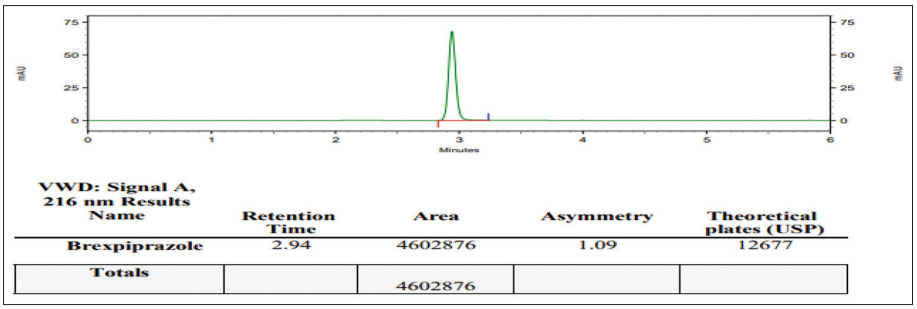
Figure 28: Chromatograms of accuracy for brexpiprazole at level of 50% -III



VWD: Signal A, 216 nm Results

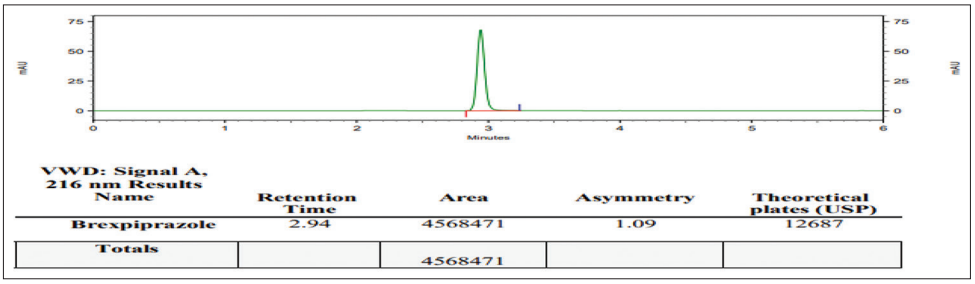
Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4518476	1.09	12685
Totals		4518476		

Figure 29: Chromatograms of accuracy for brexpiprazole at level of 100% -I



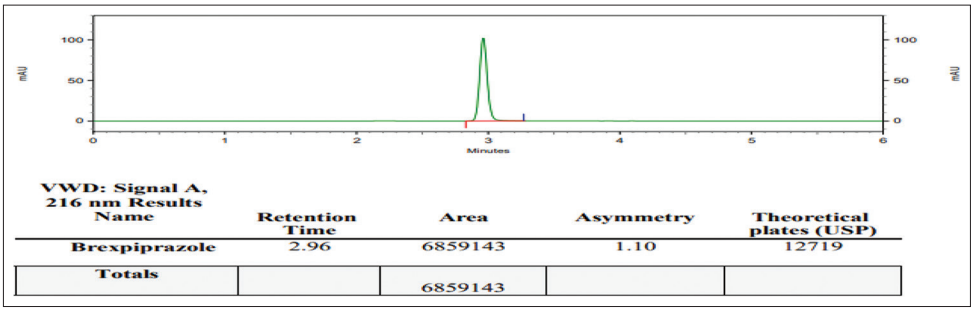
Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4602876	1.09	12677
Totals		4602876		

Figure 30: Chromatograms of accuracy for brexpiprazole at level of 100% -II



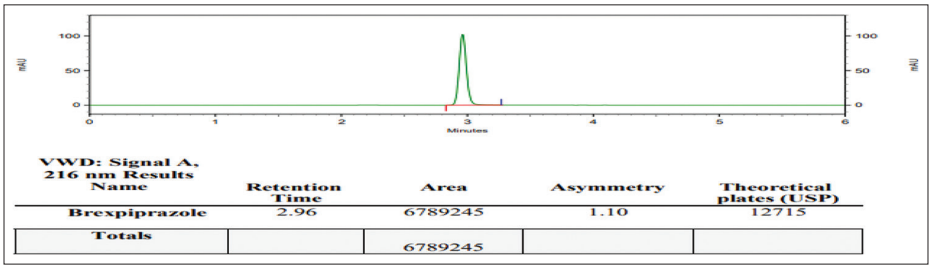
Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4568471	1.09	12687
Totals		4568471		

Figure 31: Chromatograms of accuracy for brexpiprazole at level of 100% -III



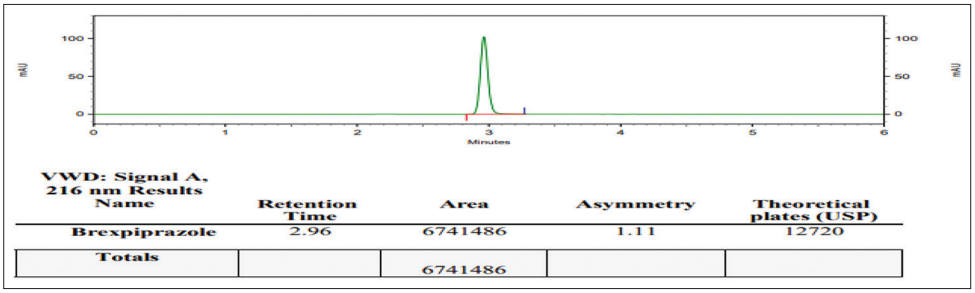
Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.96	6859143	1.10	12719
Totals		6859143		

Figure 32: Chromatograms of accuracy for brexpiprazole at level of 150% -I



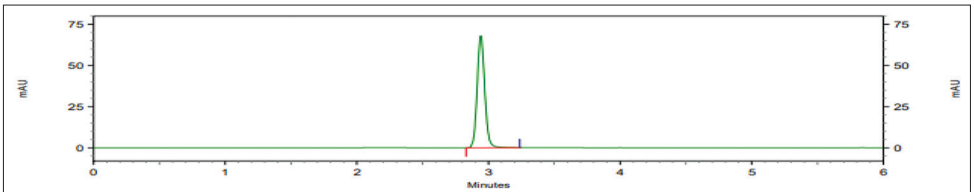
Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.96	6789245	1.10	12715
Totals		6789245		

Figure 33: Chromatograms of accuracy for brexpiprazole at a level of 150% -II



Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.96	6741486	1.11	12720
Totals		6741486		

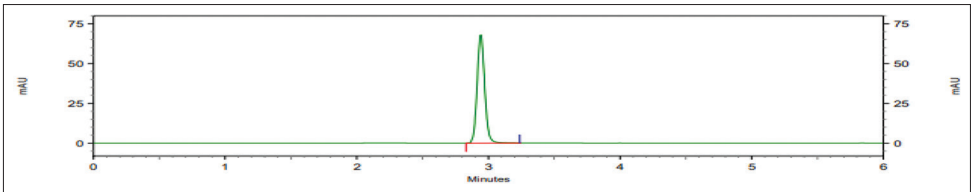
Figure 34: Chromatograms of accuracy for brexpiprazole at the level of 150% -III



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4498638	1.09	12689
Totals		4498638		

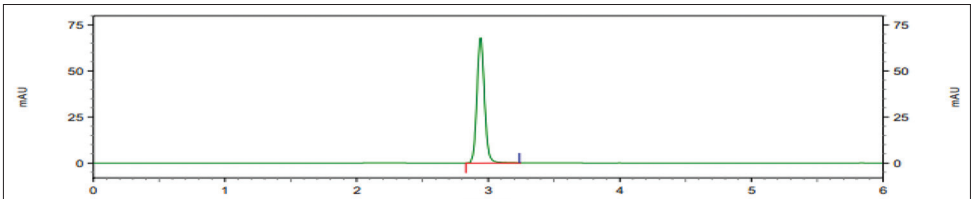
Figure 35: Chromatogram of intraday precision of brexpiprazole sample 1



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4527566	1.09	12686
Totals		4527566		

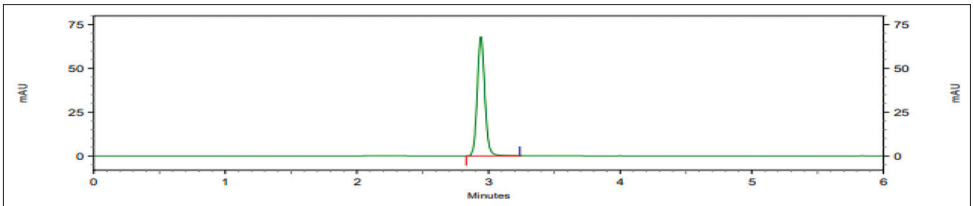
Figure 36: Chromatogram of intraday precision of brexpiprazole sample 2



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4625817	1.08	12681
Totals		4625817		

Figure 37: Chromatogram of intraday precision of brexpiprazole sample 3



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4605841	1.09	12678
Totals		4605841		

Figure 38: Chromatogram of intraday precision of brexpiprazole sample 4

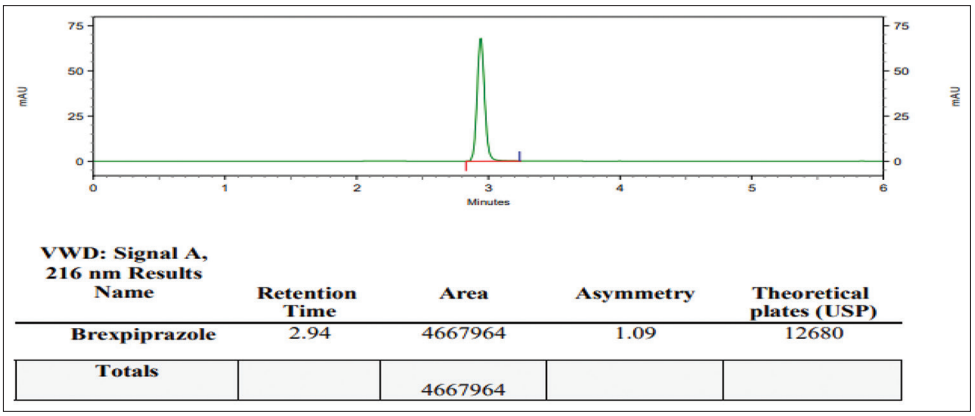


Figure 39: Chromatogram of intraday precision of brexpiprazole sample 5

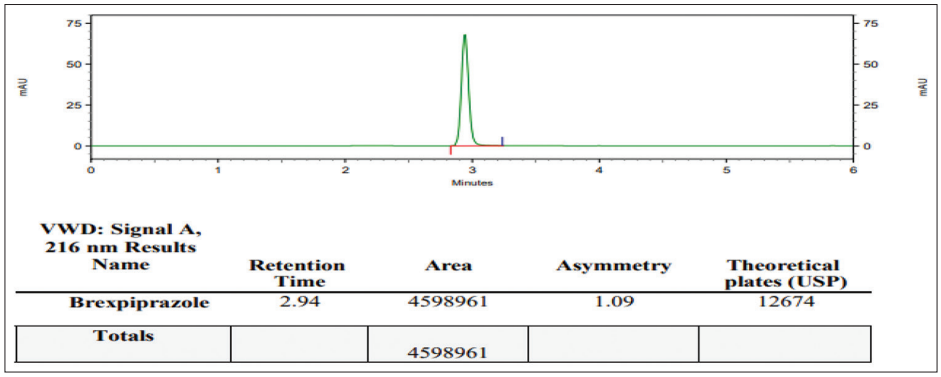


Figure 40: Chromatogram of intraday precision of brexpiprazole sample 6

Table 19: Analytical data interday precision of brexpiprazole

Sample	*Area*	*% Assay*
Sample 1	4571854	100.43
Sample 2	4520589	99.31
Sample 3	4581244	99.64
Sample 4	4628147	99.68
Sample 5	4658676	99.36
Sample 6	4541874	98.79
Mean		99.54
SD		0.542098
% RSD		0.545
Intraday plus interday precision	Mean	99.821
	SD	0.86422
	% RSD	0.866

Table 20: Result and statistical data of linearity of brexpiprazole

Level (%)	*Conc (µg/mL)*	*Area*	*Mean*	*% RSD*
10	0.51	448510	446980	0.363
		447152		
		445279		
50	2.53	2273328	2264762	0.331
		2261483		
		2259476		
100	5.05	4553284	4554584	0.147
		4561847		
		4548621		
125	6.26	5706606	5707982	0.477
		5735842		
		5681497		
150	7.58	6874760	6866853	0.110
		6859751		
		6866048		

Validation

Accuracy (%recovery): [Table 17]

Acceptance Criteria: % Recovery: 98.00–102.0%

Conclusion: % Recovery was found well within acceptance range at all three levels.

Chromatograms of accuracy for brexpiprazole [Figures 26-34].

Table 21: Data for calibration curve of brexpiprazole

Parameters	*Result*
Detection Wavelength	216 nm
Beer's law limit	5.05–75.75 µg/mL
Slope	1620101.119
Intercept	–279840.1481
Correlation coefficient (R2)	0.9999

Precision

i. Intraday precision: [Table 18]

Acceptance Criteria: The % RSD for six samples NMT 2.0

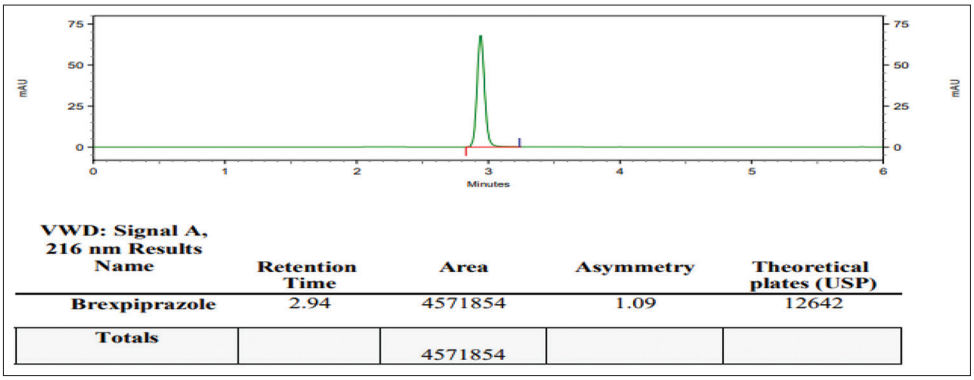
Conclusion: Precision: Precision pass the criteria, no variation found by preparing six different samples. Results are good reproducible.

Chromatogram of intraday precision of brexpiprazole: [Figures 35-40].

ii. Interday precision of brexpiprazole: [Table 19]

Acceptance criteria: The % RSD for the six samples NMT 2.0.

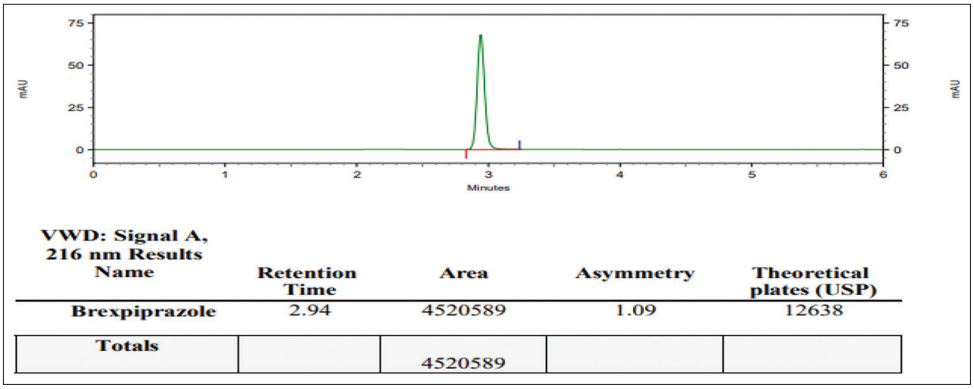
For both: % RSD for 12 sample (Precision and Intermediate



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4571854	1.09	12642
Totals		4571854		

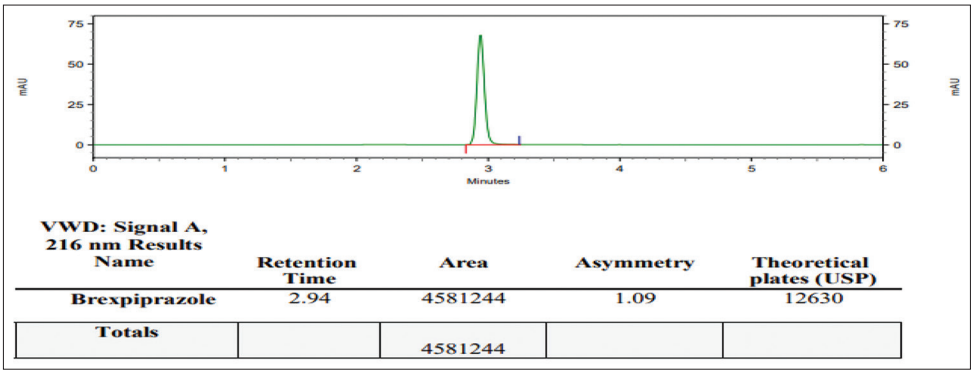
Figure 41: Chromatogram of interday precision for brexpiprazole sample 1



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4520589	1.09	12638
Totals		4520589		

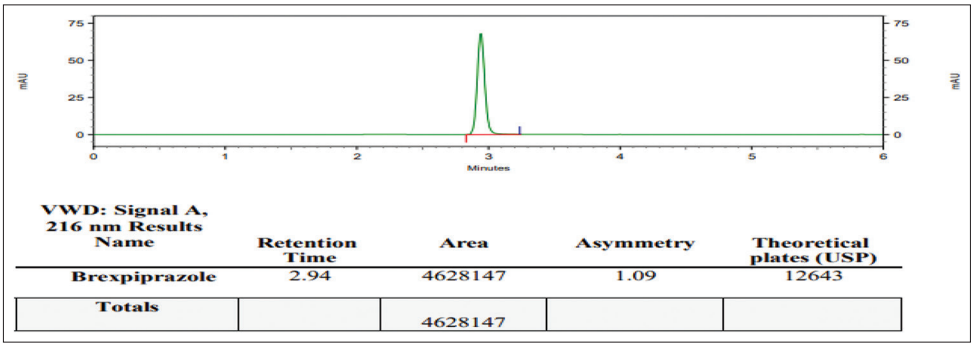
Figure 42: Chromatogram of interday precision for brexpiprazole sample 2



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4581244	1.09	12630
Totals		4581244		

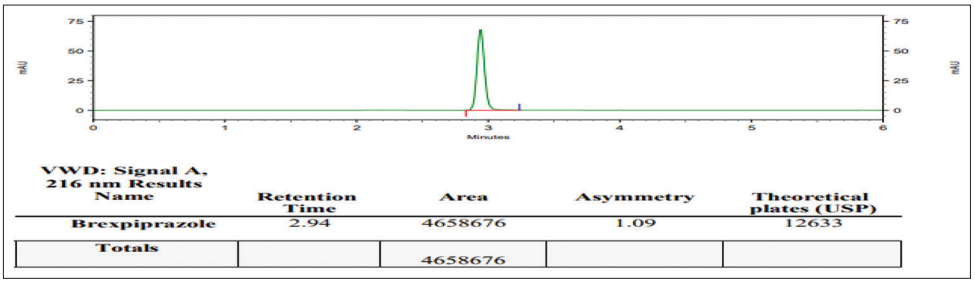
Figure 43: Chromatogram of interday precision for brexpiprazole sample 3



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4628147	1.09	12643
Totals		4628147		

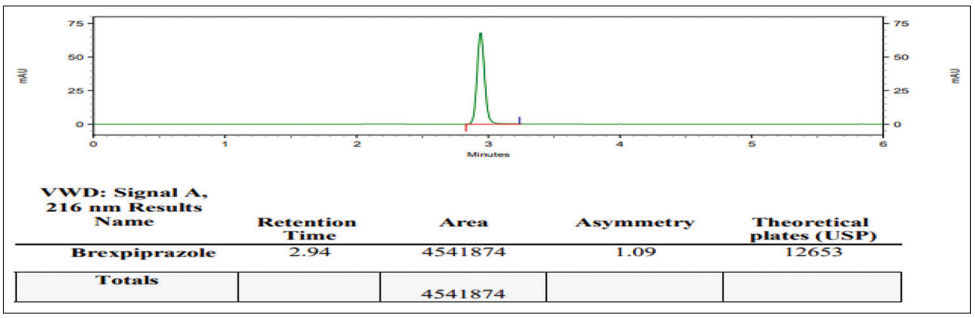
Figure 44: Chromatogram of interday precision for brexpiprazole sample 4



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4658676	1.09	12633
Totals		4658676		

Figure 45: Chromatogram of interday precision for brexpiprazole sample 5



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4541874	1.09	12653
Totals		4541874		

Figure 46: Chromatogram of interday precision for brexpiprazole sample 6

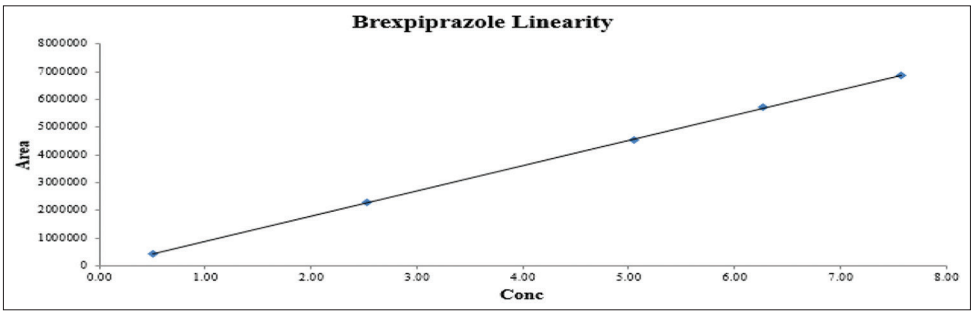
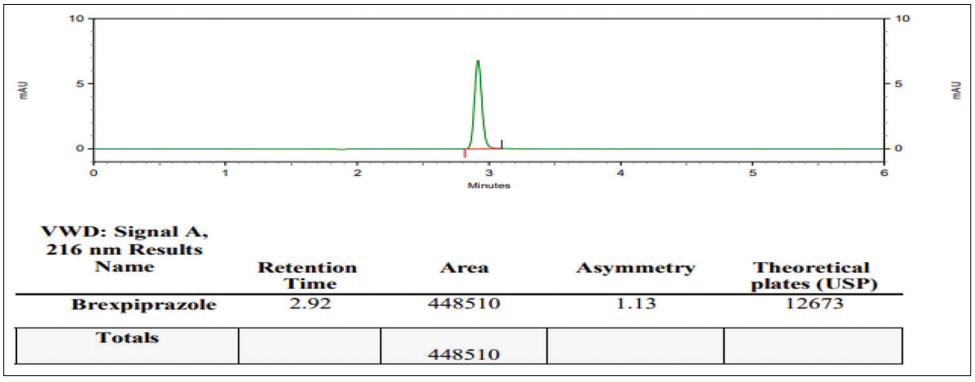


Figure 47: Linearity graph of brexpiprazole



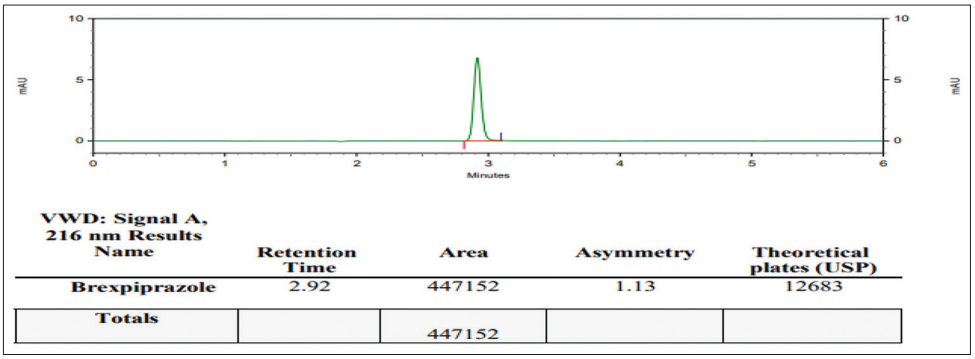
VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.92	448510	1.13	12673
Totals		448510		

Figure 48: Chromatogram of linearity for brexpiprazole sample of 10% level-I

Table 22: Data for change in flow rate [+10%] for robustness

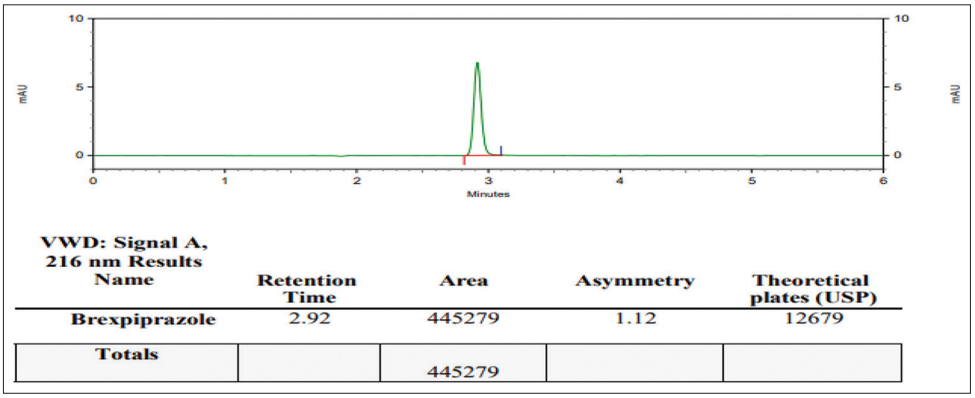
S. No.	*Standard deviation*	*Retention time*	*Area*	*Asymmetry*	*Theoretical plates*
1.	Standard	2.68	4149428	1.08	11654
2.	Sample	2.68	4115739	1.08	11643



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.92	447152	1.13	12683
Totals		447152		

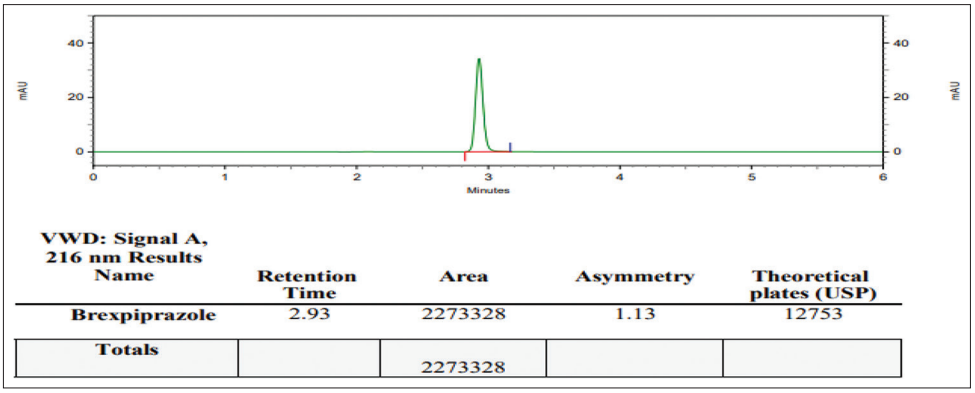
Figure 49: Chromatogram of linearity for brexpiprazole sample of 10% level-II



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.92	445279	1.12	12679
Totals		445279		

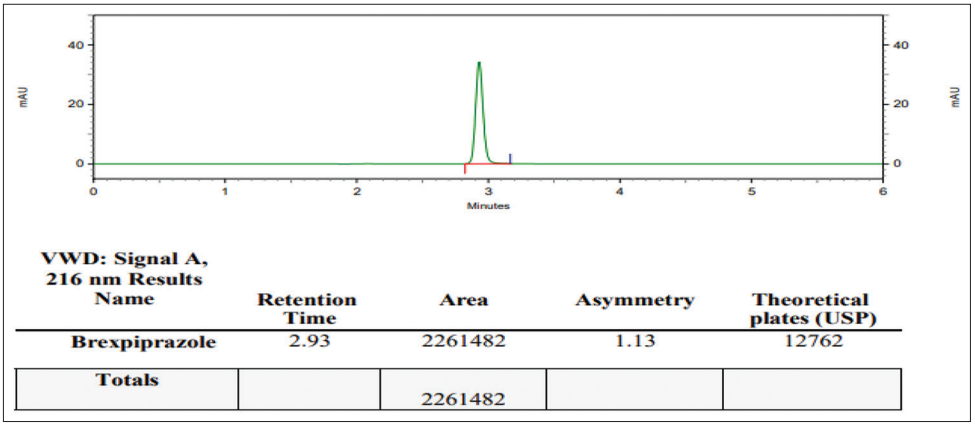
Figure 50: Chromatogram of linearity for brexpiprazole sample of 10% level-III



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.93	2273328	1.13	12753
Totals		2273328		

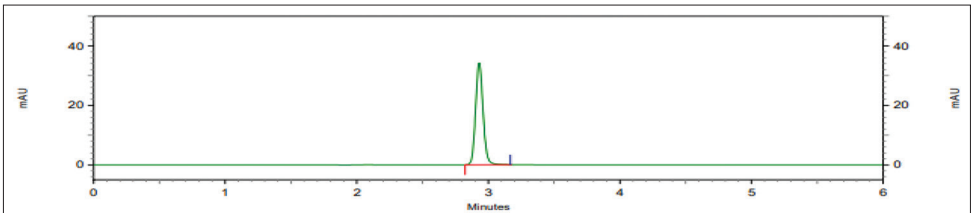
Figure 51: Chromatogram of linearity for brexpiprazole sample of 50% level-I



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.93	2261482	1.13	12762
Totals		2261482		

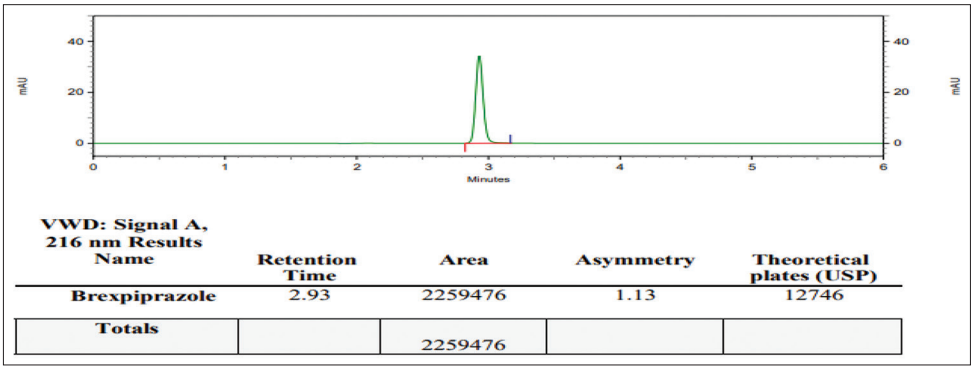
Figure 52: Chromatogram of linearity for brexpiprazole sample of 50% level-II



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.93	2261482	1.13	12762
Totals		2261482		

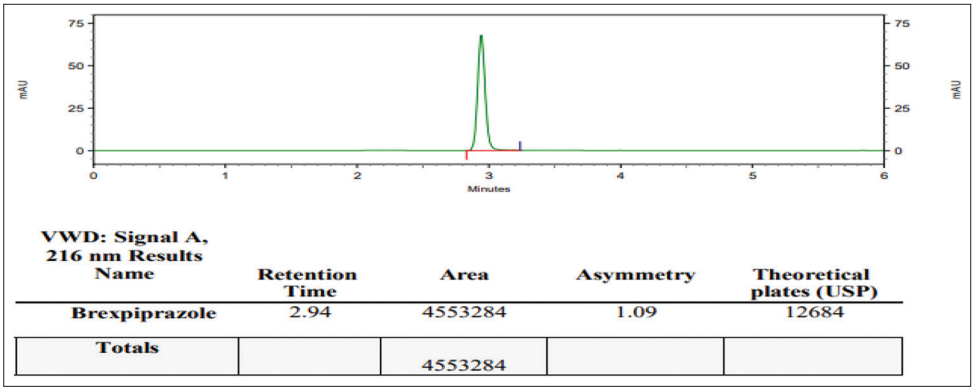
Figure 52: Chromatogram of linearity for brexpiprazole sample of 50% level-II



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.93	2259476	1.13	12746
Totals		2259476		

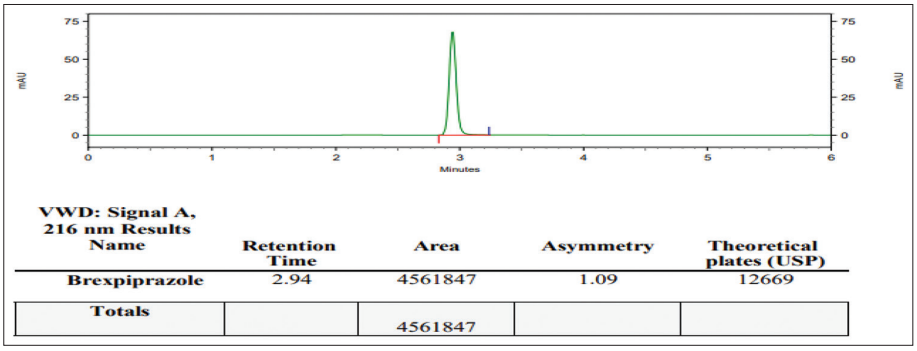
Figure 53: Chromatogram of linearity for brexpiprazole sample of 50% level-III



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4553284	1.09	12684
Totals		4553284		

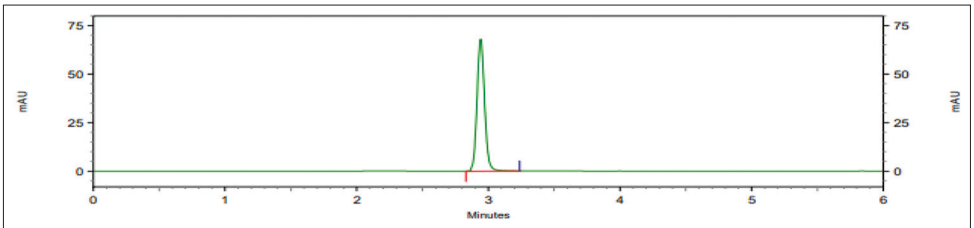
Figure 54: Chromatogram of linearity for brexpiprazole sample of 100% level-I



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4561847	1.09	12669
Totals		4561847		

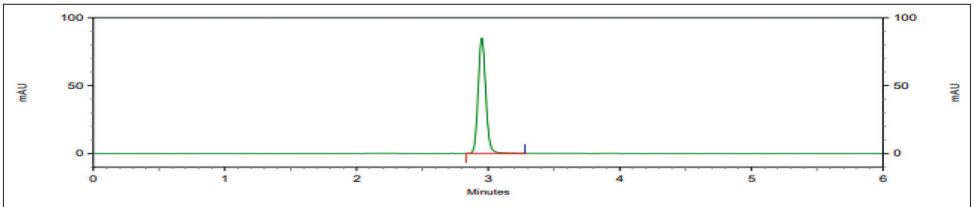
Figure 55: Chromatogram of linearity for brexpiprazole sample of 100% level-II



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.94	4548621	1.10	12677
Totals		4548621		

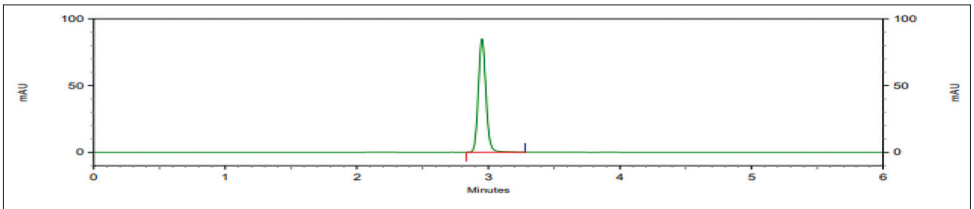
Figure 56: Chromatogram of linearity for brexpiprazole sample of 100% level-III



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.95	5706606	1.09	12689
Totals		5706606		

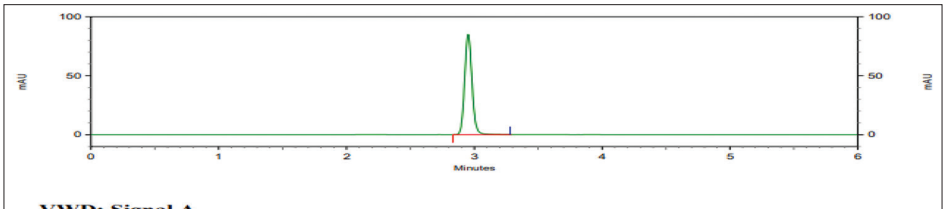
Figure 57: Chromatogram of linearity for brexpiprazole sample of 125% level-I



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.95	5735842	1.09	12686
Totals		5735842		

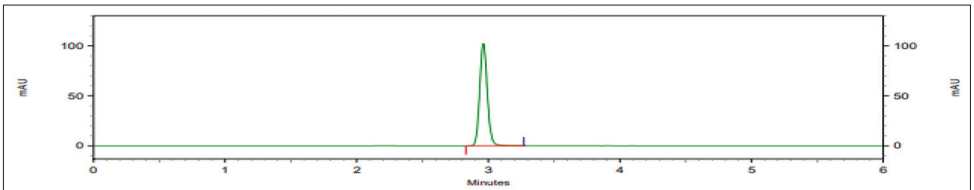
Figure 58: Chromatogram of linearity for brexpiprazole sample of 125% level-II



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.95	5681497	1.09	12691
Totals		5681497		

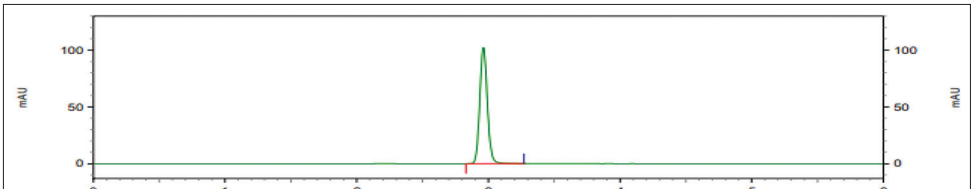
Figure 59: Chromatogram of linearity for brexpiprazole sample of 125% level-III



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.96	6874760	1.10	12705
Totals		6874760		

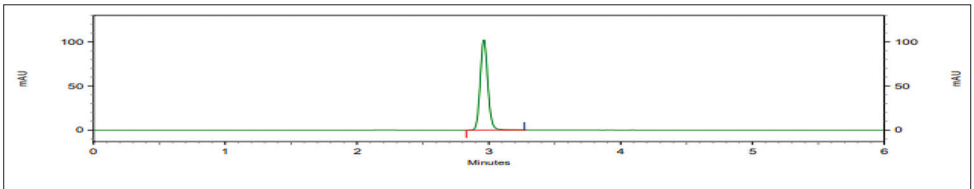
Figure 60: Chromatogram of linearity for brexpiprazole sample of 150% level-I



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.96	6859751	1.10	12710
Totals		6859751		

Figure 61: Chromatogram of linearity for brexpiprazole sample of 150% level-II



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	2.96	6866048	1.10	12701
Totals		6866048		

Figure 62: Chromatogram of linearity for brexpiprazole sample of 150% level-III

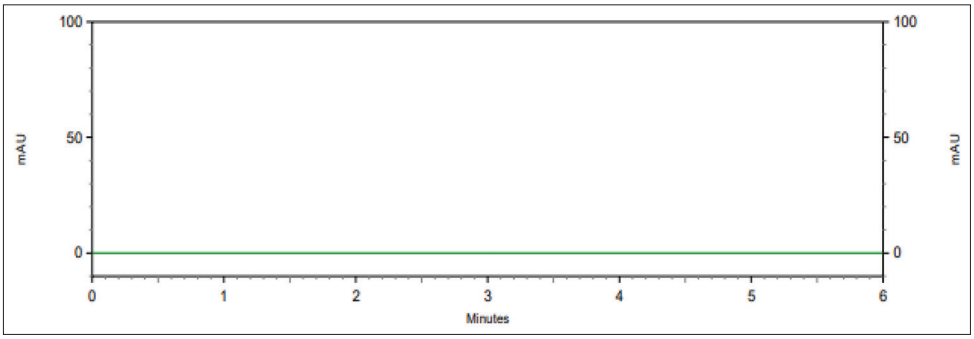


Figure 63: Chromatogram of blank for change in flow rate as such +10%

Precision samples) NMT 2.0%

Conclusion: Precision: The %RSD of method precision is 0.53 and 0.495; therefore, the HPLC method for the determination of brexpiprazole is precise.

Chromatogram of interday precision of brexpiprazole: [Figures 41-46].

Linearity [Table 20]

From the calibration curve, we had to conclude that the brexpiprazole shows linear response in the range of 5.05–75.75 µg/mL. The regression value was found well within the limit.

Linearity graph of brexpiprazole: [Figures 47].

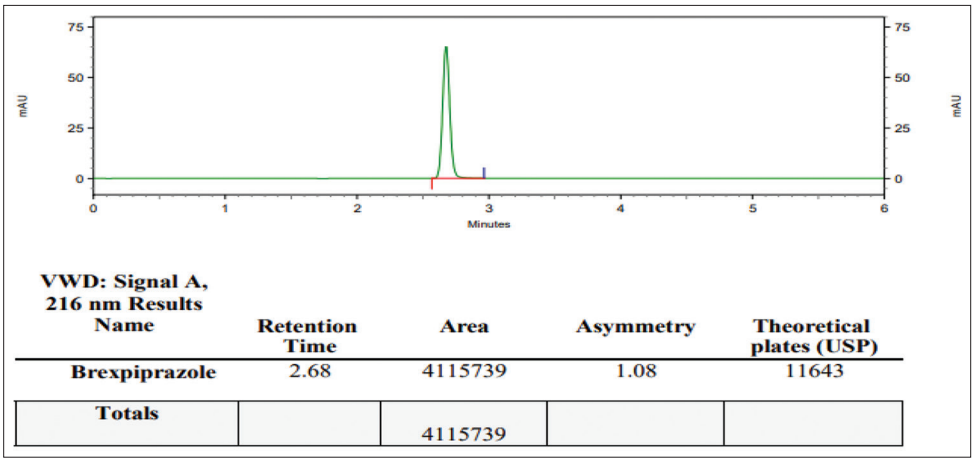


Figure 64: Chromatogram of sample for change in flow rate as such+10%

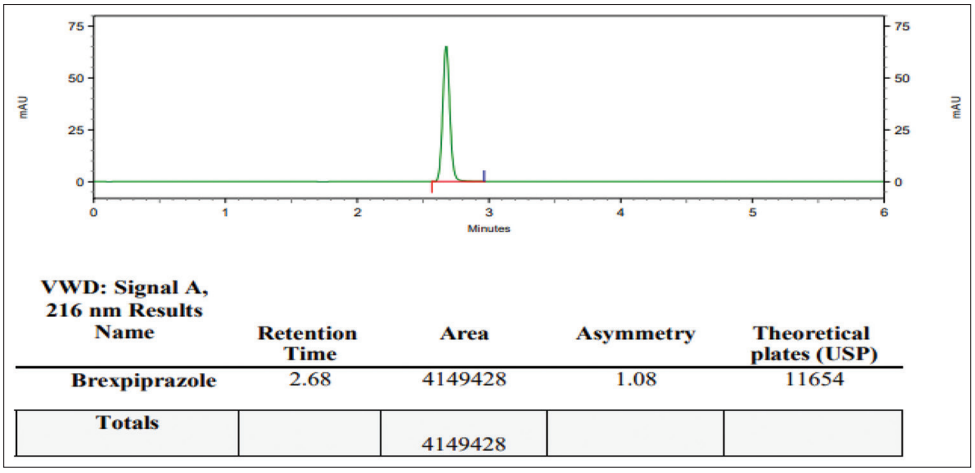


Figure 65: Chromatogram of standard for change in flow rate as such+10%

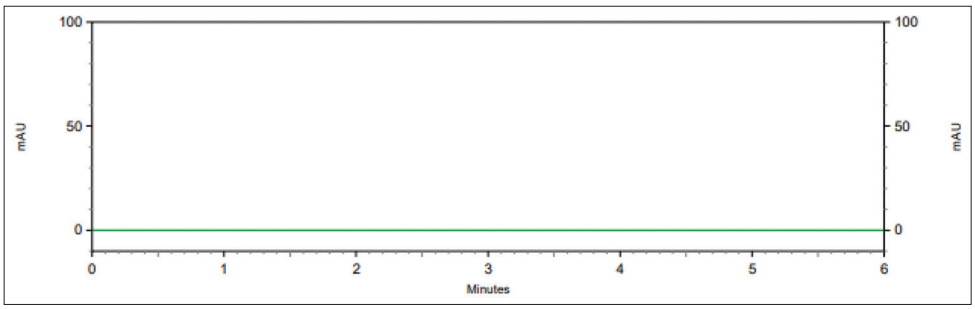
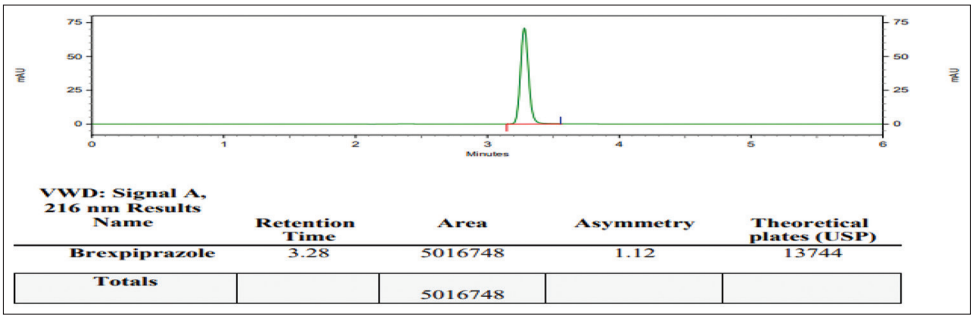


Figure 66: Chromatogram of blank for change in flow rate as such -10%

Table 23: Data for change in flow rate [−10%] for robustness

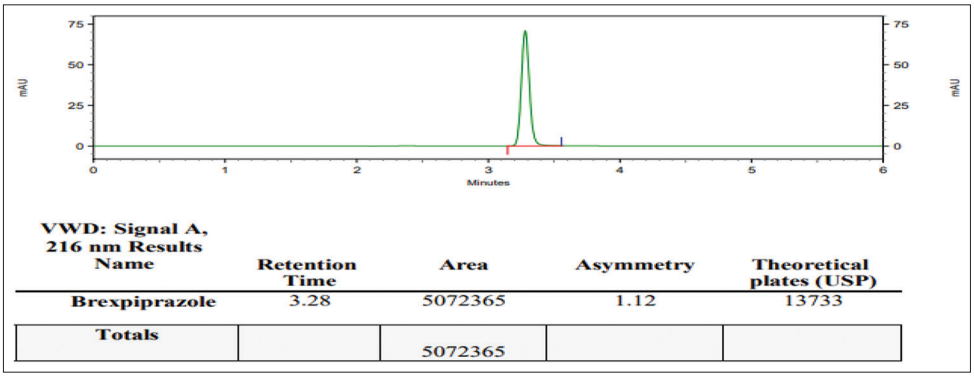
S. No.	*Standard deviation*	*Retention time*	*Area*	*Asymmetry*	*Theoretical plates*
1.	Standard	3.28	5072365	1.12	13773
2.	Sample	3.28	5016748	1.12	13747



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	3.28	5016748	1.12	13744
Totals		5016748		

Figure 67: Chromatogram of sample for change in flow rate as such -10%



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Brexpiprazole	3.28	5072365	1.12	13733
Totals		5072365		

Figure 68: Chromatogram of standard for change in flow rate as such -10%

Data for calibration curve of brexpiprazole [Table 21].
Chromatogram of linearity for brexpiprazole: [Figures 48-62].

Robustness

Effect of variation in the flow rate of the mobile phase
Change in flow rate:

i. Data for change in flow rate [+10%] for robustness [Table 22]
Chromatogram for robustness: [Figures 63-65]
ii. Data for change in flow rate [-10%] for robustness [Table 23]
Chromatogram for robustness: [Figures 66-68]

According to the above experimental results, this newly developed method for estimating brexpiprazole was found to be simple, precise, and accurate, with a shorter retention time that makes it more acceptable and cost effective, and it can be effectively applied for routine analysis in research institutions, quality control departments in industries, and approved testing laboratories.

According to the above experimental results, this newly developed method for estimating brexpiprazole was found to be simple, precise, and accurate, with a shorter retention time that makes it more acceptable and cost effective, and it can be effectively applied for routine analysis in research institutions, quality control departments in industries, and approved testing laboratories.

Conclusion

The proposed method showed acceptable precision, accuracy, sensitivity, and wide linear concentration range. The established reverse-phase high-performance liquid chromatography (RP-HPLC) method was found to be reliable for the analysis of brexpiprazole and was found to be simple, consistent, cost-effective, and precise. Therefore, this RP-HPLC method for determination of brexpiprazole can be used in quality control or routine for its quantitative determination in bulk and pharmaceutical dosage form.

Acknowledgments

The authors are thankful to Prof T. J. Sawant founder secretary Jayawant Shikshan Prasarak Mandal and Dr. V. V. Potnis Principal JSPM's Jayawantrao Sawant College of Pharmacy and Research, Handewadi, Pune, for providing all necessary facilities.

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