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GRADIENT HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR DETERMINATION OF RELATED SUBSTANCES IN BREXPIPRAZOLE API

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ABSTRACT

The purpose of this research study is to develop a novel, simple, precise, accurate and economical method for determination of related substances in Brexpiprazole API. The chromatographic method for determination of related substances was developed on Kromasil C8, (250 mm x 4.6 mm, 5 µm) Column with tertiary gradient program. The mobiles phase used for separation was (a) Dipotassium hydrogen phosphate buffer with pH 5.5 ±0.05, (b) Mix of 9 volumes of acetonitrile and 1 volume of Tetrahydrofuran and (c) Methanol. At 254nm wavelength all releted substances were detected and responses of all related substances with limit level is very good. The detection of all related substances is about 0.33ppm and quantification is 1.5ppm.This developed method was validated as per ICH guideline and found out to be linear, accurate, specific, selective, precise, and robust. Test solution was found to be stable for 24 hrs. The method can be successfully applied for the determination of related substances in Brexpiprazole API.

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INTRODUCTION

Background of the Invention: 7-(4-(4-(benzo[b]thiophen-4-yl) piperazin-1-yl)butoxy)quinolin-2(1H)-one is a novel serotonin dopamine activity modulator with partial agonist activity at serotonin -1A(5-HT1A) and D2/3 receptors, combined with potent antagonist effect on 5–HT2A, α1B, and α2C adrenergic receptors. Brexpiprazole, discovered by Otsuka, is a dopamine D2 receptor partial agonist. It has been recently approved by the FDA for the treatment of schizophrenia and as an adjunctive therapy for the treatment of major depression. Twenty antipsychotic medications are currently approved for clinical use in the US with Brexpiprazole being one of the most recent agents to become available. Despite the availability of a number of antipsychotic medications, many patients either do not benefit from or develop significant side effects to currently available agents. Brexpiprazole is used in the treatment of agitation associated with Alzheimer's disease, Attention-deficit/hyperactivity disorder, Post-traumatic stress disorder, treatment of bipolar disorder, Adjunctive Treatment of major Depressive Disorder, and Schizophrenia. Brexpiprazole is more potent than the other class of antipsychotic drug as well as aripiprazole, therefore lower dose can be used. Brexpiprazole has a higher affinity for serotonin 5HT1A receptors. Aripiprazole affects the same receptors but to a lesser extent. This may give Brexpiprazole advantage over aripiprazole. Brexpiprazole has a lower side effect like akathisia and extra pyramidal symptoms than aripiprazole and other class of antipsychotic drug. To prove his activity and product purity we had developed the high performance liquid chromatography gradient method for its related substances. The structure of API is given in Figure no 1.0

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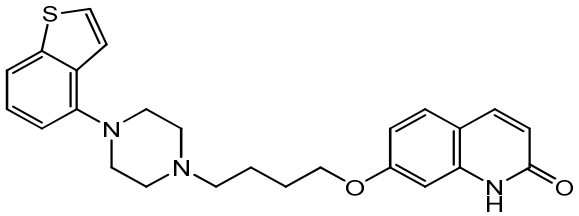
MATERIALS

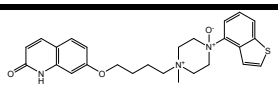
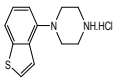
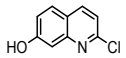
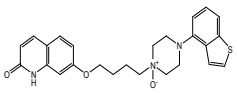
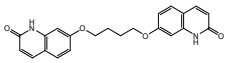
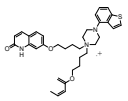
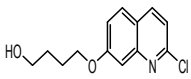
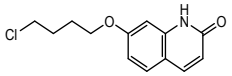
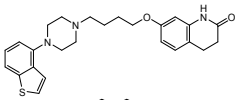
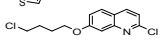
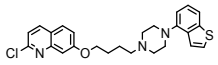
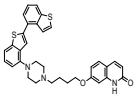
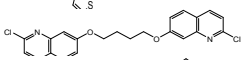
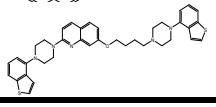
Reagent and Chemicals: Brexpiprazole sample, working standard and its related substances working standards were received from Analytical research and development department of Indoco Research Centre (Navi Mumbai).



Chemical / IUPAC name: 7-(4-(4-(benzo[b]thiophen-4-yl) piperazin-1-yl) butoxy)quinolin-2(1H)-one

Figure 1. Structure of Brexpiprazole

Table 1. Chemical name of Brexpiprazole and its related substances

Sr.No	Component Name	Chemical name	Structure
1	Impurity D	1-(benzo[b]thiophen-4-yl)-4-(4-((2-oxo-1,2-dihydroquinolin-7-yl)oxy)butyl)piperazine 1,4-dioxide.	
2	BRX1	1-(benzo[b]thiophen-4-yl)piperazine hydrochloride.	
3	BRX3	2-chloroquinolin-7-ol	
4	N-Monooxide	4-(benzo[b]thiophen-4-yl)-1-(4-((2-oxo-1,2-dihydroquinolin-7-yl)oxy)butyl) piperazine 1-oxide	
5	Impurity A	7,7'-(butane-1,4-diylbis(oxy))bis(quinolin-2(1H)-one)	
6	HDBR	7,7'-(((4-(benzo[b]thiophen-4-yl)-1l4-piperazine-1,1-diyl)bis(butane-4,1-diyl))bis(oxy))bis(quinolin-2(1H)-one).	
7	COBO	4-((2-chloroquinolin-7-yl)oxy)butan-1-ol	
8	BPZ3	7-(4-chlorobutoxy)quinolin-2(1H)-one	
9	Impurity E	7-(4-(4-(benzo[b]thiophen-4-yl)piperazin-1-yl)butoxy)-3,4-dihydroquinolin-2(1H)-one	
10	BRX4	2-chloro-7-(4-chlorobutoxy) quinoline	
11	BRX5	7-(4-(4-(benzo[b]thiophen-4-yl) piperazin-1-yl)butoxy)-2-chloroquinoline	
12	BTBQ	7-(4-(4-([2,4'-bibenzo[b]thiophen]-4-yl)piperazin-1-yl)butoxy)quinolin-2(1H)-one	
13	DBTP	1,4-bis(benzo[b]thiophen-4-yl)piperazine	
14	BCQL	1,4-bis((2-chloroquinolin-7-yl)oxy)butane	
15	BPTP	2-(4-(benzo[b]thiophen-4-yl)piperazin-1-yl)-7-(4-(4-(benzo[b]thiophen-4-yl)piperazin-1-yl)butoxy)quinoline	

HPLC grade Ortho phosphoric acid, Acetonitrile, Tetrahydrofuran, Methnaol and Dimethyl formamide were purchased from Merck (India).

Instrumentation: Waters, Alliance 2695 series HPLC system comprising a quaternary pump, an autosampler, a thermostatted column compartment, a solvent cabinet with degasser along with photodiode array (PDA) 2998 and ultraviolet (UV) 2487 detectors were used for separation and detection. Data acquisition and calculations were carried out using Waters Empower3 software (Milford). Sartorius (Germany) analytical balance was used for weighing material.

Chromatographic Conditions:

Column	Kromasil C8, (250 mm x 4.6 mm, 5 µm) or equivalent			
Column Temperature	40°C ± 2°C			
Flow Rate	1.2 mL/min			
Gradient program	Time (min)	Mobile phase-A (%)	Mobile phase-B (%)	Mobile phase-C (%)
	0	85	15	0
	16	65	35	0
	23	65	35	0
	31	35	60.3	4.7
	40	20	73	7
	55	20	80	0
	60	85	15	0
	65	85	15	0
Injection Volume	10 mL			
Detector Wavelength	254 nm			
Run Time	65 minutes			
Retention Time	Brexpiprazole about 28.9 minutes			
Needle wash	Acetonitrile: Water (80:20)			

Injection sequence

SI#	Description	No. of Injections
1	Blank	1
2	System suitability solution	1
3	Blank	1
4	Reference solution (a)	5
5	Test solution	2

MATERIALS AND METHODS

Method optimization

The Brexpiprazole API has multi known impurities. To seperrate those know impurities by hplc techenique we used verious stationary phases column like C8,C18, cyno and phenyl. Base on raw data outcome and our observation we decide that Kromasil C8, (250 mm x 4.6 mm, 5 µm) column may work for impurities separation. We injected impurities on selected column in isocratic method and observed the raw data, All impurities were not well separated and not eluted in isocratic method hence we try for gradient technology. In binary gradient, two impurities are co-eluting at same retention time. Hence we tried tertiary gradient, as well as various combinations of mobile phases and its pH with or without additive. Finally we optimize buffer as Dipotassium hydrogen phosphate with three different solvent like Tetrahydrofuran, methanol and acetonitrile. Flow was optimize to 1.2 ml/min. Details of optimized chromatographic parameters are given in below. For suitability of system check we kept Brexpiprazole API theoritical plate should not be less than 5000 and its relative standard deviation for replicate injections should not be more than 5.0%. Where as retention time conformation we injected BPTP working standard, BRX5and BRX working standard.

Preparation of Mobile Phase

Mobile Phase-A: Transfer about 0.87 g of Dipotassium hydrogen phosphate into 1L bottle, containing 1000 mL of water, dissolve in it, shake well and adjust the pH of solution to 5.5 ±0.05 with ortho phosphoric acid. Filter the solution through a 0.45 µm membrane filter and degas by sonication for 2 minutes.

Mobile Phase-B: Mix 9 volumes of acetonitrile and 1 volume of Tetrahydrofuran and degas by sonication for 2 mins.

Mobile Phase-C: Methanol

Diluent: Mix equal volumes of acetonitrile and methanol, shake well and degas by sonication for 2 mins.

Preparation of Blank: Transfer 4.0 mL of dimethyl formamide into 10 mL volumetric flask and make upto the mark with diluent.

Preparation of solution

System suitability solution: Transfer about 5 mg each of BPTP working standard, Brexpiprazole working standard and BRX5 working standard into 50 mL volumetric flask, add 4.0 mL of dimethyl formamide, dissolve in it and make upto the mark with diluent.

Reference solution (a): Transfer about 50 mg of Brexpiprazole working standard into 10 mL volumetric flask, add 4.0 mL of dimethyl formamide, dissolve in it and make upto the mark with diluent. Transfer 1.0 mL of this solution into 100 mL volumetric flask and make upto the mark with diluent. Further transfer 1.0 mL of above solution into 10 mL volumetric flask and make upto the mark with diluent.

Test solution: Transfer about 50 mg of Brexpiprazole sample into 10 mL volumetric flask, add 4.0 mL of dimethyl formamide, dissolve in it and make upto the mark with diluent.

Procedure: Equilibrate the HPLC system with the initial composition until a steady baseline is obtained. Inject blank and system suitability as per injection sequence. Ensure that system suitability parameters meet the requirements. Inject blank, reference solution (a) and test solutions as per injection sequence given into the chromatograph and record the chromatograms, make blank corrections if necessary.

Name of Impurity	Relative Retention Time	Relative Response factor
Peak Name		
Impurity D	0.28	0.52
BRX1	0.46	0.67
BRX3	0.65	0.9
N-Monoxide	0.7	0.91
Impurity A	0.73	0.81
HDBR	0.79	0.67
COBO	0.88	0.78
BPZ3	0.94	0.78
Impurity E	1.03	1.11
BRX4	1.25	0.71
BRX5	1.28	0.86
BTBQ	1.32	1.33
DBTP	1.46	0.86
BCQL	1.36	0.6
BPTP	1.57	2.97

System suitability

Acceptance criteria

Number of theoretical plates: The number of theoretical plates calculated for the peak due to BRX should not be less than 5000 in the chromatogram obtained with system suitability solution.

%RSD: The percent relative standard deviation of five replicates for the peak due to BRX in the chromatogram obtained with reference solution (a) should not be more than 5.0.

Tailing factor: The tailing factor for peak due to BRX in the chromatogram obtained with system suitability solution should not be more than 2.0.

Calculation

Calculate Impurity content by formula given below and report the average content:

$$\% \text{ Known Impurity} = \frac{AI \times WS \times 1 \times 1 \times P}{AR \times WT \times 100 \times 10 \times RRF}$$

$$\% \text{ Any unspecified impurity} = \frac{AU \times WS \times 1 \times 1}{AR \times WT \times 100 \times 10} \times P$$

$$\% \text{ Total unknown impurities} = \frac{AS \times WS \times 1 \times 1}{AR \times WT \times 100 \times 10} \times P$$

% Total impurities = [% known impurities + %Total unknown impurities]

Where,

AI = Average peak area for respective impurities in test solution.
AR = Average peak area of BRX in reference solution (a).

Analytical Method Validation

The developed Method is subjected to analytical method validation, which is conducted according to the International council for Harmonisation (ICH) guidelines. The parameter with which analytical method is validated is specificity, limit of detection, limit of quantitation, linearity, accuracy, precision, robustness and solution stability.

RESULTS AND DISCUSSION

System suitability

The System suitability test represents as an integral part of the method and used to ensure adequate performance of the chromatographic system. To check the system suitability, system suitability solution was injected and observed the retention time of BPTP and Brexpiprazol then further injecting five replicate injections of Reference solution(a) and calculate percent relative standard deviation for the peak area of Brexpraparazole and recorded the data in Table 2. The percent relative standard deviation should be less than 5.0. System suitability was checked before each validation parameter.

Table 2. System suitability data

Name	No Of Injection	Area
Reference Solution (a)	Injection-1	48430
	Injection-2	48238
	Injection-3	48017
	Injection-4	47974
	Injection-5	48127
	Avg. Area	48157
	Std. Deviation	183.77
	% RSD	0.38
Theoretical Plate	160323	
Retention Time of Brexpiprazole	28.09	
Retention Time of BRX5	37.30	
Retention Time of BPTP	45.50	

Specificity

Specificity is the capability of the method to measure the analyte response in the presence of impurities. Figure 1 shows the typical chromatograms of the blank solution, system suitability solution, reference solution (a), Test solution and impurities spiked test sample. The results indicated that all impurities are well separated under the current chromatographic conditions. Also, there was no interference of peaks from the blank solution and the samples solution within the retention time of impurities obtained. Peak purity for Brexpiprazole and its impurities were passing. The retention times of each impurity and peak purity refer Table No.03.

Limit of detection and Limit of quantitation

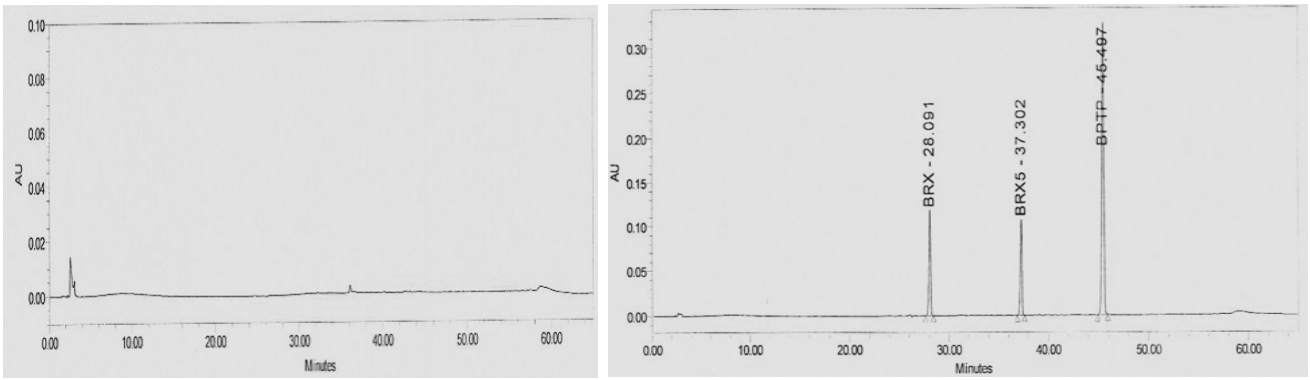
Series of standard solutions of Brexpiprazole and its Impurities were prepared and injected in concentration ranging from 50% to 150% of target concentration. Limit of detection (LOD) and Limit of quantitation (LOQ) was calculated based on residual standard deviation of regression line and slope. Both calculated LOD and LOQ were well within limit and it show below 1.54ppm LOQ for all the impurities and Brexpiprazole API (Table 04).

Linearity

Series of linearity solution of Brexpiprazole and its Impurities solution were prepared from 50% to 150% of target concentration. Linearity curves were drawn by plotting the peak areas of Brexprapozole and Impuritites against its corresponding concentration of linearity solution.

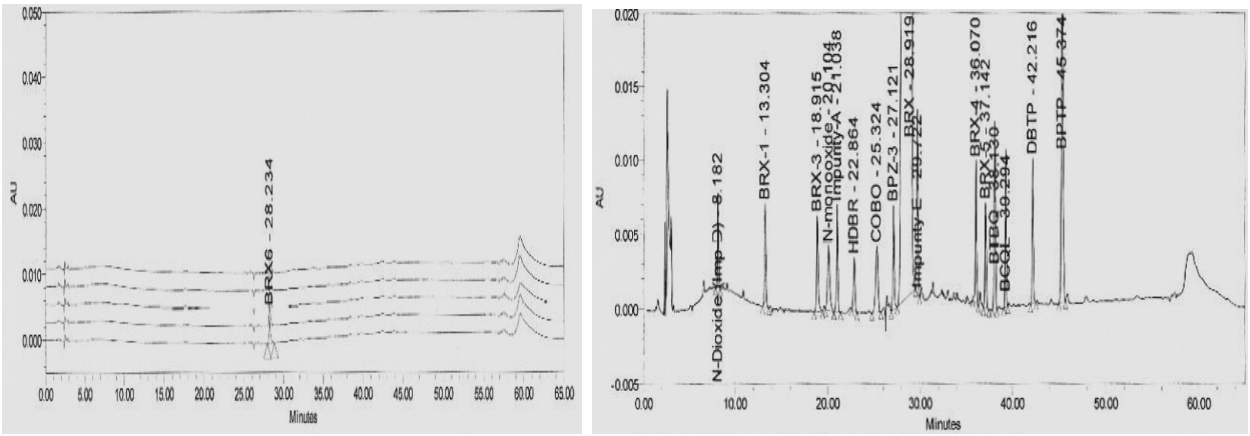
Table 3. Peak purity for spiked test solution

Sr.No	Peak Name	RT	Area	RT Ratio	Purity angle	Purity threshold
1	Imp D	8.15	50172	0.28	0.05	0.26
2	BRX1	13.3	64171	0.46	0.03	0.25
3	BRX3	18.91	77280	0.65	0.07	0.44
4	N-monooxide	20.1	70984	0.7	0.09	0.32
5	Impurity A	21.04	72689	0.73	0.11	0.37
6	HDBR	22.86	44716	0.79	0.05	0.25
7	COBO	25.32	77213	0.88	0.04	0.26
8	BPZ3	27.12	72048	0.94	0.05	0.27
9	BRX	28.92	47597731	1	13.34	76.12
10	Impurity E	29.72	105434	1.03	0.08	0.27
11	BRX4	36.07	89786	1.25	0.11	0.37
12	BRX5	37.14	69453	1.28	0.05	0.26
13	BTBQ	38.13	117291	1.32	0.06	0.24
14	BCQL	39.29	83980	1.36	0.13	0.33
15	DBTP	42.22	82712	1.46	0.15	0.28
16	BPTP	45.37	281759	1.57	0.12	0.23



A B

Figure 2. Specificity



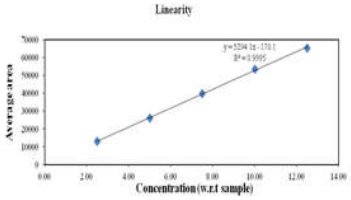
A) Blank, B) System suitability solution, C) Reference solution (a) and D) Brexprapozole spiked sample

Table 4. Limit of detection and quantitation

Sr.No	Name of impurites	Parameter	
		LOD (PPM)	LOQ (PPM)
1	Imp D	0.33	1.02
2	BRX1	0.11	0.33
3	BRX3	0.07	0.2
4	N-monooxide	0.23	1
5	Impurity A	0.33	1.01
6	HDBR	0.37	1.12
7	COBO	0.22	0.85
8	BPZ3	0.15	0.75
9	BRX	0.5	1.15
10	Impurity E	0.16	0.5
11	BRX4	0.24	0.73
12	BRX5	0.54	1.52
13	BTBQ	0.3	0.9
14	BCQL	0.6	1.54
15	DBTP	0.35	0.96
16	BPTP	0.24	0.72

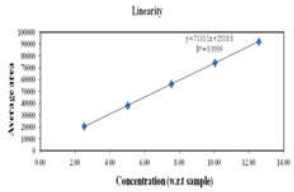
Table 5. Linearity table and Figure

Impurity-D



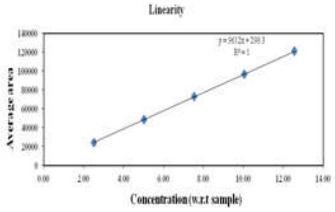
Slope	5294.12
Intercept	-170.10
%y-Intercept	0.65
Regression coefficient (R^2)	0.9995

Impurity -BRX-1



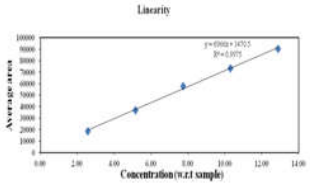
Slope	7110.10
Intercept	2518.80
%y-Intercept	-3.41
Regression coefficient (R^2)	0.9999

Impurity -BRX-3



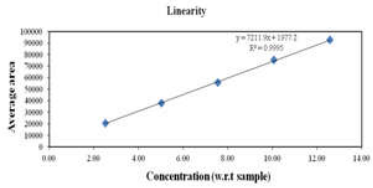
Slope	6111.9
Intercept	298.30
%y-Intercept	-0.25
Regression coefficient (R^2)	1.0000

Impurity –N-Monoxide



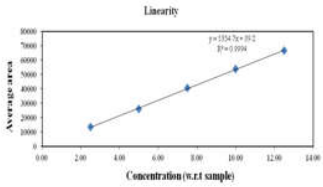
Slope	6966.0
Intercept	1470.5
%y-Intercept	-3.99
Regression coefficient (R^2)	0.9975

Impurity -A



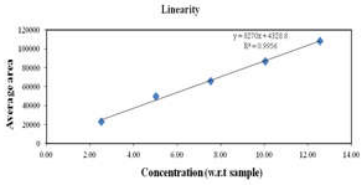
Slope	7211.9
Intercept	1977.2
%y-Intercept	-3.55
Regression coefficient (R^2)	0.9995

Impurity –HDBR



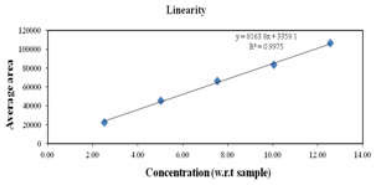
Slope	5354.7
Intercept	39.20
%y-Intercept	-0.06
Regression coefficient (R^2)	0.9994

Impurity -COBO



Slope	8269.98
Intercept	4328.80
%y-Intercept	-4.28
Regression coefficient (R^2)	0.9956

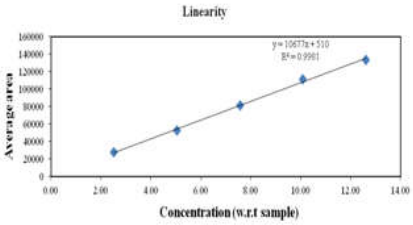
Impurity –BPZ3



Slope	8163.81
Intercept	3359.10
%y-Intercept	-4.04
Regression coefficient (R^2)	0.9975

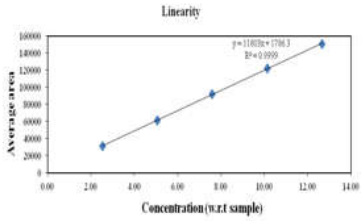
…….Continue

Impurity -Brexpiprazole



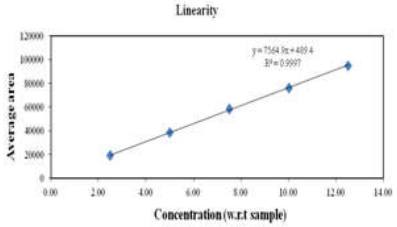
Slope	10676.84
Intercept	510.00
%y-Intercept	-0.46
Regression coefficient (R^2)	0.9981

Impurity – E



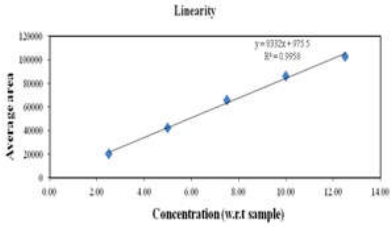
Slope	11802.64
Intercept	1786.30
%y-Intercept	-1.47
Regression coefficient (R^2)	0.9999

Impurity – BRX-4



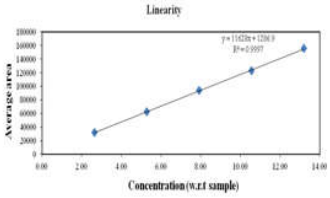
Slope	7564.88
Intercept	489.40
%y-Intercept	-0.84
Regression coefficient (R^2)	0.9997

Impurity – BRX-5



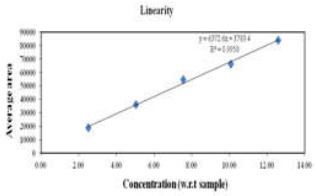
Slope	8332.04
Intercept	975.50
%y-Intercept	-0.95
Regression coefficient (R^2)	0.9958

Impurity – BTBQ



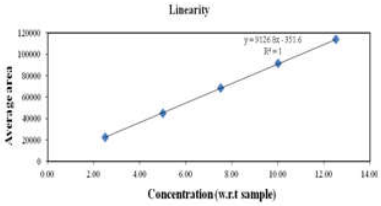
Slope	11628.37
Intercept	1286.90
%y-Intercept	-1.05
Regression coefficient (R^2)	0.9997

Impurity – BCQL



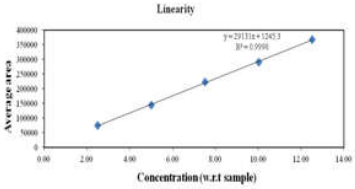
Slope	6372.62
Intercept	3783.40
%y-Intercept	-6.93
Regression coefficient (R^2)	0.9958

Impurity – DBTP



Slope	9126.84
Intercept	-351.60
%y-Intercept	0.51
Regression coefficient (R^2)	1.0000

Impurity – BPTP



Slope	2930.61
Intercept	1245.30
%y-Intercept	-0.56
Regression coefficient (R^2)	0.9998

Regression coefficient, slope and % y intercept are calculate and reported in Table 05. Observed regression coefficient was greater than 0.995 and % y intercept was less than 5.0%.

Precision: System precision was carried out by analyzing six reference solutions (a) of Brexpiprazole API at a limit level concentration.

Table 6. System precision and precision at LOQ

Parameter	Peak name	% RSD for peak area
System precision	Besifloxacin	0.25
Precision at LOQ	All the impurities	Below 3.54

Table 7. Recovery of Impurities-50%

Sr. No.	Imp Name	Test Area	Observed Area	Area of STD (0.10%)	Thereotical Added Imp (%)	Observed Imp (%)	Recovery (%)
1	IMP-D	0	11580	24628	0.05	0.044	94.04
2	BRX1	3143	19208	38840	0.05	0.041	82.72
3	BRX3	0	24555	49891	0.05	0.049	98.43
4	IMP-A	6361	25049	38833	0.04	0.04	96.25
5	COBO	6791	28193	39502	0.05	0.053	108.36
6	HDBR	0	15699	31920	0.04	0.036	98.36
7	BPZ3	0	21957	45793	0.05	0.047	95.9
8	IMP-E	0	20509	44281	0.05	0.046	92.63
9	BRX4	9674	29899	38556	0.05	0.052	104.91
10	BCQL	0	21913	42996	0.05	0.046	101.93
11	BRX5A	0	24201	46542	0.05	0.052	104
12	DBTP	0	22052	44679	0.05	0.049	98.71
13	BPTP	22685	94125	148328	0.05	0.044	96.33
14	N-monooxide	10149	28212	54645	0.05	0.05	99.55
15	BTBQ	25396	62081	54645	0.05	0.05	95.85

Table 8. Recovery of Impurities-100%

Sr. No.	Imp Name	Test Area	Observed Area	Area of STD (0.10%)	Thereotical Added Imp (%)	Observed Imp (%)	Recovery (%)
1	IMP-D	0	21152	24628	0.09	0.08	85.89
2	BRX1	3143	37916	38840	0.1	0.089	89.53
3	BRX3	0	47271	49891	0.1	0.094	94.75
4	IMP-A	6361	43490	28833	0.08	0.079	95.61
5	COBO	6791	42857	39502	0.1	0.09	91.3
6	HDBR	0	29715	31920	0.07	0.068	93.09
7	BPZ3	0	48479	45793	0.1	0.103	105.87
8	IMP-E	0	43739	44281	0.1	0.098	98.78
9	BRX4	9674	49710	38556	0.1	0.103	103.84
10	BCQL	0	41645	42996	0.09	0.087	96.86
11	BRX5A	0	48541	46542	0.1	0.104	104.3
12	DBTP	0	45047	44679	0.1	0.1	100.82
13	BPTP	22685	177204	148328	0.09	0.095	104.17
14	N-monooxide	10149	47127	54645	0.1	0.09	92.04
15	BTBQ	25396	92764	54645	0.1	0.09	87.4

Table 9. Recovery of Impurities-150%

Sr. No.	Imp Name	Test Area	Observed Area	Area of STD (0.10%)	Thereotical Added Imp (%)	Observed Imp (%)	Recovery (%)
1	IMP-D	0	31616	24628	0.14	0.12	85.58
2	BRX1	3143	55953	38840	0.15	0.136	90.65
3	BRX3	0	70269	49891	0.15	0.14	93.9
4	IMP-A	6361	59892	38833	0.12	0.114	91.9
5	COBO	6791	60407	39502	0.15	0.133	90.49
6	HDBR	0	47258	31920	0.11	0.109	98.7
7	BPZ3	0	69415	45793	0.15	0.147	101.06
8	IMP-E	0	67850	44281	0.15	0.153	102.15
9	BRX4	9674	68216	38556	0.15	0.15	101.22
10	BCQL	0	61232	42996	0.13	0.128	94.94
11	BRX5A	0	72187	46542	0.15	0.154	103.4
12	DBTP	0	75417	44679	0.15	0.167	112.53
13	BPTP	22685	258112	148328	0.14	0.144	105.81
14	N-monooxide	10149	65080	54645	0.15	0.14	91.39
15	BTBQ	25396	124225	54645	0.16	0.13	85.69

Relative standard deviation for the peak area of Brexpiprazole was calculated and found to be 2.34 %. Precision at LOQ was calculated by preparing Impurity-A solution at LOQ concentration and injecting six times. Relative standard deviation for Impurity-A peak area was 1.35 % (Table 06).

Accuracy: Accuracy of the method is established by carrying out the recovery studies of Impurities. The Test solution was spiked with Impurities solution at specific limit level concentrations 50%, 100% and 150%. Each spiked test solution was analyzed for recovery study of Impurities.

Recovery obtained for Impurities is between 80% to 120% (Table-07,08 and 09).

Solution stability: Test solution stability was established by injecting the same test solution after every six hours' time interval for 24 hours. The result obtainedin will within limit with relative standard deviationis less than 5.0 %, thus solution stability was established up to 24 hours at 25 °C (Table 10).

Table 10. Solution stability of Brexpiprazole

Sr.No.	Imp Name	% of impurities in sample			
		6 Hrs	12Hrs	18 Hrs	24 Hrs
1	IMP-D	0	0	0	0
2	BRX1	0	0	0	0.02
3	BRX3	0	0	0	0
4	IMP-A	0	0	0	0
5	COBO	0	0.02	0.02	0
6	HDBR	0	0	0	0
7	BPZ3	0	0	0	0
8	IMP-E	0.02	0.02	0.01	0.01
9	BRX4	0	0.02	0.01	0.03
10	BCQL	0.08	0.07	0.05	0.11
11	BRX5A	0	0	0	0
12	DBTP	0	0	0	0
13	BPTP	0.03	0.03	0.04	0.03
14	N-monooxide	0.09	0.09	0.09	0.1
15	BTBQ	0.06	0.07	0.03	0.02
16	Singal max	0.06	0.08	0.06	0.06
17	Total Impurities	0.35	0.39	0.38	0.37

Conclusion

The Reverse phase HPLC method was developed for quantitative determination of related substances (Impurities) of Brexpiprazole API. This method was validated and found out linear, accurate, precise specificrobust and stable. All the analytical data for all method validation parameters tested and found out to be satisfactory. The developed method can suitably use by quality control department to determine the Related substances in commercial and stability test samples of Brexpiprazole API.

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