



ISSN (E): 2277- 7695

ISSN (P): 2349-8242

NAAS Rating: 5.03

TPI 2020; 9(3): 234-238

© 2020 TPI

[www.thepharmajournal.com](http://www.thepharmajournal.com)

Received: 13-01-2020

Accepted: 17-02-2020

**Parth Patel**

Faculty of Pharmacy, G.H. Patel Building, Donor's Plaza, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

**Rajashree Mashru**

Faculty of Pharmacy, G.H. Patel Building, Donor's Plaza, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

## Validation avenues for calibration curve of Brexpiprazole: Application of statistical tests

**Parth Patel and Rajashree Mashru**

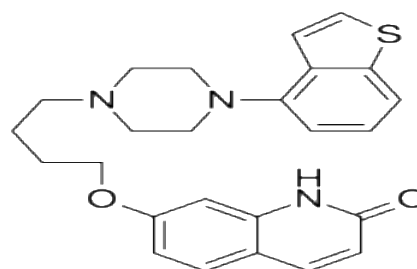
**Abstract**

The objective of a study is to validate the standard plot for a model drug Brexpiprazole by using series of statistical tests. The Study include selection of suitable assay medium and preparation of standard plot in selected medium. Hypothesis (Null and Alternative) was applied to check the suitability of data for standard plot. The Study successfully validated for standard plot of Brexpiprazole. Validation of Standard plot is of utmost importance as all the calculations including % drug release, content uniformity depend on standard plot being correct.

**Keywords:** Linearity test, null hypothesis, alternative hypothesis, ANOVA, statistical tests

**Introduction**

Brexpiprazole, an atypical antipsychotic, is available as REXULTI® (Brexpiprazole) tablets. Chemically Brexpiprazole was 7-{4-[4-(1-Benzothiophen-4-yl) piperazin-1-yl] Butoxy} quinolin- 2(1*H*)-one. The empirical formula is C<sub>25</sub>H<sub>27</sub>N<sub>3</sub>O<sub>2</sub>S and its molecular weight is 433.57 g/mol. The chemical structure was shown in figure 1. It is used in the treatment of schizophrenia and as an adjunctive therapy to antidepressants for the treatment of Major Depressive Disorder (MDD). The mechanism of action of Brexpiprazole in the treatment of major depressive disorder or schizophrenia is unknown. However, the efficacy of Brexpiprazole may be mediated through a combination of partial agonist activity at serotonin 5-HT<sub>1A</sub> and dopamine D<sub>2</sub> receptors, and antagonist activity at serotonin 5-HT<sub>2A</sub> receptors [1]. Calibration curves can be used to predict the concentration of an unknown sample. To be completely accurate, the standard samples should be run in the same matrix as the unknown sample [2]. Validation is an important ingredient in development and application of analytical methodology for assaying potency of dosage forms or drug in body fluids. Assay validation must demonstrate that the analytical procedure is able to accurately and precisely predict the concentration of unknown samples. To accomplish this several procedures are usually required. A Calibration curve is one such method, which is characterized by determining the analytical responses over a suitable range of unknown concentrations of drug. Unknown samples are then related to the calibration curve to estimate their concentrations, which in turn helps in the determination of drug release from the dosage form. Therefore validation of standard curve becomes of utmost importance. In the current study the standard curve of the model drug Brexpiprazole was subjected to various statistical test for validation of the curve [3].



**Fig 1:** Chemical structure of Brexpiprazole

**Corresponding Author:****Rajashree Mashru**

Faculty of Pharmacy, G.H. Patel Building, Donor's Plaza, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

**Materials and Methods****Instrumentation and software**

UV spectral studies were done using Shimadzu UV-1700 double beam spectrophotometer

connected to a computer loaded with Shimadzu UV probe 2.10 software. The absorbance spectra of drug were carried out in 1 cm quartz cells over the range of 200-400 nm. The samples were weighed on electronic analytical balance (A×120, Shimadzu). STATGRAPHICS 18 and Microsoft Excel for Mac Version 16.34 was used for applying various statistical tests.

### Reagents and chemicals

Methanol analytical reagent grade (Research Lab fine chem industries, Mumbai, India) was used as the solvent and diluent.

### Selection of assay medium

A detectable amount (5 mcg/ml) of the drug dissolved in Methanol. The Solution of the drug were stored in laboratory shelf at Room Temperature. The Solution of the drug were sampled periodically of 3 days and analysed for any spectral change. This monitoring helped in selection of assay medium in which standard curve was to be made. The results of the change in Absorbance have been summarized in Table 1.

**Table 1:** Absorbance Data of Brexpiprazole in Methanol (5 mcg/ml)

DAY	Methanol	
	$\lambda_{\max}(\text{nm})$	Absorbance
1	325	0.655
2	325	0.652
3	325	0.655

### Preparation of standard stock solution

10 mg of Brexpiprazole were weighed accurately and transferred into 10 ml volumetric flask. Methanol was added into the volumetric flask to dissolve the standards and finally

volume was made up to the mark with Methanol to obtain standard solutions of Brexpiprazole (1000 mcg/ml).

### Preparation of working standard solution

From the stock above solution of Brexpiprazole, Working standard solution of Brexpiprazole (100 mcg/ml) was prepared by transferring 1 ml aliquot to 10 ml volumetric flask and making up the volume with methanol.

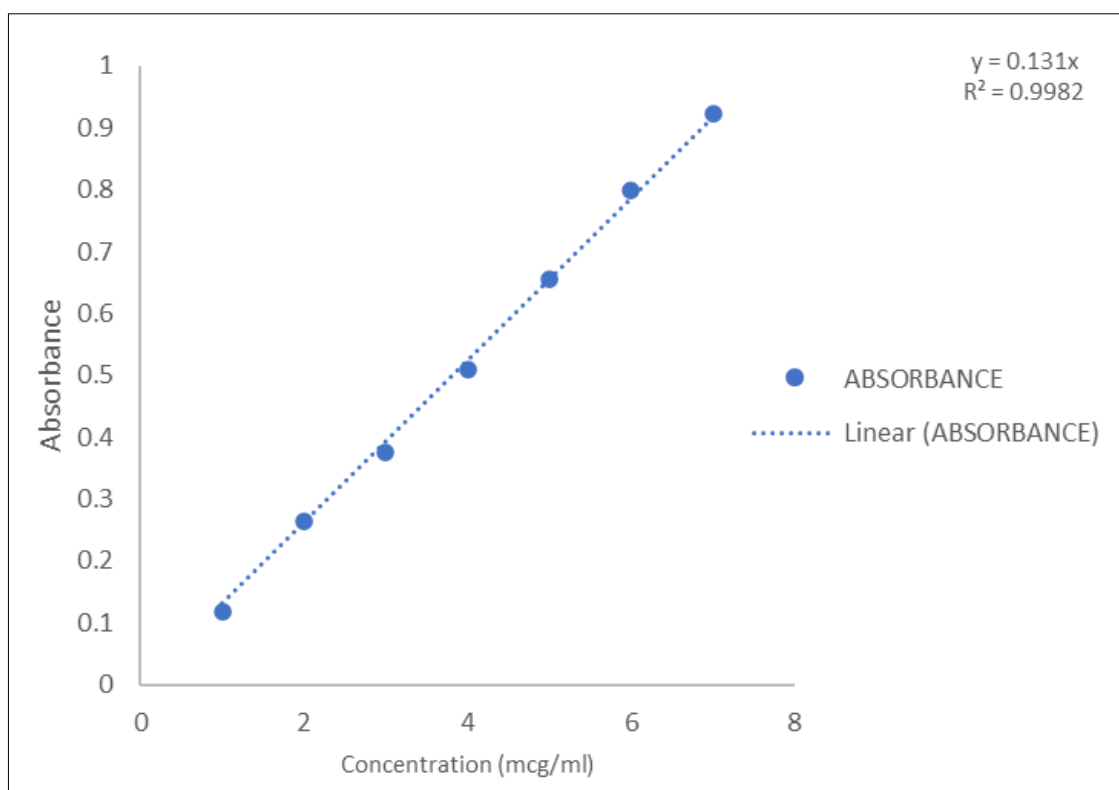
### Preparation of the standard plot

From working standard solution of Brexpiprazole(100 mcg/ml), aliquots 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, 0.6 ml and 0.7 ml were withdrawn and transferred to 10 ml volumetric flasks. Volume was made up to the mark with Methanol to produce 1 mcg/ml, 2 mcg/ml, 3 mcg/ml, 4 mcg/ml, 5 mcg/ml, 6 mcg/ml and 7 mcg/ml Respectively. Detection was done using Shimadzu double beam spectrophotometer (UV-1700) at a  $\lambda_{\max} = 325$  nm. (Table 2 and Figure 2 and 3).

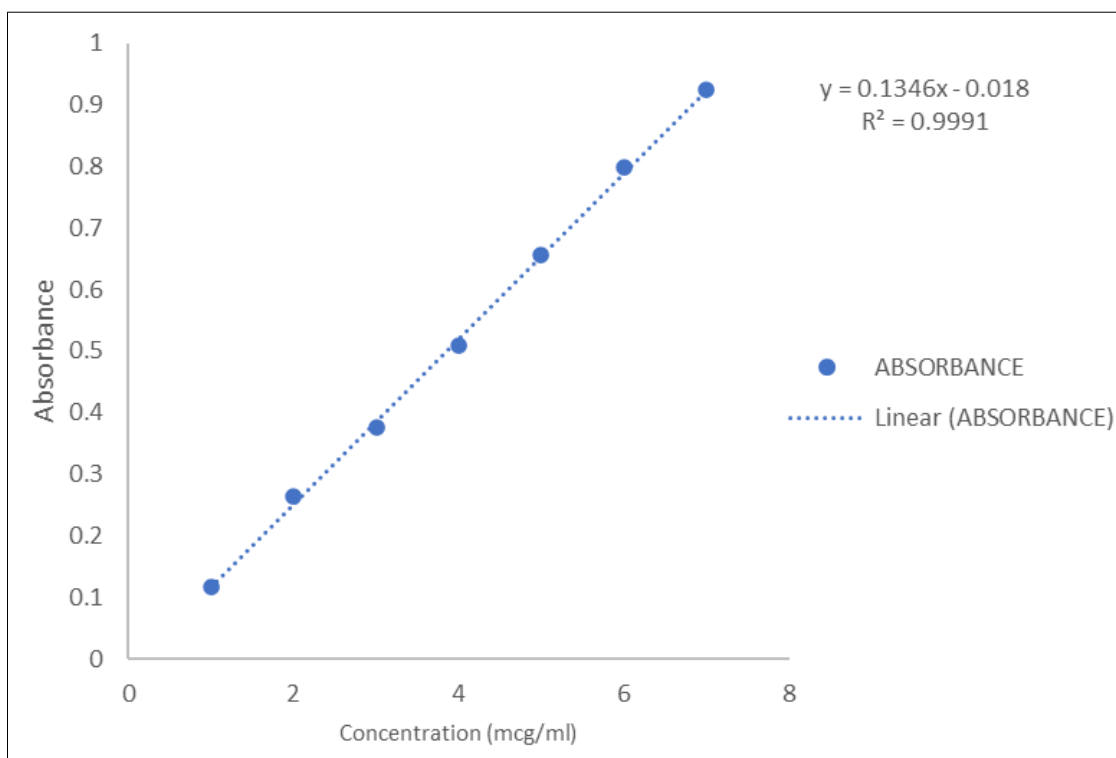
**Table 2:** Concentration v/s Absorbance relationship for Brexpiprazole dissolved in Methanol

Sr. No	Conc.(mcg/ml)	Absorbance 1*(X & Y=0)	Absorbance 2*(X=0 & Y={-0.018})
1	0	0	0.0006±0.0001
2	1	0.118±0.0011	0.118±0.002
3	2	0.263±0.0025	0.263±0.0035
4	3	0.375±0.0039	0.375±0.0025
5	4	0.51±0.0028	0.51±0.0017
6	5	0.655±0.0072	0.655±0.0081
7	6	0.799±0.0481	0.799±0.0432
8	7	0.924±0.0110	0.924±0.0125

\*Each value is an average of 3 determinations  
Standard curve for Brexpiprazole



**Fig 2:** Standard plot of Brexpiprazole showing the line of best fit with an intercept at zero



**Fig 3:** Standard plot of Brexpiprazole showing the line of best fit with a definite intercept  $C=(-0.018)$

#### Statistical test

Test for acceptability of Absorbance values was done to check whether the Absorbance values were significant and within the accepted range. If the absorbance values (A) minus the values of the mean of Absorbance (B) was less than four times the mean Standard deviation(D), the absorbance values were acceptable. Null and alternative hypothesis were applied to the resulting absorbance values obtained from the concentrations made in the Methanol for the standard plot, to validate the use of the standard plot either with the data containing a zero intercept or a data with a definite intercept.

#### Results and Discussion

##### Study for checking authenticity of using methanol as solvent

The Spectral Intensity (Absorbance) of Brexpiprazole

dissolved in Methanol were followed over a three day period. The Spectral intensity of Brexpiprazole in Methanol shown in Table 1 did not demonstrate any additional peak and hence is a suitable medium for the studies.

#### Statistical analysis

##### Test for acceptability of absorbance values

All Values of (A-B) were less than 4 times Standard Deviation (D) For Absorbance 1 & Absorbance 2.  $A-B < 4D$

##### Regression analysis <sup>[4]</sup>.

Comparison 26 models for regression analysis by STATGRAPHICS 18 software shows the highest coefficient of determination ( $R^2$ ) value for linear model, therefore this model was chosen for Regression analysis. (Table 3)

**Table 3:** Models fitted to describe regression analysis according to Statgraphics software

Sr. No	Model	$R^2$	
		Absorbance 1	Absorbance 2
1	Linear	0.9996	0.9996
2	Squared-X	0.9662	0.9991
3	Square root-Y	0.9609	0.9663
4	Squared-Y	0.9565	0.9653
5	Square root-X	0.9500	0.9565
6	Square root-Y Squared-X	0.8575	0.9498
7	Squared-Y Square root-X	0.8402	0.9217
8	Exponential	No Fit	0.8879
9	Reciprocal-Y	No Fit	0.8647
10	Double Square root	No Fit	0.8402
11	Logarithmic-Y square root-X	No Fit	0.7708
12	Reciprocal-Y square root-X	No Fit	0.6045
13	Logarithmic-X	No Fit	-0.3992
14	Square root-Y Logarithmic-X	No Fit	No Fit
15	Multiplicative	No Fit	No Fit
16	Reciprocal-Y Logarithmic-X	No Fit	No Fit
17	Squared-Y Logarithmic-X	No Fit	No Fit
18	Reciprocal-X	No Fit	No Fit

19	Square root-Y reciprocal-X	No Fit	No Fit
20	S-Curve Model	No Fit	No Fit
21	Double Reciprocal	No Fit	No Fit
22	Squared-Y reciprocal-X	No Fit	No Fit
23	Logarithmic-Y squared-X	No Fit	No Fit
24	Reciprocal-Y squared-X	No Fit	No Fit
25	Logistic	No Fit	No Fit
26	Log Probit	No Fit	No Fit

### Test for authenticity for using slope & intercept

A 't' Test was carried out for the slope & intercept. (Table 4).

The t value passed the test for using the obtained slope & intercept for both the Absorbances.

**Table 4:** Results of Various Parameters using Microsoft Excel

	Sources	Sum of squares	Df	Mean Square	F	P
Absorbance 1	Regression	0.7445	1	0.7445	6945.9049	0.0000
	Residual	0.0006	6	0.0001		
	Total	0.7451	7			
Absorbance 2	Regression	0.7439	1	0.7439	6805.1454	0.0000
	Residual	0.0006	6	0.0001		
	Total	0.7446	7			

### Anova Test <sup>[5]</sup>

This Test was carried out to test the deviation of the obtained data from Linearity. The lack of significance denoted by P values shows that the obtained data is linear (Table 5).

Since the P-value was less than 0.01 in the ANOVA Table,

there was statistically significant relationship between absorbance and concentration at 99% confidence level. Standard error of the estimate for data of Absorbance 1 & Absorbance 2 was 0.0103 & 0.0104 Respectively.

**Table 5:** Results of ANOVA test using Microsoft Excel

	Parameter	Estimate	Std. error	t-test	P-value
Absorbance 1	Intercept	0	0.0066	-1.5711	0.0002
	Slope	0.131	0.0015	83.3420	0.0000
Absorbance 2	intercept	-0.018	0.0067	-1.5186	0.0003
	slope	0.1346	0.0016	82.4933	0.0000

### Null Hypothesis <sup>[6]</sup>

Null Hypothesis ( $H_0$ ) was used to determine the authenticity of the above standard curve of Brexpiprazole from the data of concentration v/s absorbance (1). In performing null hypothesis we assumed that the intercept was zero and an alternative hypothesis ( $H_A$ ), which assumed that the intercept had some value (positive or negative) other than zero. A two-sided test (t-test) was run to establish whether the null hypothesis was to be rejected or not. (Table 6).

Null hypothesis ( $H_0$ )=0

Alternative hypothesis ( $H_A$ )<or>0

Since the P-value for the test was less than 0.05, the data at hand were not compatible with null hypothesis, but were supportive of some other hypothesis (alternative hypothesis),

therefore the null hypothesis was rejected at 95% confidence level.

**Table 6:** Result of running two-sided test by using Microsoft Excel

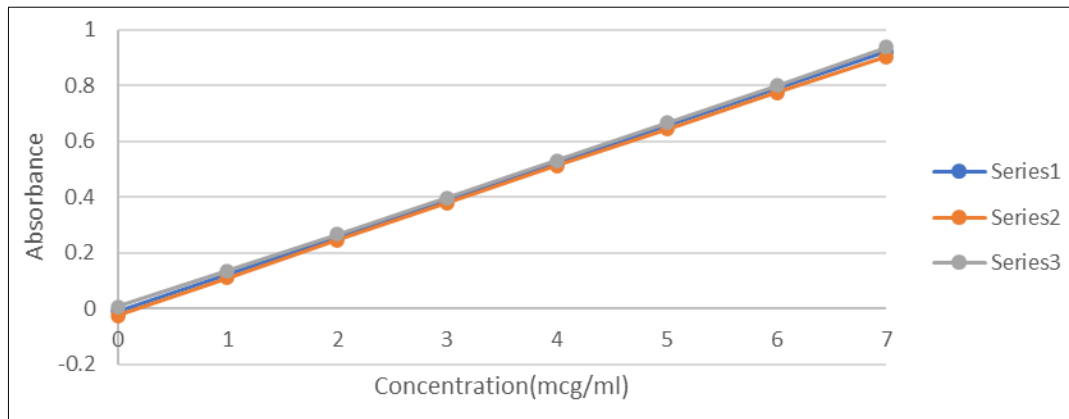
No. of Obs.	Mean	Standard dev.	t-test	P-value	$\alpha$
16	0.4555	0.2289	2.14	0.0007	0.05

### Confidence interval & prediction interval <sup>[7]</sup>.

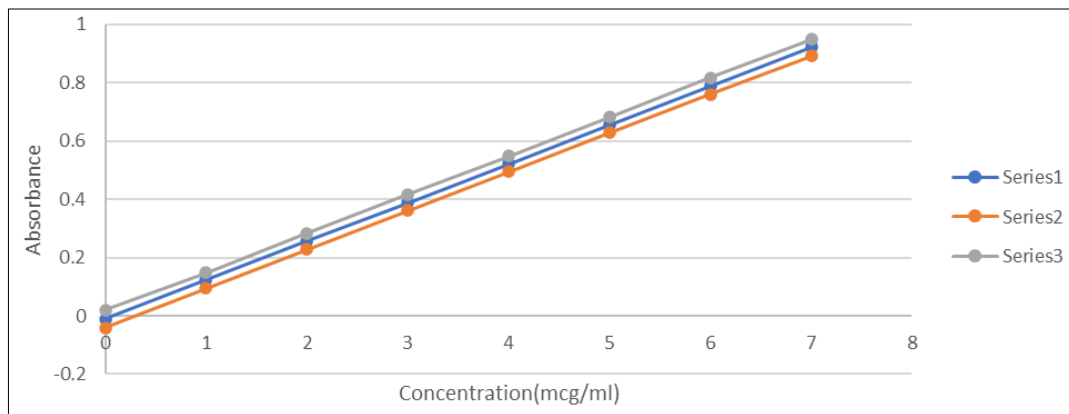
On the basis of this calculation we assure the 95% Confidence & Prediction interval. Linearity of the data is also assured by this calculation and plot. Here for the reference Absorbance graph is given in Figure 4 & 5. Data is also Represented in the Table-7.

**Table 7:** Confidence & Prediction interval of the Absorbance

Sr. No	Concentration (X)	Absorbance (Y)	Predicted Y	Confidence interval		Prediction interval	
				Min	Max	Min	Max
1	0	0	-0.0105	-0.0268	0.0058	-0.0406	0.0196
2	1	0.118	0.1226	0.1093	0.1358	0.0940	0.1482
3	2	0.263	0.2557	0.2450	0.2664	0.2282	0.2832
4	3	0.375	0.3889	0.3797	0.3980	0.3619	0.4158
5	4	0.51	0.5220	0.5129	0.5312	0.4951	0.5490
6	5	0.655	0.6552	0.6445	0.6659	0.6277	0.6827
7	6	0.799	0.7883	0.7751	0.8016	0.7597	0.8169
8	7	0.924	0.9215	0.9051	0.9378	0.8913	0.9493



**Fig 4:** Confidence interval at  $\alpha=0.05$



**Fig 5:** Prediction interval at  $\alpha=0.05$

#### Here

Series 1 = Upper Limit

Series 2 = Actual

Series 3 = Lower Limit

#### Conclusion

The standard Plot of Brexpiprazole was validated by series of statistical test. This type of statistical validation only for the standard plot apart from the other six specified in U.S.P. can help a formulator to correctly determine the various parameters he wants to find out using either the standard plot or the regression equation and eliminate the most common error of considering the standard plot can be a regressed line when in fact it is not.

#### References

1. Brexpiprazole Labelled information online available on URL: [https://www.accessdata.fda.gov/drugsatfda\\_docs/label/2015/205422Orig1Orig2s0001bl.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/205422Orig1Orig2s0001bl.pdf)
2. Calibration curve information online available on URL: <https://www.jove.com/science-education/10188/calibration-curves>
3. Philip A, Pathak K. Validation approaches for calibration curve: Application of Null Hypothesis. *Journal of pharmaceutical research* 2006; 5(2):57-60
4. Bolton S. *Pharmaceutical statistics: practical and clinical applications*. Edn 3, Marcel dekker series, new York, 80:241-243
5. Daniel WW, Cross CI. *Biostatistics basic concepts and methodology for the health sciences*, Edn 10, Wiley student edition, new Delhi, 312-322

6. Gupta SP. *Statistical methods*, Edn 28, Sultan sons & chand, new Delhi, 19-24
7. Kothari CR, garg Gaurav, *Research Methodology, Methods and techniques*, Edn 4, New age international publishers, New Delhi, 187-189