Asian Journal of Pharmaceutical and Health Sciences
www.ajphs.com

RP- HPLC method for determination of brexpiprazole in the presence of its oxidative-induced degradation product

Fathy M. Salama, Khalid A. Attia,Ragab A. Said,Ahmed El-olemy, and Ahmed M. Abdel-raoof *

Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University,11751, Nasr City, Cairo, Egypt.

ARTICLE HISTORY

Received: 16.03.2018

Accepted: 12.05.2018

Available online: 30.06.2018

Keywords:

brexpiprazole;oxidative degradation product and RP-HPLC

***Corresponding author:**

Email : ahmedmeetyazeed79@yahoo.com
Tel.: +91-.....

ABSTRACT

Objective: Accurate and selective reversed phase high performance liquid chromatography (RP-HPLC) method has been developed and validated for determination of brexpiprazole in the presence of its oxidative-induced degradation product. **Methods:** The developed method was based on the separation of the two components using methanol ,water and phosphoric acid (60:40:0.4, by volume) as a mobile phase in isocratic elution mode on ODS SUPELCO C18 (25 cm X 4.6 mm, 5 µm particle size) column at a flow rate of 1 ml min^{-1} and ultraviolet (UV) detection at 259 nm. **Result and discussion:** The components were well resolved from each other with significantly different R_t values of 4.41and 2.59 min for brexpiprazole and its oxidative-induced degradation product, respectively. **Conclusion:** The method was found to be linear in the range of (20-100 µg/mL). The developed method was validated according to the International Conference on Harmonization (ICH) guidelines demonstrated a good accuracy and precision. The results were statistically compared with those obtained by the reported method, and no significant difference was found.

INTRODUCTION

Mental health disorders affect approximately one in five adults in a given year. In the United States, approximately 1% of adults live with schizophrenia, and nearly 7% of adults have experienced at least one major depressive episode [1]

Brexpiprazole is a new dopamine D2 receptor partial agonist that received approval for the treatment of schizophrenia and for adjunctive use for the treatment of major depressive disorder (MDD) based on a clinical trial development programme that included two pivotal Phase III trials of brexpiprazole monotherapy in acute schizophrenia[2]. It is also used together with other medications to treat major depressive disorder in adults. This drug has high affinity for 5-HT1A, 5-HT2A, D2 and α1B,2C receptors. It displays partial agonism at 5-HT1A and D2 receptors and potent antagonism at 5-HT2Aand α1B,2C adrenergic receptors. It also has some affinity (antagonism) for D3, 5-HT2B, 5-HT7 and α1A, 1Dreceptors, and moderate affinity for H1 and low affinity for M1 receptors. These all lead to a favorable antipsychotic profile in terms of improvement of cognitive performance and sleep patterns, as well as effects on affective states and potential to treat core symptoms in schizophrenia and major depressive disorder, including cognitive deficits with a low risk of adverse effects (extrapyramidal symptoms, metabolic complications, weight gain, akathisia potential) that are commonly encountered with other typical and second-generation antipsychotic drugs [3]

Brexpiprazole is chemically designated as 7-{4-[4-(1-benzothiophen-4-yl) piperazin-1-yl] butoxy}-1,2-dihydroquinolin-2-one figure 1. Its molecular formula is $C_{25}H_{27}N_3O_2S$, and its molecular weight is 433.57. Brexpiprazole is a white-to-off white powder. It is freely soluble in methanol and practically insoluble in water [4]. A review of the literature revealed that only one analytical method has been described for the determination of brexpiprazole in pharmaceutical by HPLC method [5] The chromatographic separation was achieved on C18 column (Inertsil ODS 3V 150*4.6, 5um) at ambient temperature.The separation achieved employing a mobile phase consists of 0.1%v/v Formic acid in water: Methanol (35:65; V/V).

There is no stability indicating analytical method was reported for determination of brexpiprazole in presence of its degradation product.

The aim of this work is to develop and validate simple and selective chromatographic method for the determination of brexpiprazole in presence of its oxidative-induced degradation product without preliminary separation. The proposed method was found to be fast and simple and can be used for its routine analysis in quality control laboratories.

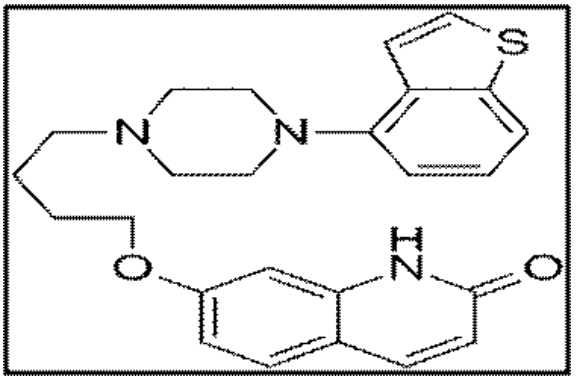


Figure 1 : Structural formula of brexpiprazole.

Experimental

Apparatus

(a) HPLC, LDC Analytical (Milton Roy, USA), equipped with Diode-array UV-visible detector and auto sampler injector, using ODS SUPELCO C18 (25 cm X 4.6 mm, 5 µm particle sizes) column. The chromatographic analysis was carried out using (EZ Chrom Elit) data analysis program.

(b) Camag Linomat autosampler (Muttenzl, Switzerland), a Camag microsyringe (100 µL) and a Camag 35/N/30319 TLC scanner with win CATS software; an ultraviolet (UV) lamp with a short wavelength at 254 nm (Desaga, Wiesloch, Germany).Aluminum TLC plates precoated with silica gel 60 GF_{254} (20×20 cm), (Merck, Darmstadt, Germany). Chromatographic tank (25 × 25 × 9 cm).

(c) Hot plate (Torrey pines Scientific, USA).

(d) Jenway, 3510 pH meter (Jenway, USA).

(e) Rotatory evaporator (Scilogex-RE 100-pro, USA).

Materials and chemicals

Pure standard

Standard brexpiprazole powder was kindly supplied by Al Andalus Pharmaceutical Company, Cairo, Egypt.

Pharmaceutical preparation

Rexulti ® tablets, each tablet claimed to contain 4 mg of brexpiprazole, (B. No.- R01230382) manufactured by Otsuka Pharmaceutical Company, purchased from USA market.

Reagents and chemicals

(a) All chemicals used were of analytical grade, solventswere of HPLC grade, water used throughoutthe procedure was freshlybidistilled.

(b) Analytical grade orthophosphoric acid was obtained from (Prolabo, Paris, France).

(c) Hydrogen peroxide (30 %), Hydrochloric acid and sodium hydroxide (El-Nasr Company, Egypt).

(d) Ethyl acetate and methanol, HPLC grade (Sigma-Aldrich, Germany).

Standard solutions

Stock standard solution of brexpiprazole (1mg/ mL) was prepared by dissolving 0.1g ofbrexpiprazole in 50 mL of methanol : water : phosphoric acid (60:40:0.4, by volume) and the volume was completed to 100 mL with the mobile phase. Working solution of brexpiprazole (100 µg/mL)was obtained by further dilution of thestock solution with the mobile phase.

Degraded sample

100 mg of pure brexpiprazole powder was dissolved in 55 mL of methanol and transferred to a 100-mL round bottomed flask to which 15 mL of 30 % H_2O_2 were added. The solution was heated under reflux for 2 hours and evaporated to dryness under vacuum. The obtained residue was extracted with methanol (2 x 10 mL), filtered into a 100-mL volumetric flask and diluted to volume with methanol to obtain a stock solution labeled to contain oxidative degradation product derived from 1 mg/mL of brexpiprazole. Working solution of oxidative degradation product (100 µg/mL) was obtained by further dilution of the stock solution with the mobile phase.

PROCEDURE

Construction of calibration curve

Aliquots of brexpiprazoleequivalent to (200-1000 µg), were accurately transferredfrom its working standard solution (100 µg/mL) into a set of 10-mLvolumetric flasks and the volume was then completed to the mark with the mobile phase in isocratic elution mode. A 20-µL aliquot of each solution was injected into aODS SUPELCO C18 (25 cm X 4.6 mm, 5 µm particle size) using the mobile phase, at flow rate 1.0 $mLmin^{-1}$ and UV detection at 259 nm. Calibration curve was constructed by plotting the peak area against the corresponding concentrations of brexpiprazole.

Application to laboratory prepared mixtures

Aliquots of brexpiprazole and itsoxidative degradation product were mixed to prepare different mixtures containing different ratios of components. The procedure mentioned under construction of calibration curve was followed and the concentrations of brexpiprazole were calculated from corresponding regression equation.

Application to pharmaceutical formulation

Ten Rexulti®4 mg tablets were accurately weighed and finely powdered, then a quantity equivalent to 10 mg of brexpiprazole was shaken with 50 mL of methanol for 10 minutes then filtered into 100 mL volumetric flask and the volume was adjusted to the mark with the mobile phase to obtain a concentration of (100 µg/mL). Proceed as described under "General Procedure". Determine the brexpiprazole content in the tablets from the corresponding regression equation.

RESULTS

Degradation of brexpiprazole

No degradation of brexpiprazole was observed by refluxing the drug using aqueous, acidic (1M HCl) or basic (1M NaOH) conditions, whereas complete degradation was attained when the drug was refluxed with 30 % H_2O_2 for 2 hours (Scheme 1). The obtained solution was tested by TLC on silica gel 60 GF254 plates. Separation of the intact drug and its corresponding oxidative degradation product was achieved by using mobile phase consisting of methanol: ethyl acetate (10: 30; V/V) as a developing system and UV detection at 254 nm.

Confirmation of degradation product using IR technique

IR for intact reveals thatpresence of characteristic peak C=O

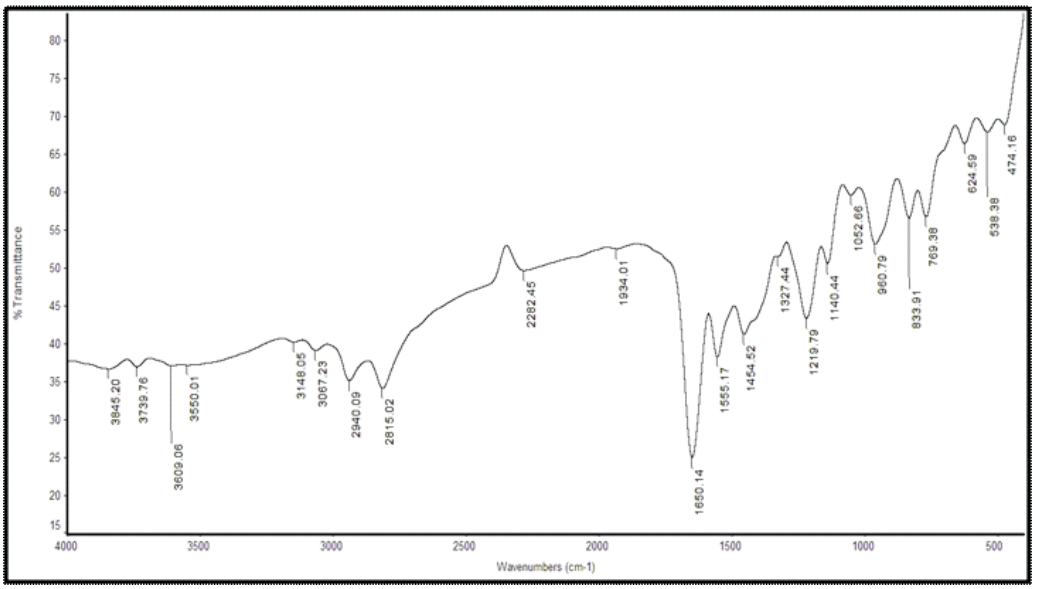


Figure 2 (a) : IR spectrum of brexpiprazole on KBr disc.

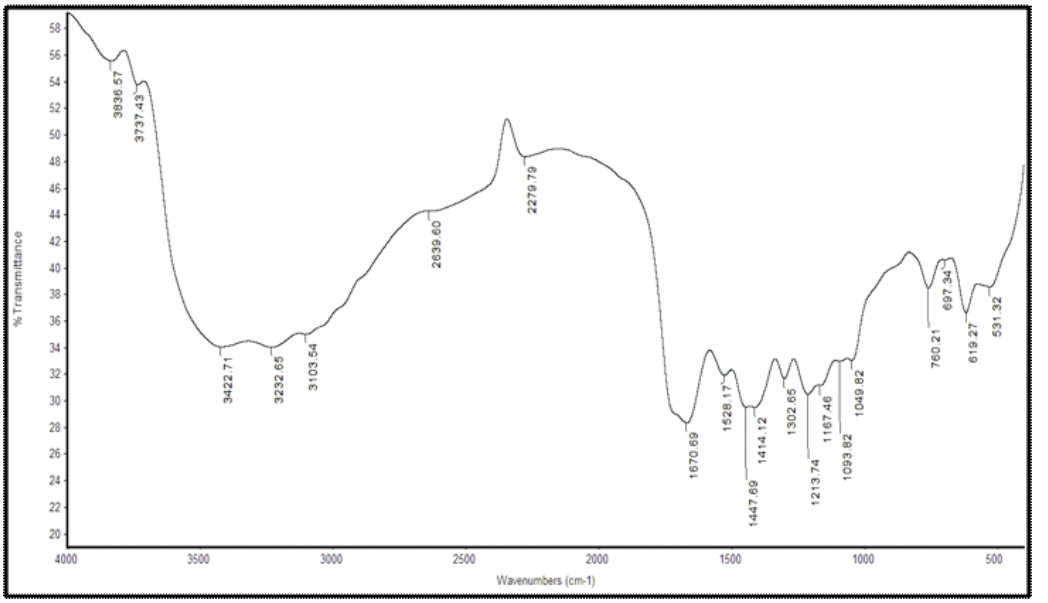
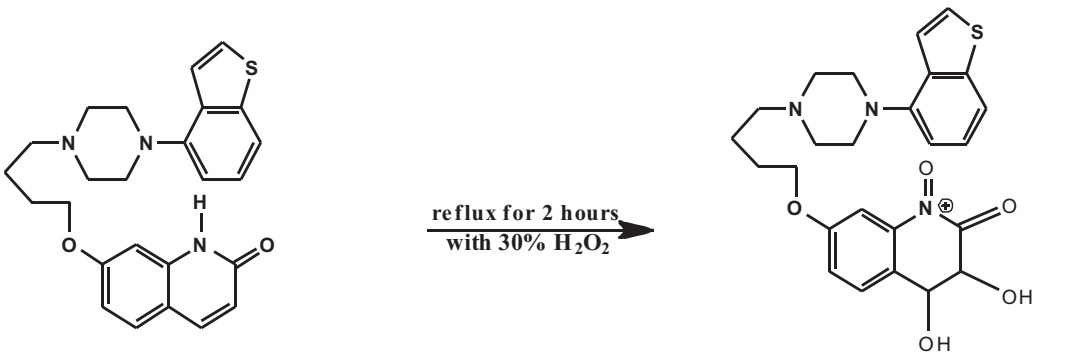


Figure 2 (b) : IR spectrum of brexpiprazole oxidative degradation product on KBr dis.



Scheme (1): Suggested degradation pathway of brexpiprazole.

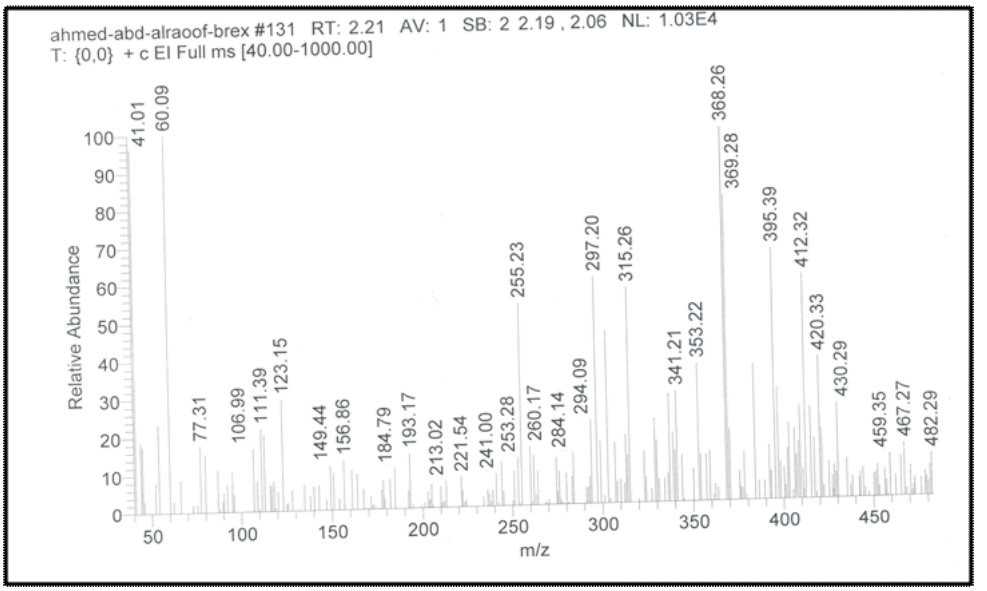


Figure 3 : Mass spectrum of brexpiprazole oxidative degradation product.

group at 1650 Cm^{-1}, Presence of-CH aromatic and CH aliphatic peaks at 3067 Cm^{-1} and 2940 Cm^{-1}respectively. While theIR for its oxidative degradation product reveals that: appearance of C=O group at 1670 Cm^{-1} and appearance of new broad peak at 3422.9 Cm^{-1} which is characteristic to (-OH) groups, while it was absent in the spectrum of pure drug. Also the spectrum showed the appearance of peaks at 1447 and 1414 cm-1 that are characteristic to (N=O) group, which were absent in the pure drug as shown in Figure 2 (a,b) .so that , this difference supported the suggested degradation pathway shown in scheme 1 by oxidation of olefinic bond to epoxide then formation of vicinal diol. Also, oxidation of secondary amine to hydroxyl amine then converted to nitroso group.

Confirmation of degradation product using mass spectrometry

Mass spectrometry was performed for brexpiprazole oxidative degradation product and molecular ion peak was obtained at m/z = 482.57 indicating that its molecular weight is 482.57 as shown in Figure 3.

DISCUSSION

Optimization of experimental conditions

Different chromatographic conditions affecting the chromatographic separation were optimized after taking in consideration the resolution between the drugs, its oxidativedegradation product. Several mobile phases were triedin order to separate the intact drug from its degradation productincluding methanol, water and phosphoric acid in differentratios. Good separation was carried out on supelcoC18 column (250 x 4.6 x 5 µm particle size) columnusing a mobile phase consists of methanol , water and phosphoric acid (60:40:0.4, by volume) at flowrate 1 mL min^{-1} and UV detection at 259 nm.HPLC chromatogram revealed that brexpiprazolewas clearly separated from its degradationproduct at retention times of 4.41 + 0.03 and 2.59 +0.05 minutes for intact brexpiprazole and its degradation product respectively, as shown in figure 4.

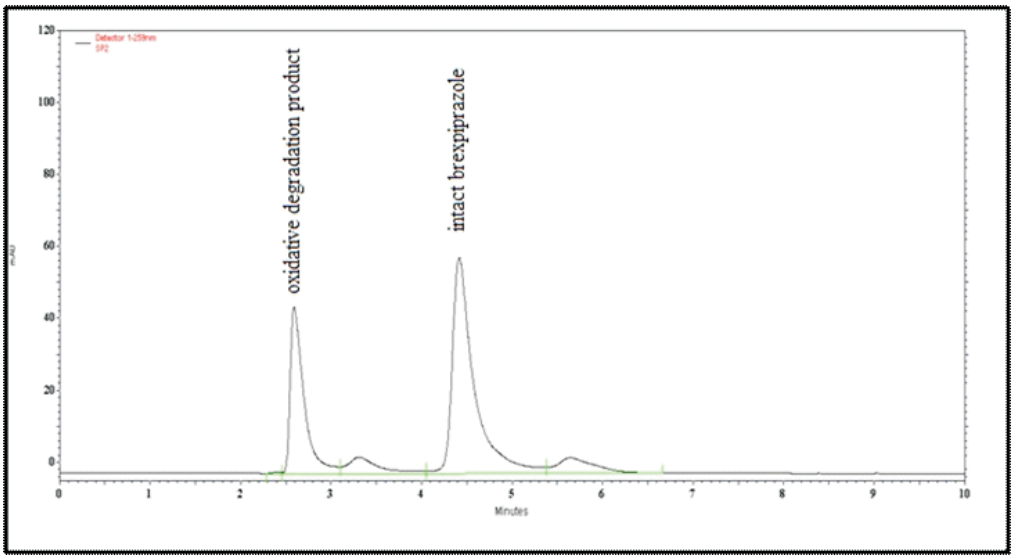


Figure 4 : HPLC chromatogram of mixture of intact brexpiprazole (50 µg/mL) anditsdegraded brexpiprazole (50 µg/mL) .

Table 1 : Regression and validation data for the determination of brexpiprazole by the proposed HPLC procedure:

Parameters	Proposed HPLC procedure
wavelength (nm)	259
Linearity range (µg/ml)	20 - 100
LOD (µg/ml)	4.77
LOQ (µg/ml)	14.4
- Regression Equation	$y^{*} = bx^{**} + a$
- Slope (b)	15762
- Intercept (a)	87676
Correlation coefficient (r)	0.9996
Accuracy (%R)	99.56
Precision(%RSD)	
Repeatability[c]	1.164
Intermediate precision[d]	0.679

y^{*} is peak area ratio.x** is concentration in g/mL.
[d]The intraday (n = 3), average of three concentrations (2, 6 and 10 µg/mL) for brexpiprazole repeated three times within the day.
[c] The interday (n = 3), average of three concentrations for brexpiprazole repeated three times in three days.

Method validation:

Validation of the proposed method was assessed according to the ICH guidelines (6)

Linearity and range

Calibration curve was constructed by plotting the area under peak versus drug concentrations in µg/mL. The regression plot was found to be linear over the range of (20-100µg/mL)the linear regression equation for the graph was:

y = 15762 x 87676..... **(r = 0.9996)**

Where **y** is the area under peak values, **x** is the drug concentration and **r** is the correlation coefficient. Linearity range, regression equation, intercept, slope and determination coefficient for the calibration data were presented in Table 1.

Limits of detection and quantitation

The limits of detection (LOD) and the limits of quantitation (LOQ) were calculated according to ICH guidelines from the following equations:

$LOD = 3.3\,\sigma / S$ $LOQ = 10\,\sigma / S$

Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.LOD and LOQ values were mentioned in Table 1.

Accuracy and precision

Accuracy and precision of the method were determined by applying the proposed procedure for determination of three different concentrations, each in triplicate, of brexpiprazole in pure form within linearity range in the same day (intraday) and in three successive days (interday).Accuracy as percent recovery (R %) and precision as percent relative standard deviation (RSD %) were calculated andresults are listed in Table 1. To ascertain the accuracy of the suggested method, recovery studies were carried out by standard addition technique at three different levels, Table2.

Specificity

The specificity of the proposed method was assured by applying to laboratory prepared mixtures of the intact brexpiprazole with its degradation product. Also, The proposed procedure was adopted for the selective determination of intact brexpiprazole in presence of of other additives and excipients and it was found that the specificity of an analytical method has an ability to measure accurately an analyte in the presence of interferences that are known to be present in the product: additives, excipients by applying standard addition technique and between the analyte of interest and its oxidativedegradant that may be present with high resolution > 1.5.

Table 2 : Recovery study of brexpiprazole by adopting standard addition technique using the proposed HPLC procedure:

Pharmaceutical taken (µg/ml)	Pure added (µg/mL)	Pure found (µg/mL)	% Recovery
20	20	20.06	100.30
	40	40.23	100.58
	60	59.46	99.10
	80	80.57	100.72
Mean			100.18
%RSD			0.737

Table 3 : System suitability parameters fordetermination of brexpiprazole by the proposed HPLC procedure:

Parameters	**Obtained value**	**Reference value**
Resolution (R)	**2.27**	**R > 1.5**
Tailing factor (T)	**1.29**	**0.8–1.5**
Capacity factor (K)	**3.41**	**1–10 acceptable**
Column efficiency (N)	**2141**	**> 2000**

System suitability

System suitability test was applied to a representative chromatogram to check various parameters such as the number of theoretical plates (N), resolution factor (R), capacity factor (k), tailingfactor (T) and selectivity factor (α). The results shown in Table 3 revealed that the chromatographic conditions described here allow complete base line separation between drug and its degradation product peaks with minimum tailing.

Robustness

The robustness of the method was evaluated by slight changes in the chromatographic parameters such as flow rate (±0.1 mL/min.), pH of the mobile phase (±0.1) and mobile phase contents ratio(±2 %). In each case only one parameter was changed while other parameters were kept constant. These minor changes did not affect the separation and resolution of brexpiprazole from its degradation product, confirming robustness of the procedure, as shownin Table 4.

Pharmaceutical applications

The proposed HPLC procedure was applied to the determination of brexpiprazole inRexulti® tablets. Satisfactory results were obtained in good agreement with the label claim, indicating no interference from excipients and additives.The obtained results were statistically compared tothose obtained by the reported method [5] indicating good accuracy and precision of the proposed method for the analysis of the studied drug in its pharmaceutical dosage form, as shown in Table 5. No significant differences were found by applying t-test and F-test at 95 % confidence level.

CONCLUSION

In this work, HPLC chromatographic method was developed and applied for the determination of brexpiprazole in the presence of its oxidative-induced degradation product. The proposed method is simple, accurate and precise and can be used for the analysis of brexpiprazole in pure form and in pharmaceutical dosage form (either alone or in the presence of its oxidative degradation product).

Table 4 : Robustness results fordetermination of brexpiprazole by the proposed HPLC procedure:

Parameters		Retention time (t_R)	Theoretical Plates (N)	Resolution* (R)	Tailing factor (T)
Flow rate (ml/min.)	0.9	4.45	2132	2.12	1.31
	1	**4.41**	**2141**	**2.27**	**1.29**
	1.1	4.1	2122	1.98	1.30
pH of mobile phase	3.9	4.31	2133	2.11	1.26
	4	**4.41**	**2141**	**2.27**	**1.29**
	4.1	4.35	2009	2.01	1.33
Mobile phase ratio (H_2O : methanol) with 0.4 mL phosphoric acid)	42:58	4.43	2111	1.97	1.34
	40:60	**4.41**	**2141**	**2.27**	**1.29**
	38:62	4.39	2134	2.21	1.30

* Relative to its degradation product.

Table 5 : Determination of brexpiprazole in Rexulti® tablets by the proposed HPLC and the reported method.

Parameters	Proposed method	Reported method[5]
n*	5	5
X'**	99.55	99.26
SD	1.034	1.031
% RSD	1.038	1.039
t***	1.171(2.306)	——
F***	4.618(6.388)	——

* Number of experiments; ** The mean of percent recovery of brexpiprazole
*** The values in parenthesis are tabulated values of "t "and "F" at (P = 0.05)

REFERENCES

1. National Alliance on Mental Illness Mental health by the numbers. Available at: www.nami.org/Learn-More/Mental-Health-By-the-Numbers [accessed on August 16, 2015].
2. Citrome,L. Int. J Clin Pract.2015:69(9): 978-97
3. Das,S.,& Barnwal,P.,& Blessed, W.A.,& Mondal,S., & Saha, I. Ther Adv Psychopharmacol. 2016: 6(1): 3954
4. Wikipedia [internet], [reviewed 2017 Sep 2; cited 2017 Sep 27]. Available from:https://en.wikipedia.org/wiki/Brexpiprazole, oct. 4th, 2017
5. Sravani, A.,& Durga, C.H.N.,& Divya,U.,& Suneetha, C.H., & Suresh,P., & Rao,B.T.,& Sudheer, C. Indo Am J Pharm Res.2017: 7: 8560-8565
6. ICH Guideline Q2(R1) (1996) on Validation of Analytical Procedures: Text and Methodology, International Conference on Harmonization, Geneva, 6 Nov 1996, incorp. Nov 2005