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Special Article – RP-HPLC

Quantification of Brexpiprazole in Bulk and Its Pharmaceutical Dosage Form by UV - Visible Spectroscopic and SIAM RP-LC Method

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Abstract

A sensitive, selective, rapid, precise, and economical UV- Visible Spectroscopic method and stability indicating Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method were developed for the quantification of Brexpiprazole in bulk and pharmaceutical dosage form. UV – Visible Spectroscopic determination was carried out at absorption maxima of 215nm using methanol as a solvent. The linearities were in the range of 1-6µg/ml for UV-Visible Spectroscopic method and 0.01-10µg/ml for RP-HPLC method, respectively. The detection and quantitation limits were 0.33µg/ml and 1µg/ml and 0.003µg/ml and 0.01µg/ml for RP-HPLC method, respectively indicating sensitivity of the method. HPLC method was carried out on a Sun fire C18 (250x0.46)mm; 5 µm with a mobile phase consisting of Acetonitrile: Methanol in the ratio of (60:40 v/v) at a flow rate of 1.0ml/min. Detection was carried out at 215nm with help of photodiode array (PDA) detector. The retention time of Brexpiprazole was 3.89min. The stock solution of Brexpiprazole (1000µg/ml) were subjected to acid and alkali hydrolysis, chemical oxidation, dry heat degradation and photo degradation. The drug was found to be susceptible to acid and alkali hydrolysis, chemical oxidation, photo degradation and dry heat. The degraded product peaks were well resolved from the pure drug peak with significant difference in their Rt values. Stressed samples were assayed using proposed developed RP-LC method. The developed method was validated according to ICH guidelines for evaluation of accuracy, precision, linearity, limit of detection, limit of quantitation and robustness. The proposed method can be used for the estimation of the drug in bulk and pharmaceutical dosage form.

Keywords: Brexpiprazole; Stability Indicating RP-HPLC; UV-Visible Spectroscopy; Validation

Abbreviations

BREX: Brexpiprazole; SIAM: Stability Indicating Analytical Method; UV: Ultraviolet; RP-HPLC: Reversed Phase High Performance liquid Chromatography; FDA: Food and Drug Administration; ICH: International Conference on Harmonization; RSD: Relative Standard Deviation

Introduction

Brexpiprazole (BREX) is an atypical antipsychotic chemically 7- [4-[4-(1-benzothiophen-4-yl) piperazin-1-yl] butoxy]-1H-quinolin-2-one Chemical structure is depicted in (Figure 1). It is a dopamine D_2 receptor partial agonist and has been described as a "Serotonin Dopamine Activity Modulator" (SDAM). The drug received FDA approval on July 10, 2015 for the treatment of Schizophrenia, and as an adjunctive treatment for depression.

Partial agonists have both blocking properties and stimulating properties at the receptor they bind to. The ratio of blocking activity to stimulating activity determines a portion of its clinical effects. BREX has more blocking and less stimulating activity than its predecessor, aripiprazole, which may decrease its risk for agitation and restlessness. It is also an antagonist of the serotonin 5-HT_{2A}, 5-HT_{2B}, and 5-HT_7 receptors and the α_{1A}-, α_{1B}-, α_{1D}-, and α_{2C}-adrenergic receptors. The drug has negligible affinity for the muscarinic acetylcholine receptors, and hence has no anticholinergic effects.

Only RP-HPLC method has been reported for estimation of BREX bulk and not in tablet dosage forms. In the present work, a successful attempt has been made to estimate BREX in bulk and pharmaceutical dosage form by using UV - Visible Spectroscopic method and stability indicating RP-HPLC method which is more sensitive, accurate and Economic as compared to published one. This study attempts to develop a simple, accurate and precise analytical chromatographic method, which can quantify the drug from a tablet dosage form. The developed method was validated as per ICH guidelines and found to comply with the acceptance Criteria.

Materials and Methods Experimental work

Apparatus of UV-Visible Spectrophotometry

Spectrophotometer: All the absorption spectra and derivative spectra were recorded on UV-visible double beam spectrophotometer (UV-1700, Shimadzu Corp., Japan) with 1cm quartz cell.

Electronic balance: All the drugs and chemicals were weighed on Shimadzu electronic balance (AX 200, Shimadzu Corp., and Japan).

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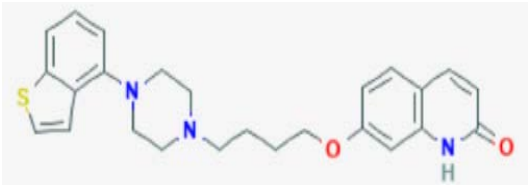

Figure 1: Structure of BREX.

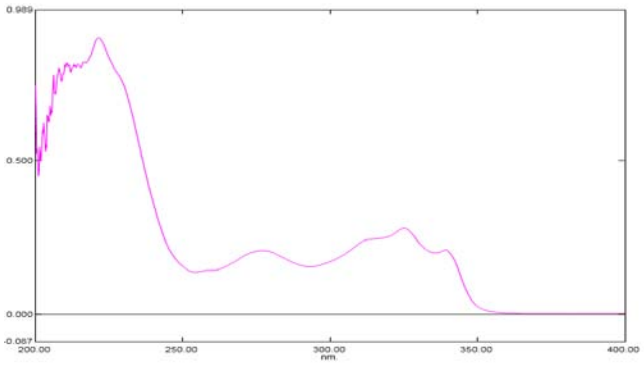

Figure 2: UV- spectra of BREX (6µg/ml) in Methanol.

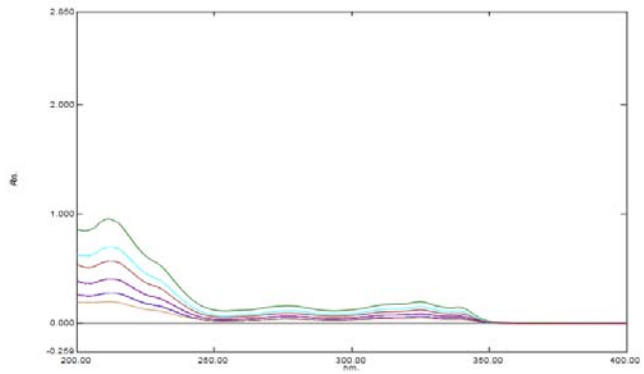

Figure 3: Overlay spectra of BREX in Methanol (1-6µg/ml).

Materials and reagents: Methanol was used throughout UV – Visible spectroscopic method development and validation.

Apparatus: UV spectrophotometric method was performed on double beam UV-visible spectrophotometer (Shimadzu, model 1700) having two matched quartz cells with 1cm light path.

Selection of solvent: BREX is soluble in methanol; therefore, methanol was chosen as a solvent for stock solution preparation.

Preparation of standard stock solution (100µg/ml): Accurately weighed BREX (10mg) was transferred into 10ml volumetric flask having few ml of methanol and swirl to dissolve and diluted up to the mark with methanol to obtain solution having concentration of BREX (1000µg/ml). From the above solution, pipette out 1ml of aliquot and transfer into another 10ml volumetric flask diluted up to the mark with methanol to obtain standard stock solution of (100µg/ml).

Selection of analytical wavelength: The solution of BREX was

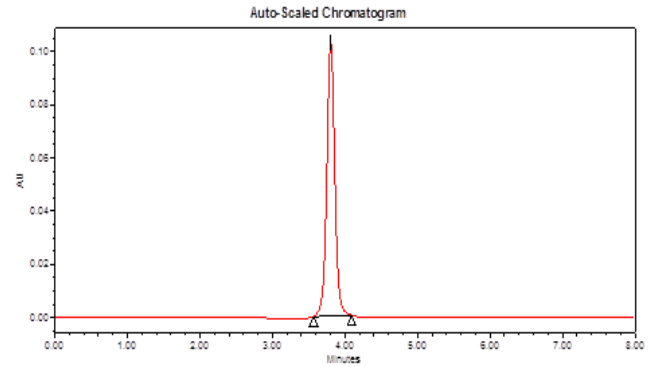

Figure 4: Chromatogram for the standard solution of BREX (5 µg/ml) with mobile phase ACN: Methanol (60:40 v/v).

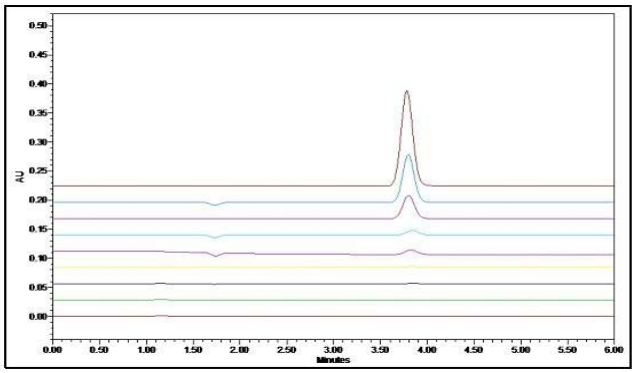

Figure 5: Overlay of chromatograms of different concentrations of BREX (0.01 – 10 µg/ml).

prepared in methanol at a concentration of 6µg/ml. It was scanned in the wavelength range of 200-400nm. Analytical wavelength of 215nm was selected for determination of BREX.

Calibration Curve: Appropriate aliquots of stock solution were taken in six different 10ml volumetric flask. Volume was made up to the mark with methanol to obtain final concentration of 1, 2, 3, 4, 5 and 6 µg/ml.

Apparatus and chromatographic conditions of HPLC

Chromatographic separation was performed on a model of Waters 2998 containing PDA detector and Empower 2 software. A Sun fire C_{18} (250x0.46)mm; 5µm was used for the separation, mobile phase of a mixture of acetonitrile and methanol in the ratio of 60:40 v/v was delivered at a flow rate of 1.0ml/min with detection at 215nm. The mobile phase was filtered through a 125mm membrane filter and degassed. The injection volume was 20µl; Analysis was performed at ambient temperature.

Chemicals and solution: Acetonitrile (HPLC grade) and Methanol (HPLC grade) – SRL Pvt. Ltd., Mumbai, India and Distilled water (HPLC grade) was procured from all Quartz Double Distiller (Bhanu)™. Reference standards of BREX obtained as gratis sample and Marketed tablet dosage form was procured from reputed pharma company, India.

Preparation of standard solutions: Accurately weigh 10mg of

BREX and transfer to 10ml volumetric flask containing few ml of methanol and makeup the volume up to the mark with methanol which gives the solution having concentration of 1000µg/ml of BREX. Take an aliquot from the stock solution and dilute with mobile phase to obtain working standard of 100µg/ml of BREX.

Preparation of sample solution: Twenty tablets were weighed and powdered; powder equivalent to 2mg of BREX was transferred in to a 10ml volumetric flask containing few ml of methanol and sonicated for 20 min. The solution was filtered through what man filter paper No.41 and the volume was adjusted up to the mark with methanol. This will produce sample solution containing BREX 200µg/ml. From the above solution 1ml aliquots was pipette out and transfer in to another 10ml volumetric flask and diluted to the mark with methanol. This will produce sample solution containing BREX 20µg/ml (Stock solution A). From the stock solution A, 2ml was transferred to another 10ml volumetric flask and volume was made up to the mark with methanol to give a solution containing 4µg/ml BREX. The Rt of solution was measured at 215nm and the quantification was carried out by keeping this value to straight line of calibration curve.

Calibration Curve of BREX: Pipette out appropriate volume of aliquot from standard stock solution and transferred to seven different volumetric flask of 10ml and volume was adjusted with the mark with the mobile phase to give a solution containing 0.01, 0.05, 0.1, 0.5, 1, 5, and 10µg/ml of BREX.

Each solution was analyzed by the proposed method and the chromatogram was recorded. The standard solution was run for 10 min. using mobile phase at a flow rate of 1ml/min.

Calibration curve was constructed by plotting concentration v/s peak area and regression equation was computed.

Chromatographic separation: Standard solutions of 0.01-10 µg/ml were injected in column with injection volume 20µl. The chromatogram was run for appropriate minutes with mobile phase Acetonitrile: Methanol (60:40 v/v). The detection was carried out at wavelength 215nm. The chromatogram was stopped after separation achieved completely. Data related to peak like area, height, retention time, resolution etc. maintained.

System suitability test: System suitability tests are based on the concept that the equipment, electronics, Analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability was performed and calculated as the Start of study of each validation parameter. The values of system suitability results obtained during the entire study and results were recorded in terms of capacity factor 1.04, Theoretical plates 8292.87 and asymmetry factor 1.04.

Method validation

As per ICH guidelines Q_2R_1, the method validation parameters studied were linearity, range, accuracy, precision, limit of detection, limit of quantification, and robustness.

Method Validation of UV-Spectrophotometric method

Linearity and range: Linearity of the method was performed by constructing calibration curves at six different concentration levels over a range of 1-6µg/ml for BREX. The calibration curve was constructed by plotting absorbance versus concentration (n=5).

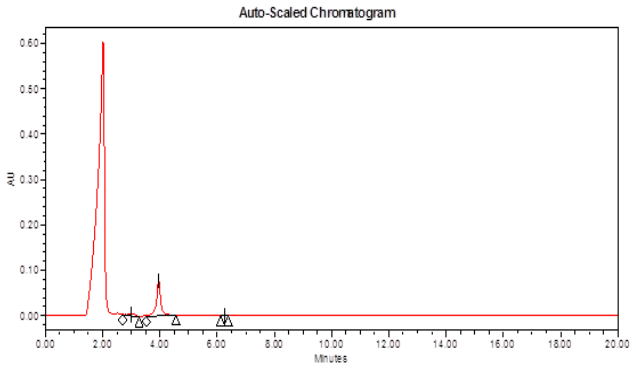

Figure 6: Chromatogram of Acid Degradation of BREX (0.1N HCl /80°C).

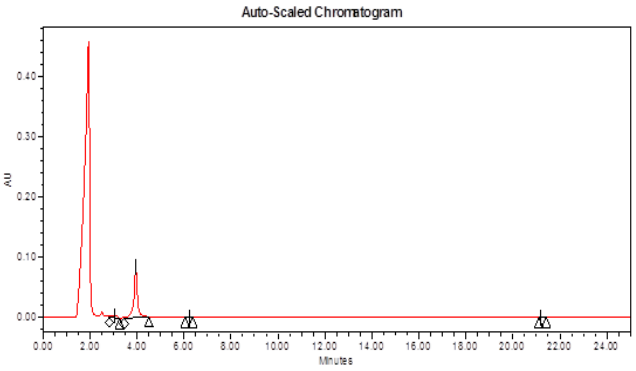

Figure 7: Chromatogram of Base Degradation of BREX (0.1N NaOH /80° C).

Precision

Repeatability: Standard solution of BREX (3µg/ml) was prepared and spectra were recorded. Absorbance was measured at 215nm using methanol as a blank. The absorbance of the same concentration of BREX solution was measured six times and %RSD was calculated.

Intra and inter day precision: Difference in the results of three different concentrations (1, 3 and 6µg/ml) within the same day (intra-day) and difference in the results between different days (inter-day) were analyzed. Intra-day precision was performed by analyzing each concentration of BREX for three times in the same day. Inter-day precision was performed by analyzing each concentration of BREX for three times for three different days.

Limit of detection: From the linearity curve equation, the Standard Deviation (SD) of the Y-intercepts (response) was calculated. Then LOD was measured by using Visual method

Limit of quantification: From the linearity curve equation, the Standard Deviation (SD) of the Y-intercepts (response) was calculated. Then LOQ was measured by using Visual method

Accuracy: The accuracy study of the method was performed by calculating recoveries of BREX by standard addition method. Known amount of BREX (0, 1, 2, and 3µg/ml) were taken from the working standard solution (100µg/ml of BREX). It was added to pre-quantified sample solutions to obtain 2, 3, 4, 5µg/ml solutions. The amount of BREX was determined by measuring the absorbance and by fitting these values to the straight-line equation of calibration curve.

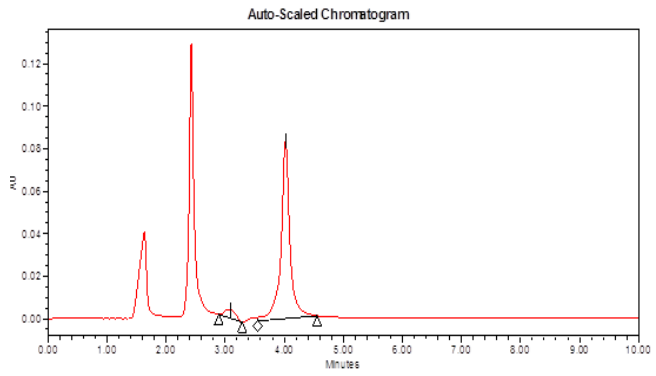

Figure 8: Chromatogram of Oxidative Degradation of BREX (3%H_2O_2 /80° C).

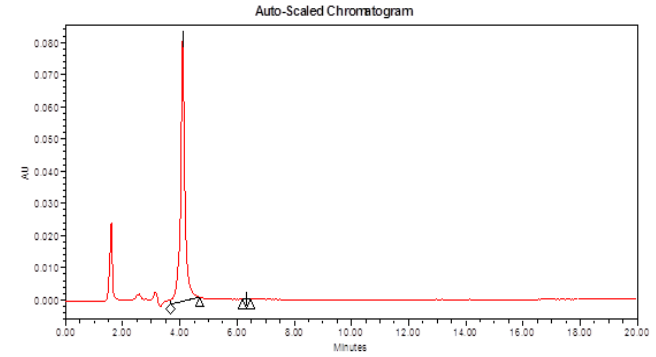

Figure 9: Chromatogram of Photolytic Degradation.

Ruggedness: It is the degree of reproducibility of test results obtained by analyzing the drug under variety of normal test conditions such as different analysts, instruments, days, reagents and columns.

Robustness: The Robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provide an indication of its reliability during normal usage.

Analysis of marketed formulation: Twenty tablets were weighed and powdered; powder equivalent to 2 mg of BREX was transferred in to a 10ml volumetric flask containing few ml of methanol and sonicated for 20 min.The solution was filtered through what man filter paper No.41 and the volume was adjusted up to the mark with methanol. This will produce sample solution containing BREX 200µg/ml. From the above solution 1ml of aliquot was pipette out and transfers in to another 10ml volumetric flask and diluted to the mark with methanol. This will produce sample solution containing BREX 20µg/ml (Stock solution A). From the stock solution A, 1.5ml was transferred to another 10ml volumetric flask and volume was made up to the mark with methanol to give a solution containing 3µg/ml BREX. The absorbance of solution was measured at 215nm and the quantification was carried out by keeping this value to straight line equation of calibration curve.

Method validation of HPLC

Linearity: Linearity was studied by preparing standard solution of 6 different concentration of 0.01, 0.05, 0.1, 0.5, 1,5, 10µg/ml for B.

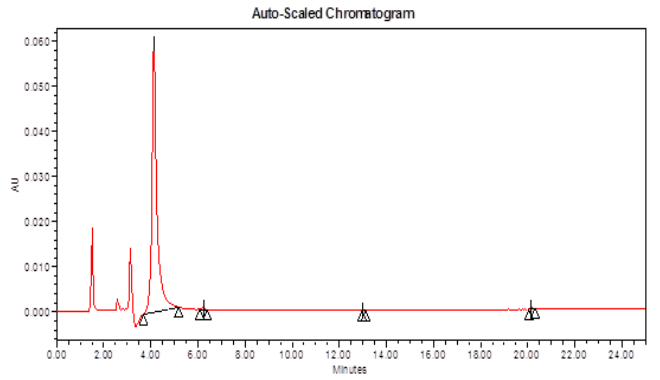

Figure 10: Chromatogram of thermal degradation (Dry Heat) of BREX.

Each concentration was repeated 5 times and linearity was assessed in terms of slope, intercept and correlation coefficient of BREX. The calibration curves were developed by plotting concentration v/s peak area (n=5).

Precision: Precision was calculated in terms of intraday and intraday precisions. Intraday precision was determined by analyzing sample solution of BREX (0.01, 0.5 and 10) µg/ml at three levels covering low, medium and high concentration of the calibration curve three times on the same day (n=3).

Now, intraday precision were determined by analyzing sample solution of BREX (0.01, 0.5 and 10µg/ml) at three levels covering low, medium and high concentration over a period of three days (n=3). The peak areas obtained were used to calculate mean and %RSD values. The repeatability studies were carried out by estimating the response of 0.5µg/ml of BREX 6 times and result are reported in terms of % RSD.

Limits of detection and Quantification: According to ICH, Limit of Detection (LOD) is the lowest concentration of the analytes that can be detected and Limit of Quantification (LOQ) is the lowest concentration of analytes that can be detected with acceptable accuracy and precision. LOD and LOQ are calculated by using Visual method.

Recovery studies: To study the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels. Known amount of the drug was added to pre-analyzed tablet powder and percentage recoveries were calculated.

Robustness: It should show the reliability of an analysis with respect to deliberate variations in method parameters.

In case of liquid chromatography, examples of typical variations are:

1. Proportion of mobile phase,
2. Flow rate. (±0.1)

Table 1: Precision parameters for BREX.

Parameter		BREX (U.V)	BREX (HPLC)
Repeatability n=6	%RSD	0.62	0.93
Interday precision (n=3)	%RSD	0.25-0.71	0.39-1.25
Intraday precision (n=3)	%RSD	0.21-0.36	0.15-1.20

Table 2: Accuracy data of BREX (U.V).

Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (µg/ml)	Total Conc. Found (µg/ml)	% Recovery ± S.D. (n=3)
BREX At 215 nm	50 %	2	1	3.00	100.12±0.82
	100 %	2	2	3.99	99.50±0.56
	150 %	2	3	4.99	99.66±0.18

Table 3: Accuracy data of BREX (HPLC).

Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (µg/ml)	Total Conc. Found (µg/ml)	% Recovery ± S.D. (n=3)
BREX At 215 nm	50 %	4	2	5.96	99.04±0.62
	100 %	4	4	7.96	99.15±0.97
	150 %	4	6	9.92	98.19±0.36

3. Wavelength (±2%)

System suitability: System suitability was established in order to determine the adequate resolution and reproducibility of the proposed method. Suitability parameters including retention factor, resolution, asymmetry factor, and plate number were investigated.

Assay of the marketed formulation: The developed method was applied for the determination of BREX in pharmaceutical formulations. Sample was analyzed by performing three independent determinations

Forced degradation study: Forced degradation study using acid and alkali hydrolysis, chemical oxidation, photolytic degradation and dry heat degradation were carried out and interference of the degradation products was investigated. Accurately weighed BREX (10.0mg) was transferred into 10ml volumetric flasks and expose to different stress conditions.

Heat induced alkali hydrolysis: Accurately weighed 10.0mg of BREX was taken into the 10ml volumetric flask and 2.0ml of 0.1N NaOH was added to perform heat induced base hydrolysis. The flask was heated in a water bath at 80°C for 2 hrs and allowed to cool to room temperature. The solution was neutralized by addition of required amount of 0.1 NHCl and volume was made up to the mark with methanol. From the above solution 1ml of aliquot was pipette out and transferred in to another 10ml volumetric flask and diluted to the mark with methanol. This will produce sample solution containing BREX 100µg/ml. Pipette out aliquot of (0.5ml) solution and transfer into another 10ml volumetric flask and make up the volume up to the mark with mobile phase to obtain final concentration of 5µg/mL of BREX.

Heat induced acid hydrolysis: Accurately weighed 10.0mg of BREX was taken into the 10ml volumetric flask and 2.0ml of 0.5N HCl was added to perform heat induced acid hydrolysis. The flask was heated in a water bath at 80°C for 2 hrs and allowed to cool to room temperature. The solution was neutralized by addition of required amount of 0.5N NaOH and volume was made up to the mark with methanol. From the above solution 1ml of aliquot was pipette out and transferred in to another 10ml volumetric flask and diluted to the mark with methanol. This will produce sample solution containing BREX 100µg/ml. Pipette out aliquot of (0.5ml) solution and transfer into another 10ml volumetric flask and make up the volume up to the mark with mobile phase to obtain final concentration of 5µg/ml of BREX.

Table 4: Robustness study data of BREX (HPLC).

Parameter			BREX	
	Proportion of M.P 58:42		1.22	
	Proportion of M.P 62:38		1.61	
Robustness %RSD	Flow rate (+0.1 units)		1.36	
	Flow rate (-0.1 units)		1.87	
	U.V.	HPLC		
	Wavelength (+1%)	Wavelength (+2%)	0.73	1.74
	Wavelength (-1%)	Wavelength (-2%)	0.85	1.18

Table 5: Summary of validation parameters.

Parameters	BREX(U.V.)		(HPLC)
Range(µg/ml)	1-6		0.01-10
Detection limit (µg/ml)	0.33		0.003
Quantisation limit (µg/ml)	1		0.01
Intra-day (n=3)	0.21-0.36		0.15-1.20
Inter-day (n=3)	0.25-0.71		0.39-1.25
Repeatability (n=6)	0.621		0.93-93
Accuracy (%) (n=3)	99.5-100.12		98.19-99.68
Ruggedness (%RSD)	UV 1800:0.76	Reagent 1:0.68	-
	Elico:1.66	Reagent 2:0.95	-
Robustness (%RSD)	214:0.73		213:1.74
	216:0.85		217:1.18

Heat induced oxidative stress degradation: To perform heat induced oxidative stress degradation, 10.0mg of BREX was transferred in 10ml volumetric flask and 2.0ml of 3% hydrogen peroxide was added. The mixture was heated in a water bath at 80°C for 2 hrs and allowed to cool to room temperature and volume was made up to the mark with methanol. From the above solution 1ml of aliquot was pipette out and transferred in to another 10ml volumetric flask and diluted to the mark with methanol. This will produce sample solution containing BREX 100 µg/ml. Pipette out aliquot of (0.5ml) solution and transfer into another 10ml volumetric flask and make up the volume up to the mark with mobile phase to obtain final concentration of 5µg/ml of BREX.

Dry heat degradation: Accurately weighed analytically pure 10mg sample of BREX was exposed in oven at 80°C for 1 hr. The drug

Table 6: Analysis of marketed formulation.

		Amount of drug taken(µg/ml)	Amount of drug found (µg/ml) (n=3)	% of drug found (n=3) ± SD
Formulation (Rexulti)	UV Method	3	2.98	99.49 ± 0.12
	HPLC	4	3.99	99.88± 0.32

*Rexulti, Otsuka ltd. Each tablet contains 2 mg of Brexpiprazole.

Table 7: Results of Forced degradation study by Proposed HPLC Method.

Stress Conditions	Time (h)	Assay of active substance (%)	Peak Purity
0.1 N HCl/80°C/2ml	2	82.36	1
0.1 N NaOH/80°C/2ml	2	93.05	1
3%H_2O_2/80°C/2ml	2	84.27	0.99
Photo degradation/U.V. light	24	86.02	1
Thermal /80°C	1	91.45	1

powder was allowed to cool and transferred into 10ml volumetric flask containing few ml of methanol and volume was made up to the mark with the methanol (1000µg/ml). From the above solution 1ml of aliquot was pipette out and transferred in to another 10ml volumetric flask and diluted to the mark with methanol. This will produce sample solution containing BREX 100µg/ml. Pipette out aliquot of (0.5ml) solution and transfer into another 10ml volumetric flask and make up the volume up to the mark with mobile phase to obtain final concentration of 5µg/ml of BREX.

Study of photolytic degradation: In Petri dish, accurately weighed analytically pure 10mg of drug was exposed to UV light for 24 hrs. The powder content was transfer into 10ml volumetric flask containing few ml of methanol and volume was made up to the mark with the methanol (1000µg/ml). From the above solution 1ml of aliquot was pipette out and transferred in to another 10ml volumetric flask and diluted to the mark with methanol. This will produce sample solution containing BREX 100µg/ml. Pipette out aliquot of (0.5ml) solution and transfer into another 10ml volumetric flask and make up the volume up to the mark with mobile phase to obtain final concentration of 5µg/ml of BREX [1-12].

The final concentrations of all the BREX solutions 5µg/ml was injected into the column and chromatograms were recorded.

Results and Discussion

Result of UV – Visible Spectrophotometric method

Selection of solvent: BREX is soluble in Organic solvent. It is soluble in methanol; therefore, methanol was chosen as a solvent for stock solution.

Selection analytical wavelength: The solution of BREX was prepared in methanol at a concentration of 6µg/ml. It was scanned within the wavelength range of 200-400nm. Data was recorded at an interval of 1nm. The wavelength of 215nm was selected for estimation of BREX (Figure 2).

Validation of developed UV Visible method

Linearity and range: The method was found to be linear over the range of 1–6µg/ml. Overlay spectra of BREX is shown in (Figure 3).

Precision

Repeatability: Repeatability studies were carried out and data was reported in (Table 1). The %RSD is <2 for BREX which indicates that the method is repeatable.

Intra and interday precision: Variation of results within the same day (intra-day), variation of results between days (inter-day) was analyzed. For intra-day (n=3) % RSD was found to be 0.21-0.35 and % RSD for inter-day (n=3) was 0.24 - 0.71 for BREX (Table 1). The % RSD is <2 for BREX which indicates that the proposed method is precise.

Limit of detection and limit of quantification: Under the experimental conditions used, the minimum amount of drug that could be detected (LOD) for BREX was found to be 0.330. The Limit of Quantification (LOQ) for BREX was found to be 1.00.

Accuracy: Accuracy study was performed by calculating the recovery. The % recovery was found to be 98.19-99.68 % for BREX (Table 2), which indicates that method is accurate.

Ruggedness: Ruggedness of the method was analyzed by deliberate change different parameters like different brand of reagent and different UV – Visible spectrophotometer model. The % RSD was < 2 indicates that developed method is rugged.

Robustness: Robustness of the method was analyzed by deliberate variation in method parameters like change in wavelength (Table 4). The % RSD was < 2 indicates that developed method is robust.

Summary of validation parameters are shown in (Table 5).

Analysis of marketed formulation: The proposed method was applied to determine BREX, in their dosage form. The % amount of drug was found to be 99.49-100.12 % for BREX (Table 6).

Results and Discussion of HPLC

Selection of elution mode: Reverse phase chromatography was chosen because of its recommended use for ionic and moderate to non-polar compounds. Reverse phase chromatography is not only simple, convenient but also better performing in terms of efficiency, stability and reproducibility. Hence C18, 250 × 0.46mm column of 5µm particle packing was selected for separation of BREX.

Selection of wavelength: BREX show reasonably good response at 215nm mention in Figure 2.

Mobile phase optimization: Chromatographic parameters were optimized to develop a HPLC method for simultaneous determination of BREX with short analysis time (< 10 min), and acceptable resolution (R_S < 2). Various compositions of mobile phases like Methanol: Water; Acetonitrile (ACN): Water and Acetonitrile: Methanol in different ratios was tried. But with mixed ACN: Methanol in the ratio of (60:40 v/v) at a flow rate of 1ml/min, symmetrical peaks with good resolution were obtained. The optimum wavelength for detection was set at 215nm at which better detector response for drug was obtained. The retention time was 3.89 min for BREX (Figure 4).

Validation

Calibration graphs were constructed by plotting the peak area versus their corresponding concentrations. Good linearity was obtained in the range of 0.01-10 µg/ml for BREX Overlay is shown in

(Figure 5). LOD and LOQ were calculated by using the visual method. The precision of the method and instrument precision was evaluated and Relative Standard Deviation (RSD) values were calculated. The RSD values for BREX showed that the precision of the method was satisfactory. The results are shown in (Table 1). The accuracy of the method was determined by recovery studies. The recovery was found to be more than 98 % for BREX. The results are shown in (Table 3). Developed method was found to be robust when the mobile phase ratio, flow rate and wavelength were changed. The results are shown in (Table 4).

Summary of validation parameters are shown in (Table 5).

Assay of marketed formulation

The tablet powder equivalent to 2mg of BREX was taken in 100ml volumetric flask containing few ml of methanol and sonicated for 20 min. That content is diluted with mobile phase and mark up to 100ml with same solution. It gives 200 (µg/ml) of BREX. The prepared solution was filtered through what man filter paper No.41. From the above solution 1ml aliquot was pipette out and transfers in to another 10ml volumetric flask and diluted to the mark with methanol. This will produce sample solution containing BREX 20µg/ml. From the stock solution A, 2ml was transferred to another 10ml volumetric flask and volume was made up to the mark with methanol to give a solution containing 4µg/ml BREX. The diluted solution was analyzed under optimized chromatographic conditions. The areas of resulting peak were measured at 215nm and the quantification was carried out by keeping these values to straight line of calibration curve. Shown in (Table 6).

Forced degradation study

Acid and base hydrolysis study, photolytic degradation, dry heat degradation and oxidative stress degradation studies were carried out and the degraded samples were analyzed by the developed method.

Chromatogram of acid hydrolysis was performed at 80°C for 2 hrs reflux showed degradation of BREX with degradation product peak at retention time (Rt) 6.26 (Figure 6). Chromatogram of base hydrolysis was performed at 80°C for 2 hrs. Reflux showed degradation of BREX with degradation product peak at retention time (Rt) 3.03 (Figure 7). The chromatogram of oxidized BREX with 3% hydrogen peroxide was performed at 80°C for 2 hrs reflux, showed degradation of BREX with degradation product peak at retention time (Rt) 3.15 (Figure 8). The chromatogram of ultraviolet light exposed BREX was performed for 24 hrs, reflux showed degradation of BREX with degradation product peak at retention time (Rt) 6.23 (Figure 9). The chromatogram of BREX exposed to dry heat at 80°C for 1 hr, showed degradation of BREX with degradation product peak at retention time (Rt) 20.13 (Figure 10).

The degradation study thereby indicated that BREX was found to be susceptible to acid, base hydrolysis, oxidation (3% hydrogen peroxide), photolytic degradation and dry heat degradation. The degradation peaks are well resolved from the drug peak and no degradation products from different stress conditions interfere in the determination of BREX which indicate that the method is selective and specific.

Summary of Forced Degradation Study is shown in (Table 7).

Conclusion

A specific, accurate, precise and robust isocratic and stability indicating RP-HPLC and UV-Visible Spectroscopic methods have been developed for the quantification of BREX in bulk and Pharmaceutical Dosage Form. In RP – HPLC method, the drug was found to be susceptible in acidic, basic, oxidative stress, thermal degradation and photolytic degradation. The proposed both methods can be used for the drug analysis in routine quality control. The drug was subjected to acid, alkali, oxidative stress, dry heat and UV exposure. From all above exposure conditions, degrading peak was well resolved from the parent peak. Comparison with recently published HPLC article have been shows simple and less sensitive with respect to linearity and range, method for quantification BREX while in proposed method is stability indicating HPLC method for quantification of BREX and its impurities. In addition, the proposed stability indicating RP-HPLC procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiration dates of pharmaceuticals.

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