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A NOVEL STABILITY-INDICATING UHPLC–MS/MS METHOD FOR QUANTITATIVE DETERMINATION OF BREXPIPRAZOLE IN BULK DRUG SUBSTANCE: DEVELOPMENT, VALIDATION, AND FORCED DEGRADATION STUDIES

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ABSTRACT:
Background: Brexpiprazole is an atypical antipsychotic drug used to treat schizophrenia and agitation induced by Alzheimer's disease.
Aim: The present study aimed to develop and validate a simple, rapid, sensitive, and stability-indicating UHPLC-MS/MS method for measuring Brexpiprazole by using a dilute-and-shoot sample preparation technique.
Method: Chromatographic separation was performed on a Shim-pack GIST C18 column using a mobile phase at 0.1% formic acid in water and acetonitrile. Electrospray ionization was performed in multiple reaction monitoring mode to detect the transition m/z 434.15 → 273.10.
Result: The method showed excellent linearity between 1-100 ng/mL, with a correlation coefficient (r^2) of 0.9976. Brexpiprazole has a retention period of 2.18 minutes, that allowed for rapid analysis. The limits of detection and quantification were found to be 0.45 and 1.0 ng/mL, respectively. Accuracy studies showed recoveries ranging from 89.0% to 96.8%, while precision tests achieved acceptable %RSD values. The Stability tests found that Brexpiprazole was stable under the investigated conditions, with no significant degradation.
Conclusion: The novel UHPLC-MS/MS technique is rapid, sensitive, and reliable, excellent for routine pharmaceutical analysis, bioanalytical investigation, stability assessments, and pharmacokinetic applications.

KEYWORDS: Brexpiprazole, UHPLC–MS/MS, Stability-indicating method, Method validation, Dilute-and-shoot, Bioanalytical analysis, Quantification.

INTRODUCTION

Brexpiprazole is classified as Atypical antipsychotic drug is approved by US in July 2025(1) and FDA approved by August 2022 (2). The first treatment of agitation associated with dementia due to alzhmeir disease(3). Brexpiprazole is a successive aripiprazole derivative (4) It is a partial agonist of 5HT (Serotonin), D2 and D3 receptor. Brexpiprazole is a less stimulating partial agonist of the dopamine receptor than the aripiprazole potentially decreasing its risk of agitation and restlessness(5). Compared to aripiprazole brexpiprazole have high affinity and lower intrinsic activity. Also aripiprazole may cause weight gain it is a common side effect(6). The chemical name of brexpiprazole is 7-[4-[4(1-benzothiphen-4-yl) piperazine-1-yl]butoxyl]quinolin-2(1H)-one [FigureNo.1]. Molecular formula is C23H27N3O2S2 and the molecular weight of brexpiprazole 433.57g(7). The chemical structure of brexpiprazole is shown in Figure 1.

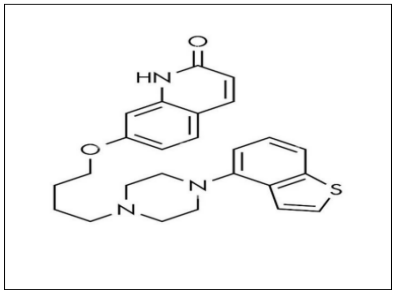


Figure.1 Chemical structure of Brexpiprazole

Source: Adapted from reference 13

Among the study LCMS have a highly selective and sensitive technique for the quantification of the drug. It is more sensitive technique for the detection of identify the complete separated technique (8). This method is used to detect unidentify the constituents compared to other chromatographic technique they give superior selectivity, resolution, precise mass measurement and specificity. The analytical technique can be widely used for pharmaceutical and biopharmaceutical research purpose (9). According to Internation conference harmonization (ICH) guidelines, a validated for the suitable procedure for qualitative and quantitative detection for bulk formulation(10). The recent study thin layer chromatographic technique brexpiprazole shows a sharp peak and get different Rf value to produce selective, precise, repeatable statistical analysis(11). Among our study they using a highly purity of organic solvent in mobile phase to acidified the bulk drug to optimized the sharp peak and better resolution also they stable in standard sample spiked with plasma it is stored in normal temperature at 20-25 days to get sharp peak.
The aim of the study was to developed and validate the stability indicating technique using UHPLC/MS/MS method for the quantification and purity of brexpiprazole bulk formulation.

Experimental technique

MATERIAL AND METHODS
The gifted sample of brexpiprazole was purchased from Triveni inter chem Pvt, Gujarat, India. The following analytical solvents purchased from precision chemicals, Coimbatore Acetonitrile, Formic acid, Methanol in HPLC grade

2.2 Instrumentation technique
Shimadzu's UHPLC system included a DGU-405 degasser, LC-40D×s solvent delivery pump, SIL-40C×s auto sampler, and CTO-40 column oven. The shim pack GIST C Column (2.1×150mm, 3µm) was used for chromatographic separation at 40ºC. The mobile phase consisted of 0.1% formic acid in water and acetonitrile applied via gradient elution. The flow rate was set to 0.5ml/min and the injection volume was 1µl.
A Shimadzu LCMS 8045 triple quadrapole mass spectrometer equipped with an electrospray ionisation (ESI) source in multiple reaction monitoring mode was used to detect the samples. The optimal transition for brexpiprazole was m/z 434.15 to 273.1 with a collosion energy of -30V. The source parameters were as follows, and the result shown interface is (Table-1): temperature 300ºC, desolvation lines 280ºC, and heat block 35ºC. Nitrogen was utilised as a nebulising, drying, and heating gas. The optimized MRM transition parameters are presented in Table 1.

Table:1 MRM transition energy for the determination of brexpiprazole

Sample	Transition product ion	Transition precursor ion	Collosion energy (eV)
Brexpiprazole	273.10	434.15	-30

Preparation of sample solution
In the present work, 0.1g of brexpiprazole was taken and added to a 100 mL measuring jar. The mixture was then dissolved in 100 mL of acetonitrile under sonication for 15 minutes, followed by the addition of 1mL of the sample solution. The solution was then centrifuged at 1500 RPM for 5 minutes at room temperature. Finally, the supernatant solution was transferred to an HPLC Vial and analysed using UHPLC-MS/MS

Preparation of standard solution
A standard solution of 1000 µg/mL brexpiprazole was prepared in mass spectrometry-grade acetonitrile and diluted further with acetonitrile to create secondary stock and calibrants, which were then used for analysis. From the standard stock solution was further diluted at 1 ng/ml to 100 ng/ml using (HPLC grade) acetonitrile.

METHOD VALIDATION (LC-MS/MS)
To make sure the developed LC–MS/MS technique for the quantitative determination of Brexpiprazole is accurate, repeatable, dependable, and appropriate for its intended use, method validation was carried out. Internationally recognized regulatory requirements for bioanalytical technique validation were followed during the validation process. Selectivity, linearity, sensitivity, accuracy, precision, recovery, matrix effect, and stability were among the validation criteria that were methodically assessed

Specificity
Specificity was confirmed by tracking the analyte's characteristic precursor-to-product ion transitions utilizing multiple reaction monitoring mode. The absence of considerable interference proved the method's specificity for Brexpiprazole.

Linearity
The calibration curve is constructed by plotting peak area against concentration using eight standard solutions in the concentration range of 1-100 ng/ml. The linearity was evaluated by using least regression they showed the correlation co-efficient (r) of 0.998.

3.3 LOD and LOQ

LOD and LOQ were calculated as the 3rd and 10th times of standard deviations of sample measurement, respectively. The limits of detection and quantitation were 0.45 and 1.0 ng/ml, respectively. Thus the validation results indicate that the presented method is simple, sensitive and efficient for the analysis of the brexpiprazole in human plasma.

Accuracy and precision

Accuracy and precision were evaluated by three replicates of human plasma spiked with low(2.5ng/ml), medium(25ng/ml), high(50ng/ml) in the same day in three consecutive days . The %RSD was calculated. Accuracy was expressed as percent relative error (%RE) and precision of each concentration as relative standard deviation

3.4 Stability

The stability test was evaluated in human plasma using hydrolysis such as neutral, acidic, alkaline and standard sample spiked with human plasma. This solution was kept in normal temperature and sharp peak was obtained. The calibration and purity of the sample were plotted to analyse all the stability testing of standard.

3.5 System Suitability

System suitability tests were performed prior to analysis to ensure the LC-MS/MS system was functioning correctly. Among the factors evaluated were retention time, peak form, signal intensity, and repeatability. Sufficient instrument performance for reliable analysis was ensured by system suitability.

3.6 Research on Stability

The stability of Brexpiprazole in plasma was evaluated under several situations, including benchtop stability, freeze-thaw stability, long-term storage stability, and autosampler stability. Stability tests ensured the analyte's stability during sample collection, storage, preparation, and analysis. In order to confirm sample integrity throughout the analytical process, these investigations are essential (12,13).

4. RESULT AND DISCUSSION

4.1 Optimization of chromatographic condition

The optimized chromatographic conditions achieved remarkable separation and peak properties for brexpiprazole. A consistently sharp, symmetrical, and well defined peak was observed at a retention time of 2.18 minutes, highlighting the effectiveness of the optimized chromatographic methodology. Utilizing a C18 reverse-phase column along with an isocratic mobile phase (70:30, A: B) ensured consistent retention and reduced variability between injections. No overlapping peaks were detected at the retention time of brexpiprazole in blank plasma samples, which confirms the excellent specificity of the method. Additionally, chromatograms from LOD, LOQ, and spiked plasma samples exhibited clean baselines with minimal background noise, suggesting efficient separation from endogenous components in plasma. These findings validate that the developed technique is appropriate for direct plasma analysis and facilitates reliable quantification without the need for extensive sample preparation. The optimized chromatogram obtained at LOD level is shown in Figure 2. The chromatogram at LOQ level is shown in Figure 3. The chromatogram of 100 ng/ml sample is shown in Figure 4.

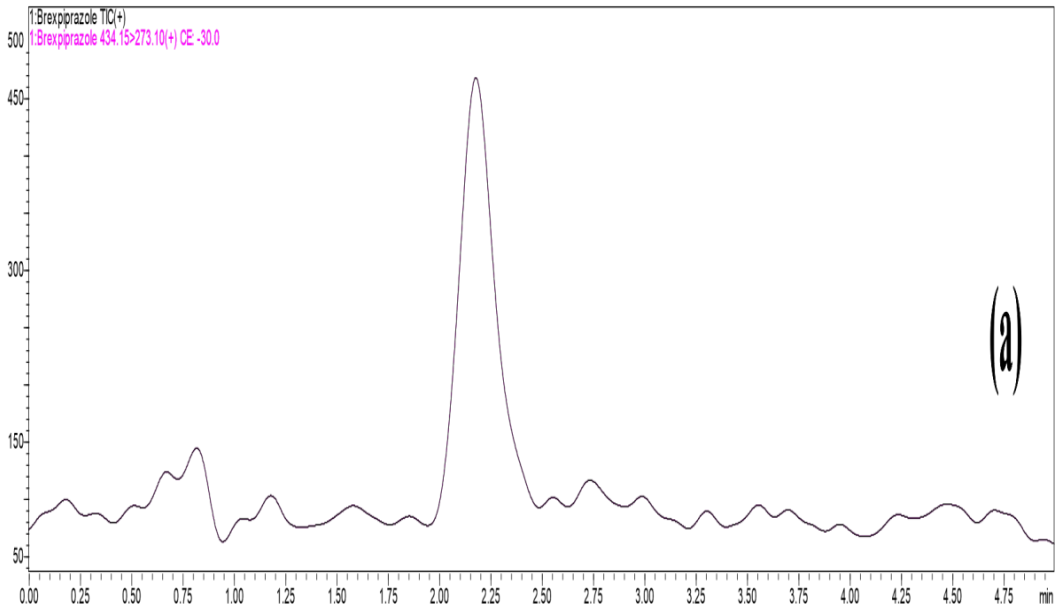


Figure.2 Representative chromatogram of brexpiprazole at the retention time of 2.18 min in the LOD level in UHPLC-MS/MS.

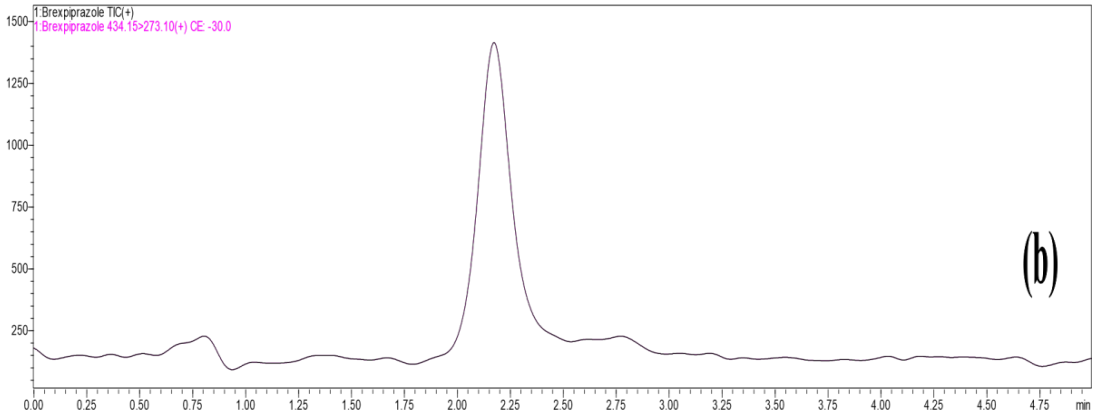


Figure.3 Representative chromatogram of brexpiprazole at the retention time of 2.18 min in the LOQ level in UHPLC-MS/MS.

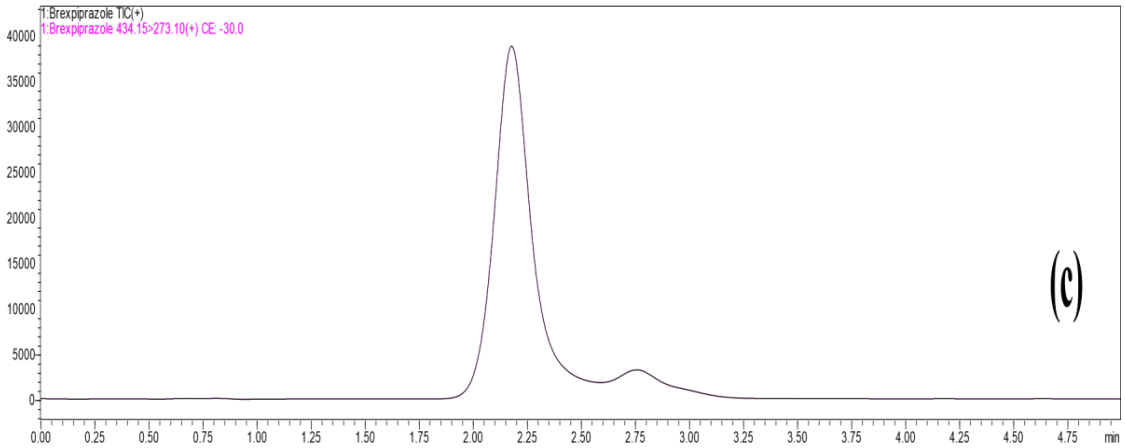


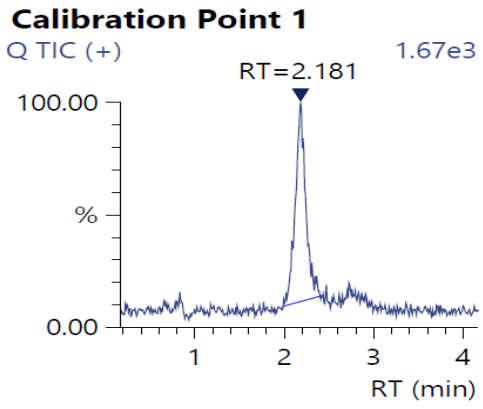
Figure.4 Representative chromatogram of brexpiprazole at the retention time of 2.18 min in a 100 ng/g spiked UHPLCMS/MS.

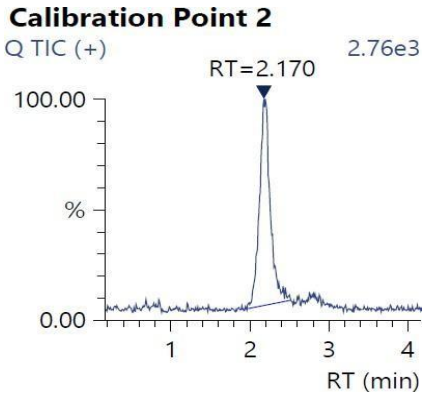
4.2 Calibration Curve

The calibration data obtained for brexpiprazole are summarized in Table 2. The calibration chromatogram of Brexpiprazole obtained under optimized UHPLC-MS/MS conditions is shown in figure 5. The chromatogram exhibited a well-defined peak with good detector response across the calibration range.

Table:2 Calibration of brexpiprazole

Sample name	Found rt	Peak area	Concentration
Calibration point 1	2.181	12369	-0.0076
Calibration point 2	2.170	25468	1.8321
Calibration point 3	2.175	42400	4.2101
Calibration point 4	2.171	77358	9.1200
Calibration point 5	2.180	204880	27.0303
Calibration point 6	2.180	392761	53.4180
Calibration point 7	2.183	726899	100.3475





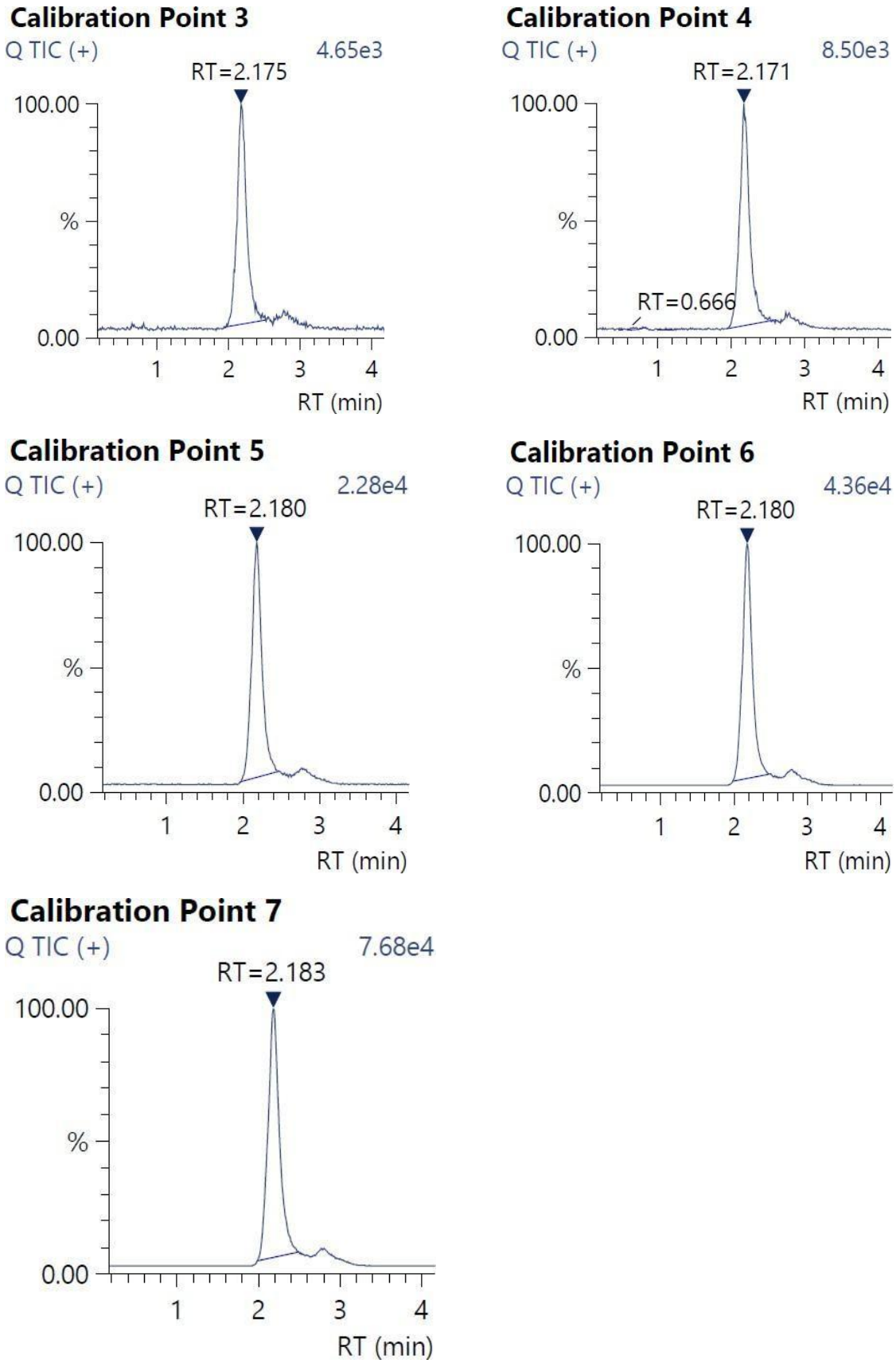


Figure.5 Calibration chromatogram of brexpiprazole

4.3 LINEARITY

The analytical approach demonstrated excellent linearity within the concentration range of 1–100 ng/mL, effectively encompassing the anticipated plasma levels of brexpiprazole. The calibration curve displayed a strong correlation coefficient ($r^2 = 0.9976$), suggesting a direct proportional relationship between the concentration of the analyte and the detector's response. The regression equation $y = 7528.9x + 9973.7$ further validates the method's consistency and reliability throughout the examined range, as illustrated in Figure 6.

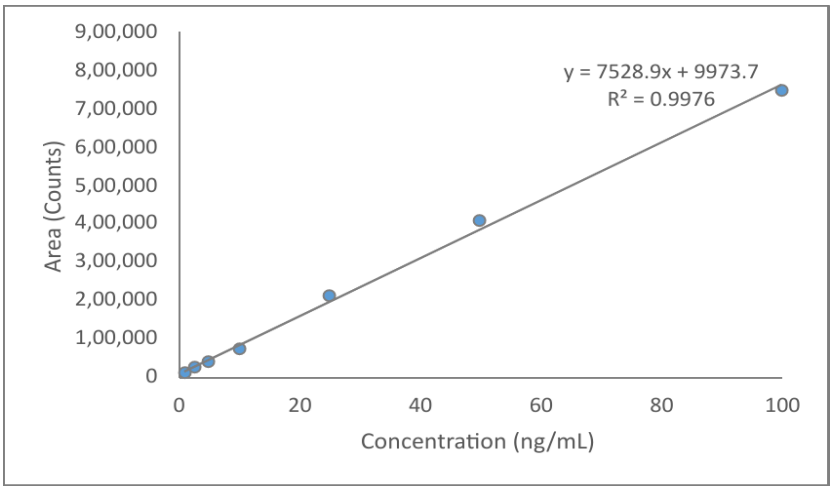


Figure.6 Linearity graph (calibration curve) of Brexpiprazole showing peak area versus concentration.

4.4 LOD AND LOQ

The determined limits of detection (LOD) at 0.45 ng/mL and limits of quantification (LOQ) at 1.0 ng/mL demonstrate the exceptional sensitivity of the method, allowing for the detection and measurement of brexpiprazole at very low concentrations. Although a simplified dilute-and-shoot approach was utilized for sample preparation, which typically leads to decreased sensitivity, the method still exhibited outstanding detection performance (6). This underscores the efficacy of the optimized chromatographic and detection parameters in offsetting the effects of minimal sample processing.

4.5 ACCURACY AND PRECISION

The method's accuracy was determined via recovery studies conducted at three quality control levels (2.5, 25, and 100 ng/mL), which correspond to low, medium, and high concentrations within the calibration range. Recovery percentages ranging from 89.0% to 96.80% indicate effective extraction of brexpiprazole from the plasma matrix and verify that the sample preparation process does not significantly compromise the analyte. Precision was evaluated through both intraday and inter-day studies, yielding RSD values between 1.28% and 9.57%, all falling within acceptable analytical thresholds. These low variability figures signify remarkable repeatability and intermediate precision of the method. Taken together, the accuracy and precision findings validate that the method generates reliable and consistent data, rendering it appropriate for routine bio analytical use. The accuracy and precision results obtained at low, medium, high quality control levels are summarized in Table 3.

Table 3: Precision of the dilute and shot method for brexpiprazole under acetonitrile as a solvent for UHPLC-MS/MS.

Day	Spike Conc. (ng/mL)	Obtained Conc. (ng/mL)	ER (%)	RSD (%)
Inter day	Blank	-	-	-
	2.5	2.234	89.36	1.28
	25	24.078	96.31	3.29
	100	89.003	89.00	1.99
Intra day	Blank	-	-	-
	2.5	2.39	95.53	9.57
	25	24.20	96.80	4.34
	100	87.60	87.60	2.25

4.6 METHOD RELIABILITY AND ROBUSTNESS

The reliability of the method was further confirmed by consistent retention times, stable peak area responses, and the absence of interference from the matrix in all samples analysed. The optimized detection settings, which included the chosen MRM transition (434.15 → 273.10 m/z), offered high selectivity and significantly minimized background noise,

resulting in clearer signals and enhanced quantification accuracy. The method's robustness was demonstrated by its consistent performance across multiple analytical runs, showing no significant variation. Moreover, the streamlined sample-preparation process did not adversely affect sensitivity or precision, validating that the method is both effective and resilient. These attributes render the developed method particularly well-suited for high-throughput analysis, encompassing clinical studies and pharmacokinetic research.

4.7 STABILITY STUDY

Stability study was carried out to evaluate the stability of the analyte Brexpiprazole under different analytical conditions and to confirm that the developed UHPLC–MS/MS method can reliably quantify the drug without degradation. Stability studies are an important part of method validation to ensure that the analyte remains stable during sample preparation, storage, and analysis.

In this study, quality control (QC) samples at different concentration levels were analyzed in different days to determine the stability of Brexpiprazole. The chromatographic results showed that the retention time of Brexpiprazole remained consistent at approximately 2.16–2.19 minutes, indicating that the analyte remained stable during the analytical process. The chromatograms obtained during the stability study showed well-defined peaks with no additional degradation peaks or interference. The measured concentrations of QC samples during stability analysis were close to their nominal concentrations, demonstrating that the analyte did not undergo significant degradation. The peak areas and calculated concentrations remained consistent across different runs, confirming the stability of Brexpiprazole under the tested conditions. The absence of interfering peaks at the retention time of Brexpiprazole indicates that the developed UHPLC–MS/MS method is capable of distinguishing the analyte from potential degradation products. This confirms that the method is stability-indicating and suitable for the determination of Brexpiprazole in analytical studies.

Overall, the stability study results demonstrate that Brexpiprazole remains stable during the analytical procedure, and the developed UHPLC–MS/MS method is reliable for accurate and precise quantification of the drug. The stability of Brexpiprazole was evaluated using three Quality Control (QC) concentration levels: Low QC (LQC), Middle QC (MQC), and High QC (HQC). These QC levels represent the lower, middle, and higher regions of the calibration range to ensure that the analyte remains stable across the entire concentration range during analysis.

The Low QC (LQC) sample was prepared at approximately 2 ng/mL concentration. The chromatographic analysis showed a consistent peak at a retention time around 2.18 minutes with calculated concentrations close to the nominal value (around 1.15–1.51 ng/mL). These results indicate that the analyte remained stable at low concentration levels during the analytical process (12).

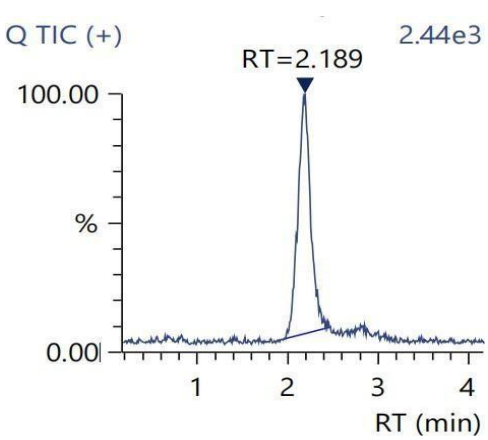
The Middle QC (MQC) sample was prepared at approximately 25–30 ng/ml. The observed concentrations during the analysis ranged between 26.17 ng/mL and 27.68 ng/mL, which were close to the expected concentration. The chromatograms showed well-defined peaks without any additional degradation peaks, confirming that the drug remained stable at the middle concentration level(12).

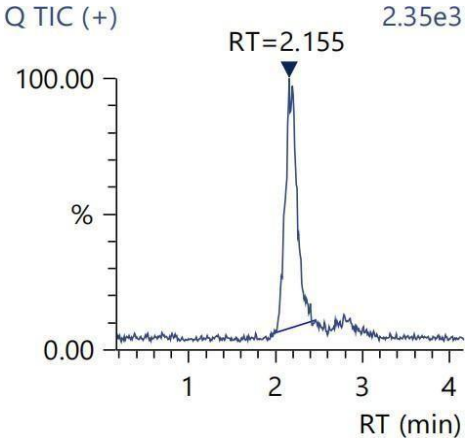
The High QC (HQC) sample was prepared at approximately 100 ng/mL concentration. The measured concentrations during the stability evaluation ranged from 98.72 ng/mL to 101.86 ng/mL, indicating minimal variation from the nominal value. The retention time remained consistent and the peak response was stable, demonstrating that the analyte maintained its stability even at higher concentration levels (13).

Overall, the results obtained from the LQC, MQC, and HQC samples indicate that Brexpiprazole is stable under the analytical conditions used in this study. The consistency in retention time, peak area, and calculated concentrations confirms that the developed UHPLC–MS/MS method is reliable for stability analysis across different concentration levels. The stability results obtained for the low-quality control (LQC) sample of brexpiprazole are presented in Table 4. The chromatograms obtained for the Low-quality control (LQC) samples during the inter-day stability study are shown in Figure 7. The stability results obtained for the Middle-quality control (MQC) sample of brexpiprazole are presented in Table 5. The chromatograms obtained for the Middle-quality control (MQC) samples during the inter-day stability study are shown in Figure 8. The stability results obtained for the high-quality control (HQC) sample of brexpiprazole are presented in Table 6. The chromatograms obtained for the High-quality control(HQC) samples during the inter-day stability study are shown in Figure 9.

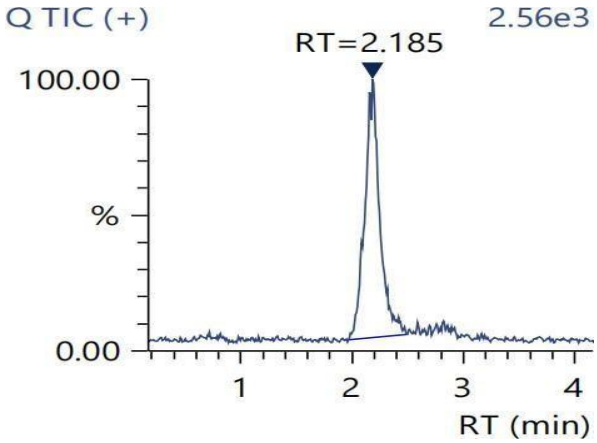
Table 4: LOW QC sample of brexpiprazole

DAY	CONCENTRATION	RT	AREA
Inter day 1	1.4451 ng/mL	2.189	22713
Inter day 2	1.1575 ng/ml	2.155	20665
Inter day 3	1.5107 ng/mL	2.185	23180





QC- Inter day 1 **QC- Inter day 2**

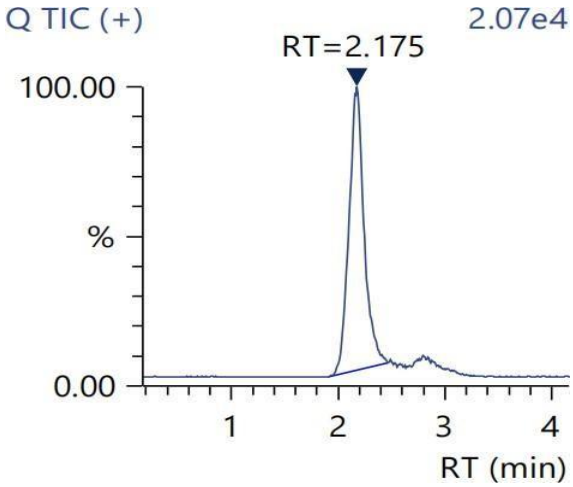


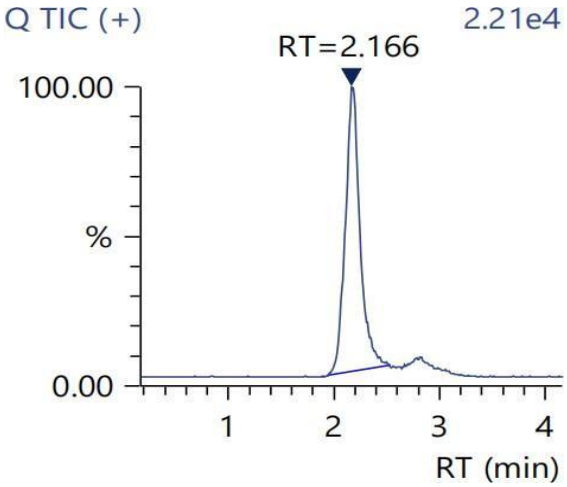
QC- Inter day 3

Figure.7: Representative Chromatograms of LOW QC sample of brexpiprazole

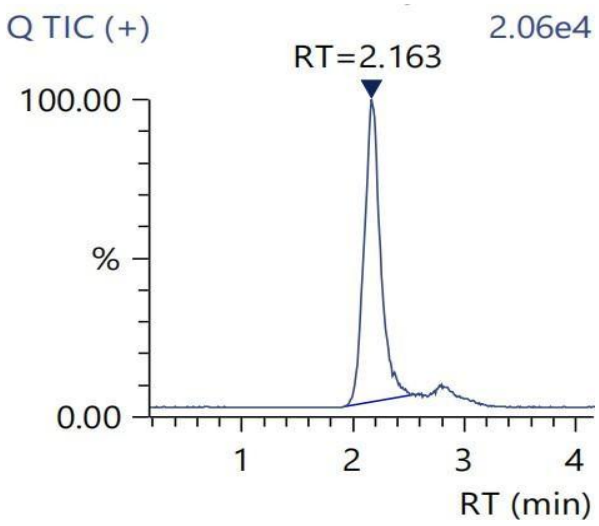
Table 5: MID QC sample of brexpiprazole

DAY	CONCENTRATION	RT	AREA
Inter day 1	26.1786 ng/ml	2.175	198815
Inter day 2	27.6862 ng/Ml	2.166	209549
Inter day 3	27.2780 ng/mL	2.163	206643





QC- Inter day 1 **QC- Inter day 2**

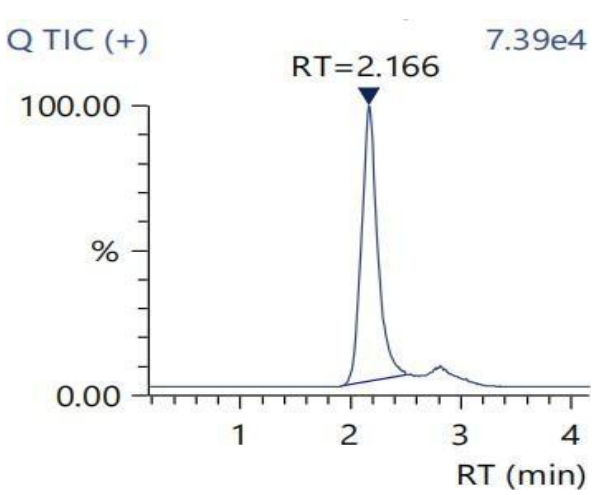


QC- Inter day 3

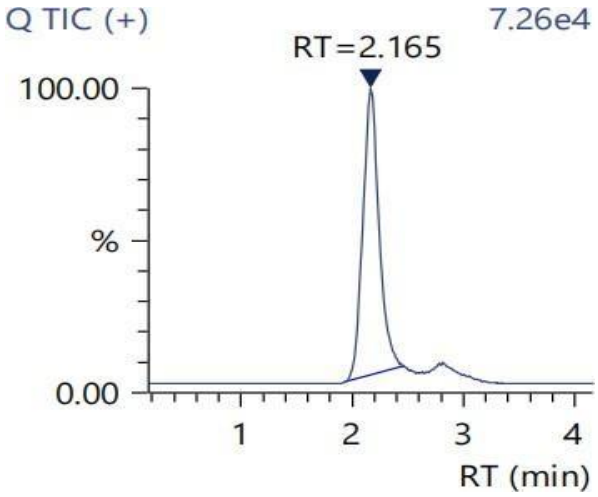
Figure.8: Representative Chromatograms of MID QC sample of brexpiprazole

Table 6: HIGH QC sample of brexpiprazole

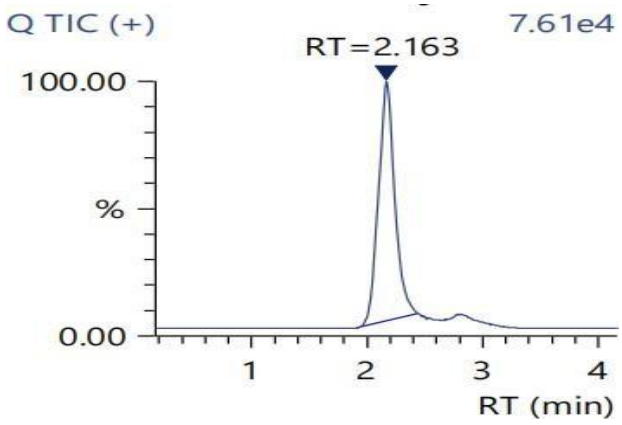
DAY	CONCENTRATION	RT	AREA
Inter day 1	101.8635 ng/Ml	2.166	737693
Inter day 2	98.7201 ng/mL	2.165	715312
Inter day 3	99.9353 ng/Ml	2.163	723964



QC- Inter day 1



QC- Inter day 2



QC- Inter day 3

Figure.9: Representative Chromatograms of HIGH QC sample of brexpiprazole

CONCLUSION:

A simple, rapid, sensitive and stability indicating UHPLC–MS/MS method was developed and validated successfully for the quantification of Brexpiprazole in plasma samples using dilute-and-shoot sample preparation approach. The optimized chromatographic conditions showed excellent peak symmetry, high selectivity and short retention time (~2.18 min) for efficient and high throughput analysis. The developed method exhibited excellent linearity with strongcorrelation coefficient (r2 = 0.9976) in the concentration range of 1–100 ng/mL.

The method was found to be highly sensitive with LOD and LOQ values of 0.45 ng/mL and 1.0 ng/mL respectively, indicating its suitability for quantification of brexpiprazole at trace levels in biological matrices. The acceptable recovery and low %RSD values obtained during the accuracy and precision studies confirmed the reliability and reproducibility of the method for routine bioanalytical applications.

The novelty of present UHPLC–MS/MS method provided excellent sensitivity, short run time and reliable quantification suitable for routine pharmaceutical analysis, bioanalytical studies and pharmacokinetic applications using a simple dilute-and-shoot sample preparation technique

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Conflicts of interests

All the authors have contributed equally. The authors declare there is no conflicts of interest.

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