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A Review on Quantification of Brexpiprazole in Its Bulk and Pharmaceutical Dosage Form by Various Analytical Methods.

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ABSTRACT: Brexpiprazole is an atypical antipsychotic that works as a partial agonist at serotonin 5-hydroxytryptamine1A and dopamine D2 receptors and an antagonist at serotonin 5-hydroxytryptamine2A. It has US Food and Drug Administration approval for monotherapy treatment of schizophrenia and adjunctive treatment to antidepressants for major depressive disorder. Some HPLC assay methods were used to monitor Brexpiprazole according to literature survey, such as UV-Spectroscopic, High-Performance Thin Layer Chromatography (HPTLC), Bioanalytical Method development of Brexpiprazole by UPLC-MS/MS, Analytical Method Development of Brexpiprazole by HPLC in that RP-HPLC Stability Indicating Assay Method, UV - Visible Spectroscopic and SIAM RP-LC Method, RP-HPLC Method Development and Validation. The proposed method was validated for selectivity, precision, linearity and accuracy. All validation parameters were within the acceptable range. Many methods for determination of Brexpiprazole have been reported.

Keywords: Brexpiprazole, HPLC, Antidepressant, Schizophrenia, Development, Validation.

I. INTRODUCTION:

Schizophrenia is a serious mental illness that interferes with a person's ability to think clearly, manage emotions, make decisions and relate to others. It is a complex, long-term medical illness. The exact prevalence of schizophrenia is difficult to measure, but estimates range from0.25% to 0.64% of U.S. adults. Although schizophrenia can occur at any age, the average age of onset tends to be in the late teens to the early 20s for men, and the late 20s to early 30s for women. It is uncommon for schizophrenia to be diagnosed in a person younger than 12 or older than 40. It is possible to live well with schizophrenia. [1,2]

In July 2015, the US FDA approved disorder (MDD) and for the treatment schizophrenia. The approval was based on data from four randomized, placebo-controlled phase III trials; two studies in patients with MDD receiving antidepressant therapy (NCT01360632; POLARIS and NCT01360645; PYXIS) and two in patients with acute schizophrenia (NCT01396421; VECTOR and NCT01393613; BEACON).[3]

II. CHEMISTRY:

Brexpiprazole is chemically designated as 7-{4-[4-(1-benzothiophen-4-yl) piperazin-1-yl] butoxy}-1,2-dihydroquinolin-2- one. 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.

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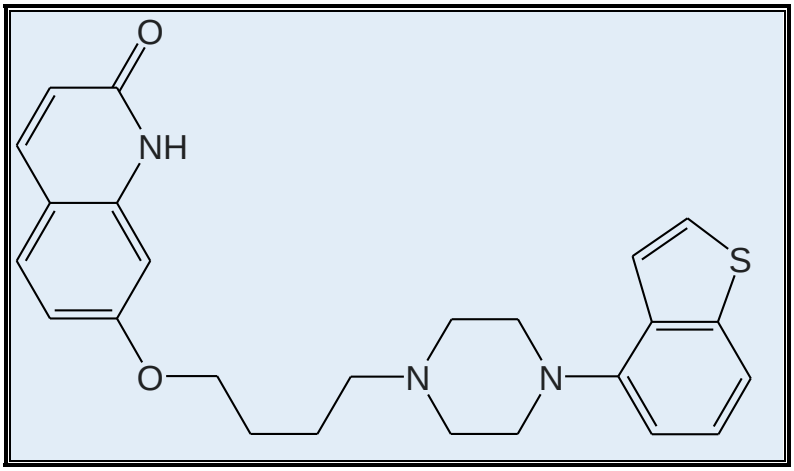

Fig.no.1 Structure of Brexpiprazole.

Brexpiprazole is an antipsychotic medication. It works by changing the actions of chemicals in the brain. Brexpiprazole is used to treat the symptoms of schizophrenia. It is also used together with other medications to treat major depressive disorder in adults. Brexpiprazole is a novel D2 dopamine and serotonin 1A partial agonist, called serotonin-dopamine activity modulator (SDAM), and a potent antagonist of serotonin 2A receptors, noradrenergic alpha 1B and 2C receptors.[4-13]

Properties	Description
Chemical Name	7-[4-[4-(1-benzo[b]thiophen-4-yl) piperazin-1-yl] butoxy] quinolin-2(1H)-one.
Generic Names	Brexpiprazole
Brand Names	Rexulti
Empirical Formula	$C_{25}H_{27}N_3O_2S$
Molecular mass	433.6
CAS Registry No.	913611-97-9
Melting Point	272-274°C
Storage	Store in a cool and dry place
U.V spectrum	λmax 216 nm
Density	1.2 ± 0.1 g/cm^3
Refractive index	1.646
pKa	13.56 (strongest acidic) and 8.4 (strongest basic)

Table no. 01: Physicochemical properties of Brexpiprazole[4-13]

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III. PHARMACOLOGICAL PROPERTIES:

A. Pharmacodynamics- Brexpiprazole has affinity (expressed as Ki) for multiple monoaminergic receptors including serotonin 5-HT1A (0.12 nM), 5-HT2A (0.47 nM), 5-HT2B (1.9 nM), 5-HT7 (3.7 nM), dopamine D2 (0.30 nM), D3 (1.1 nM), and noradrenergic α1A (3.8 nM), α1B (0.17 nM), α1D (2.6 nM), and α2C (0.59 nM) receptors. Brexpiprazole acts as a partial agonist at the 5-HT1A, D2, and D3 receptors and as an antagonist at 5-HT2A, 5-HT2B, 5-HT7, α1A, α1B, α1D, and α2C receptors. Brexpiprazole also exhibits affinity for the histamine H1 receptor (19 nM) and muscarinic M1 receptor (67% inhibition at 10 µM)

B. Pharmacokinetics:

a) Absorption: Brexpiprazole is well absorbed after administration of REXULTI tablets, with peak plasma concentrations occurring within 4.0 hours after single-dose administration; the absolute oral bioavailability of the tablet formulation is 95.1%. Brexpiprazole steady-state concentrations are attained within 10-12 days of dosing. REXULTI can be administered with or without food. Administration of a REXULTI 4 mg tablet with a standard high-fat meal did not significantly affect the Cmax or AUC of brexpiprazole. After single and multiple once-daily dose administration, brexpiprazole exposure (Cmax and AUC) increased in proportion to the dose administered.

b) Distribution: The volume of distribution of brexpiprazole following intravenous administration is high (1.56±0.418 L/kg), indicating extravascular distribution. Brexpiprazole is highly protein-bound in plasma (greater than 99%) to serum albumin and α1-acid glycoprotein, and its protein binding is not affected by renal or hepatic impairment

c) Metabolism: Brexpiprazole is mainly metabolized by cytochrome P450 (CYP) 2D6 and CYP3A4 and has demonstrated no effect on inducing or inhibiting any CYP 450 isoenzymes. These CYP enzymes metabolize brexpiprazole into its major metabolite DM3411. No therapeutic effects have been attributed to DM-3411. The half-life of brexpiprazole is 91 hours.

d) Elimination: Brexpiprazole is excreted in the urine (25.00%) and faces (46.00%) with a negligible amount excreted unchanged in the urine.

C. Contraindication: REXULTI is contraindicated in patients with a known hypersensitivity to brexpiprazole or any of its components. Reactions have included rash, facial swelling, drowsiness, urticaria, and anaphylaxis. [14-19]

Table. No. 02. Key pharmacokinetic measures of Brexpiprazole[14-19]

Parameter	Indication
Oral bioavailability	**95.00%**
Time to peak plasma concentration	4 hours
Metabolic pathway	CYP3A4, CYP2D6, CYP1A1/1A2, CYP2A6, CYP2B6, CYP2C8/2C9, CYP2C19, CYP2E1
Terminal half-life	91 hours
Protein binding	>99.00%
Elimination	Approximately 25% in urine and 46% in feces

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IV. VALIDATION OF RP-HPLC METHOD:[11,20,21]

The developed method for estimation of Brexpiprazole was validated as per ICH guidelines for the following parameters.

4.1. Filtration Study: Filtration study of an analytical procedure checks the interference of extraneous components from the filter, deposition on filter bed and compatibility of the filter with the sample. This study will be conducted with a sample of Rexulti tablet.

4.2. Specificity: Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present.

4.3. Linearity and Range: The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of an analyte in samples within a given range.

Determination The linearity of the analytical method is determined by the mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range.

The area is plotted graphically as a function of analyte concentration. Percentage of curve fittings are calculated. Acceptance Criteria: The plot should be linear passing through the origin. Correlation Coefficient (r^2) should not be less than 0.999

4.4. Accuracy (% Recovery): The accuracy of the analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value of the value found.

Acceptance Criteria: Mean recovery should be in the range of 98.00-102.00% The Relative Standard Deviation should not be more than 2.0%.

4.5. Precision: The precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple Samplings of a homogenous sample. The precision of an analytical method is usually expressed as a standard deviation or relative standard deviation. Precision is of two types, Repeatability and Intermediate precision. It is performed on an API sample. Prepare six different test solution of the 100% test concentration from the same sample matrix. Inject duplicate injections of each test solution.

4.6. Intermediate precision:(Interdayprecision): It is performed by analysing by another analyst on another day to check the reproducibility of results. Samples prepared in the same manner as that of Repeatability parameter (6 Samples prepared).

Acceptance criteria: % RSD of 6 samples NMT 2.0% for test results.

% RSD of Total 12 samples NMT 2.0% for test results

(6 of Repeatability and 6 of Intermediate precision)

4.7. 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 provides an indication of its reliability during normal usage.

4.8. Detection:

I. **Limit of Detection (LOD):** The lowest conc. of the analyte in the sample that the method can detect but not necessarily quantify under the stated experimental conditions simply indicates that the sample is below or above a certain level. Limit test prescribed as a percentage or as parts per million. The limit of detection will not only depend on the procedure of analysis but also the type of instrument.

S/N= 2/1 or 3/1

Where, S= Signal, N=Noise

It may be calculated based on the standard deviation (SD) of the response and slope of the curve(S).

LOD= 3.3 (SD)/S

Where, SD= Standard deviation, S= Slope

II. **Limit of Quantitation (LOQ):** The limit of quantitation (LOQ) is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. It is expressed as the conc. of analyte (e.g., percentage, parts per billion) in the sample. The S/N ratio should not less than 10 and RSD ≤ 3%.

S/N= 10/1

Where S= Signal N=Noise

It may be calculated based on the standard deviation (SD) of the response and slope of thecurve(S).

LOQ= 10 (SD)/S

Where, SD= Standard deviation, S= Slope

V. EXPERIMENTAL WORK:

Literature survey revealed that Brexpiprazole was determined by UV-visible spectroscopy and HPLC. In the current work, the authors have proposed a simple, specific, valid and robust RP-HPLC

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method for the estimation of Brexpiprazole in pharmaceutical active substance form.

Various Analytical methods:

Table.No.3. Analytical Method Development of Brexpiprazole by various analytical techniques:

Sr. No.	Name of Author	Name of Journal	Title of Article	Analytical Conditions
UV SPECTROPHOTOMETRIC				
1.	**S. Mondal et.al** [9]	International Journal of Pharmaceutical Sciences and Research. **(2018)**	"New spectrophotometric techniques for the estimation of brexpiprazole in the tablet dosage form."	**Method A** **Solvent-**0.1N HCl **λmax -** 214 nm **Beer-Lambert's limits (µg/mL) –** **0.002**-0.02 **linear regression equation-** y = 15.4517x + 0.0221 **correlation coefficient**-0.9990 **% RSD-** 0.12 **% Recovery**-99.18% **LOD-** 0.002 µg/mL **LOQ**-0.006 µg/mL
				Method B **Solvent-** Sodium Acetate buffer pH 4.5 **λmax -** 214 nm **Beer-Lambert's limits (µg/mL) -** 0.005-0.1 **linear regression equation-** y =23.4576x - 0.0235 **correlation coefficient =** 0.9992 **% RSD-** 0.48 **% Recovery-** 99.28% **LOD-** 0.03 µg/mL **LOQ**-0.0099 µg/mL
				Method C **Solvent-** 0.1N HCl and Sodium Acetate pH 4.5 **λmax -** 339.49-341.49 **Beer-Lambert's limits (µg/mL) - 0.002**-0.02 **linear regression equation-** y = 4.9605x + 0.0003 **correlation coefficient -** 0.9990 **% RSD-** 0.24 **% Recovery-** 99.54% **LOD-** 0.002 µg/mL **LOQ**-0.006 µg/mL
				Method D **Solvent-** 0.1N HCl and Sodium Acetate pH 4.5 **λmax -** 335.16-341.15 **Beer-Lambert's limits (µg/mL) - 0.005**-0.1

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<table>
<tr><td></td><td></td><td></td><td></td><td>linear regression equation-
y = 1.9990x - 0.0009
correlation coefficient - 0.9991
% RSD-0.65
% Recovery- 99.08%
LOD- 0.03 µg/mL
LOQ-0.0099 µg/mL</td></tr>
<tr><td>2.</td><td>P. Patel et.al [22]</td><td>The Pharma Innovation Journal.
(2020)</td><td>“Design, optimization, and validation of chemometrics assisted spectrophotometric methods for simultaneous determination of Brexpiprazole and Aripiprazole.”</td><td>Method- Multivariate calibration methods (CLS, ILS, PCR &PLS)
Method.1.CLS-
<table>
<tr><th>Parameter</th><th>Brexpiprazole</th><th>A
o</th></tr>
<tr><td>Concentration range</td><td>1-5 mcg/ml</td><td>5-
m</td></tr>
<tr><td>Spectral region (nm)</td><td>240-350 nm</td><td>2</td></tr>
<tr><td>R^2</td><td>0.999</td><td>0</td></tr>
</table>
Method 2. ILS-
<table>
<tr><th>Parameter</th><th>Brexpiprazole</th><th>Ari</th></tr>
<tr><td>Concentration range</td><td>1-5 mcg/ml</td><td>5-2</td></tr>
<tr><td>Spectral region (nm)</td><td>240-350 nm</td><td>240</td></tr>
<tr><td>R^2</td><td>0.999</td><td>0.9</td></tr>
</table>
Method 3. PCR -
<table>
<tr><th>Parameter</th><th>Brexpiprazole</th><th>A
o</th></tr>
</table></td></tr>
</table>

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				Concentration range	1-5 mcg/ml
				Spectral region (nm)	240-350 nm
				R^2	0.999
				Method 4. PLS –	
				Parameter	**Brexpiprazole**
				Concentration range	1-5 mcg/ml
				Spectral region (nm)	240-350 nm
				R^2	0.998
High-Performance Thin Layer Chromatography (HPTLC)					
3.	**A. M. Thakkar et.al.[23]**	Journal of Chromatographic Science. **(2019)**	"Stability Indicating TLC Method for Quantification of Brexpiprazole in Bulk and Its Pharmaceutical Dosage Form and Determination of Content Uniformity."	**stationary phase-** HPTLC Aluminium Plates (Pre-Coated with silica gel 60 F254) **mobile phase-** n-butanol **Rf value- 0.38** **densitometric analysis was done on UV-** **λmax** - 215 nm **concentration range** - 200–1,600 ng band^{-1} **LOD-** 66 ng band^{-1} **LOQ-** 100ng band^{-1} **Accuracy-** 99.34–101.08% **Precision- (% RSD)** **Intra-day (n = 3) -**1.03–2.46 **Inter-day (n = 3) -** 1.15–2.57 **Repeatability study (n = 6) -** 1.30	

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Table.No.4. Bioanalytical Method development of Brexpiprazole by UPLC-MS/MS

Sr. No.	Name of Author	Name of Journal	Title of Article	Analytical Conditions
1.	**Q. Zou et.al.**[24]	Journal of Chromatographic Science. **(2018)**	"A Validated Quantification Method for Brexpiprazole in Dog Plasma."	**Specimen-** Dog Plasma **Extraction –** LLE **Column-**UPLC BEH C18 (particle size 1.7um, 2.1 × 50 mm, Column, Waters Corp) **Mob. Phase-** Ammonium acetate: Methanol **Internal Std-** Brexpiprazole **Flow Rate-** 5µL/min **Detection-** UPLC- MS-MS
2.	**Meng-yuan WU et.al.** [25]	Latin American Journal of Pharmacy. **(2020)**	"Development and Validation of the UPLC-MS/MS Method for Determination of Brexpiprazole in Rat Plasma."	**Specimen-** Rat Plasma **Extraction–**Protein PrecipitationExtraction (PPE) **Column-** UPLC BEH C18 column (2.1 × 50 mm, 1.7µm) **Mob. Phase-** Acetonitrile and 0.1% formic acid in water **Linearity range-** 5-1000 ng/mL **Internal Std-** Carbamazepine **Flow Rate-** 0.40 mL/min. **LOQ-** 5 ng/mL **Detection-** UPLC- MS-MS

Table.no.5. Analytical Method Development of Brexpiprazole by HPLC:

Sr. No.	Name of Author	Name of Journal	Title of Article	Analytical Conditions
RP-HPLC STABILITY INDICATING ASSAY METHOD				
1.	**N. P. Bhatt et.al.**[6]	Journal of Chemical and Pharmaceutical Research. **(2018)**	"Development and Validation of Stability Indicating Assay Method and Characterization of Degradation Product for Brexpiprazole Bulk by RP-HPLC."	**Column -**Inertsil ODS 3V (150 cm × 4.6 mm × 5 µm) **Mob. Phase-** 20 mM Potassium Hydrogen Phosphate buffer at pH 6.8 and Acetonitrile (50:50 v/v) **flow rate-** 1.5 mL/min **R.T.-** 5.95 min **λmax** - 220 nm **Linearity range-** 0.96

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				-71 µg/mL **% Recovery - 95-** 105% **(% RSD)-** **Repeatability** **Intraday** **Interday** **Different analyst** **correlation coefficient ->**0.999 **LOQ -** 0.9688 µg/mL **Degradation -** Acid, Alkali, Neutral, Thermal, Photolytic, Accelerated stress study.
2.	**V. G. Kumar et.al.[10]**	Journal of Drug Delivery & Therapeutics. **(2019)**	"A new stability-indicating RP-HPLC method for estimation of Brexpiprazole."	**Column** – Phenomenex C18 (250 mm × 4.6 mm i.d., 5 µm particle size **Mob. Phase-** 0.1% Acetic Acid and Methanol (65:35 v/v) **flow rate -** 0.9 mL/min **R.T.-** 2.17 ± 0.03 min **λmax-** 214nm **Linearity range -** 0.1–250µg/mL **correlation coefficient** (R^2)- 0.9999 **regression equation- y** = 39617.94x + 3300.8. **(% RSD) -** 0. 24-0.65 **LOQ –** 0.0614µg/mL **LOD –** 0.0203µg/mL **Degradation-** Acidic, Alkaline, Oxidation, and Thermal Degradation.
3.	**F.M. Salama et.al. [2]**	Asian Journal of Pharmaceutical and Health Sciences. **(2018)**	"RP- HPLC method for determination of brexpiprazole in the presence of its oxidative-induced degradation product."	**Column** – ODS SUPELCO C18 (25 cm X 4.6 mm, 5 µm particle size) **Mob. Phase-** Methanol, Water and Phosphoric Acid (60:40:0.4, by volume) **flow rate -** 1 mL /min **R.T.-** 4.4 min

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				λmax- 259 nm. **Linearity range-** (20-100 µg/mL) **correlation coefficient-** 0.9996 **(% RSD) - 0.737** Repeatability **Intermediate Precision** **LOQ** –14.4 **LOD–** 4.77
4.	**C. C. Jaiswal et.al.[26]**	World Journal of Pharmacy and Pharmaceutical Sciences. **(2020)**	"Development and validation of stability indicating RP-HPLC method for estimation of Brexpiprazole in the tablet."	**Column** –Cosmosil (250mm x 4.6 mm) **Mob. Phase-** Buffer (pH 4.0): Methanol (40:60) **flow rate-** 1 mL/min **R.T.-** 4.307 min **λmax-** 248 nm **Linearity range-** 10-30 µg/mL **% RSD-** Repeatability Intraday Inter day **correlation coefficient** - 0.9996 **LOQ –** 1.827 µg/mL **LOD–** 0.603 µg/mL **Degradation-** Hydrolysis, Oxidation, Photolysis, and Thermal Degradation.
5.	**H.S. Bhawar et.al. [5]**	Journal of Drug Delivery & Therapeutics. **(2019)**	"Development and validation of stability indicating RP-HPLC method for estimation of Brexpiprazole from bulk and tablet form."	**Column** –Grace C8 (250mm x 4.6 i.d., particle size: 5 µm) **Mob. Phase-** Methanol and Water (90:10, v/v) with OPA **flow rate-** 0.9 mL/min **R.T.-** 5.099 min **λmax-** 215 nm **Linearity range-** 10–50 µg/mL **% Recovery-** 98% - 102% **correlation coefficient-** 0.9989 **% RSD** – **Intraday** 0.25 **Interday** 0.40

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				LOQ –0.55 µg/ mL **LOD**– 1.68 µg/ mL **Degradation**-Acid, Base, Neutral Hydrolysis, Oxidation, Dry heat and Photolysis
6.	**Dr. A. Gosar et.al.** [27]	International Journal of Development Research. **(2018)**	"Gradient High-Performance Liquid Chromatography (HPLC) method for determination of related substances in Brexpiprazole API."	**Column** – n-Kromasil C8, (250 mm x 4.6 mm, 5 µm) **Mob. Phase-** **a**. Dipotassium Hydrogen Phosphate buffer with pH 5.5 ±0.05 **b.** A mix of 9 volumes of Acetonitrile (ACN) and 1 volume of Tetrahydrofuran (THF) **c.** Methanol **λmax**- 254nm **LOQ** – 1.5ppm **LOD** –0.33ppm
UV - Visible Spectroscopic and SIAM RP-LC Method				
7.	**A.M. Thakkar et.al.**[28]	Austin Chromatography. **(2018)**	"Quantification of Brexpiprazole in Bulk and Its Pharmaceutical Dosage Form by UV - Visible Spectroscopic and SIAM RP-LC Method."	**UV – Visible Spectroscopic-** **λmax** - 215nm **solvent-** Methanol **Linearity range-** 1-6µg/mL **% Recovery-** 99.66-100.12 **% RSD** – **Repeatability (n=6)** **0.62** **Intraday (n=3)** 0.25 **Interday (n=3)** 0.21 **LOQ** – 1µg/mL **LOD-** 0.33µg/mL **SIAM RP-LC Method-** **Column** –Sun fire C18 (250x0.46mm; 5 µm particle size) **Mob. Phase** -Acetonitrile: Methanol (60:40 v/v) **flow rate**-1.0mL/min **λmax** - 215nm **R.T.-** 3.89min

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<table>
<tr><td></td><td></td><td></td><td></td><td>Linearity range- 0.01-10µg/mL
% Recovery- 99.04-99.19%
%RSD-<table><tr><td>Repeatability (n=6)</td><td>0.93</td></tr><tr><td>Intraday (n=3)</td><td>0.39</td></tr><tr><td>Interday (n=3)</td><td>0.15</td></tr></table>LOQ – 0.003µg/mL
LOD-0.01µg/mL
Forced degradation- Acid and Alkali hydrolysis, Chemical Oxidation, Photolytic degradation, and Dry heat degradation</td></tr>
<tr><td colspan="5">RP-HPLC METHOD DEVELOPMENT AND VALIDATION</td></tr>
<tr><td>8.</td><td>B. Sowjanya et.al [8]</td><td>European Journal of Biomedical and Pharmaceutical sciences.
(2018)</td><td>“Development and validation for the simultaneous estimation of Brexpiprazole and Fluoxetine in drug substance by RP-HPLC.”</td><td>Column-Inertsil ODS 3V C18 (5 µ, 250 cm X 4.6 mm i.d.)
Mob. Phase- 0.1%v/v Formic acid in water: Methanol (35:65)
flow rate- 0.8 mL/ min
λmax- 263nm</td></tr>
<tr><td></td><td></td><td></td><td></td><td>BREXPIPRAZOLE
Linearity range-50% to 150%
% Recovery- 99.0-100.4
correlation coefficient- 0.9993
% RSD –20.0µg/mL
LOQ –0.004
LOD– 0.001</td></tr>
<tr><td></td><td></td><td></td><td></td><td>FLUOXETINE
Linearity range-50% to 150%
% Recovery-98.6-99.6
correlation coefficient-0.9998
% RSD –20.0µg/mL
LOQ –0.001
LOD–0.0002</td></tr>
<tr><td>9.</td><td>V. S. Pulusu et.al. [11]</td><td>Pharmaceutical Analytical Acta.
(2019)</td><td>“Quantitative Determination of Brexpiprazole by RP-HPLC Method.”</td><td>Column – C18 column Waters (150 mm×4.6 mm, 5 µm)
Mob. Phase- 500 mL of 10 mM Monobasic</td></tr>
</table>

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				Potassium Phosphate buffer adjusted pH 2.0 with 85% Orthophosphoric Acid and 500 mL of HPLC grade Acetonitrile. **flow rate-** 1.0 mL/min **R.T.-** 2.5 min **λmax-** 213 nm **Linearity range-** 0.01-0.06 mg/mL **% Recovery-** 99.8-100.0 **correlation coefficient**-0.999 **% RSD** –0.10 **LOQ** –0.3 µg/mL **LOD**–0.1 µg/mL **Degradation-** Hydrolytic, Oxidative, Heat and Photolytic degradation
10.	**A. Sravani et.al. [4]**	Indo American Journal of Pharmaceutical Research. **(2017)**	"Method development and validation for the estimation of Brexpiprazole in drug substance by RP-HPLC method."	**Column –** C_{18} column (Inertsil ODS 3V 150*4.6, 5um) **Mob. Phase-** 0.1%v/v Formic Acid in water: Methanol (35:65) **flow rate-** 0.8 mL/ min **R.T.-** 2.27 min **λmax-** 315nm **Linearity range-** 50-150µg/mL **% Recovery-** 98.8 to 100.8% **% RSD** – 0.70% **LOQ –** 0.013 **LOD**– 0.004 **Ruggedness-** 0.14%

VI. CONCLUSION:

Brexpiprazole is an antipsychotic that works as a partial agonist at serotonin 5-hydroxytryptamine.Various analytical methods such as UV Spectrophotometric, HPTLC, UV and SIAM RP-LCMethod, bioanalytical, LC-MS-MS, UPLC etc. for determination of Brexpiprazole have been reported. Some HPLC assay methods were used to monitor Brexpiprazole. Methods for the analysis of active and inactive metabolites of Brexpiprazole in plasma have also been reported under bioanalytical methods. Validation of the developed method was done as per the ICH Q2(R1) guidelines.

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