Atomic Spectroscopy

Article

Stability indicating Modern HPLC-UV method development and authentication of human made Allopregnanolone used to treat postpartum depression – Brexanolone injection

Talluri Sarada Mrinalini[1,2], Kilaru Naveen Babu[3]

[1]Research Scholar, School of health science and pharmacy, Career Point University, Alaniya, kota, 325003, Rajasthan, INDIA.

[2]Assistant professor, Department of Pharmaceutical Analysis, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, 520010, Andhra Pradesh, INDIA.

[3]Professor, Department of pharmaceutics and biotechnology, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, 520010, Andhra Pradesh, INDIA

ABSTRACT: Background and Objective: Allopregnanolone is a naturally occurring neurosteroid which is synthesized in our body from progesterone hormone. Brexanolone, a synthetic form of allopregnanolone inhibitory neurosteroid, a class of Anti-depressants, finds uses in treating severe postpartum depression. The present study aimed for the development and validation of RP-HPLC method, the detection of Brexanolone followed by forced degradation studies.

Materials and Methods: Brexanolone was separated on an Agilent C18, 150x 4.6mm, 5µm, 180Ao column using with isocratic elution of mobile phase of 0.1% ortho phosphoric acid and acetonitrile in the ratio of 60:40 at 30°C. The chromatographic detection was done using UV-VIS spectrophotometer at 245.0 nm. The developed method was validated for accuracy, precision, selectivity, linearity, solution stability, LOD and LOQ as per ICH guidelines.

Results: The method precision was found to be 99.58% with an RSD of 0.68%. The established LOD and LOQ are 1.10 and 3.33 µg/mL respectively. Oxidation of drug with 20% hydrogen peroxide (H2O2), acid degradation with 2N hydrochloric acid, alkali degradation using 2N sodium hydroxide, dry heat degradation, neutral degradation, and photo stability studies were evaluated as part of degradation studies with %recovery more than 98%.

Conclusion: current method is accurate and easy for the estimation in labs and validated for all the parameters as per ICH guidelines with a high percentage recovery under various conditions of degradation.

Keywords: Neuroactive steroid, Allopregnanolone, Postpartum depression, Brexanolone, HPLC-UV Method, Development, Validation, Forced degradation studies.

INTRODUCTION

The IoT idea has currently come into existence as globalization as well as smart device connectivity with skyrocketed. Despite involving humans, IoT devices are utilized in sensing, controlling, monitoring intelligent data sharing, and other functions. The idea of the IoTs has been used in smart grids for transportation and agriculture, smart cities, smart public safety systems and smart healthcare among other smart global networks. Featuring billions of gadgets capable of sending important data about the physical environment and carrying out basic tasks of IoT has begun to pass and provide rise to the vision of anytime and anywhere connectivity with anything [1]. There are several areas like smart building, supply chain of logistics and transportation, industrial production plant management and e-health have developed recent applications with large amounts of data flow using IoT. In addition, there are some IoT applications that are created for an architecture of service-oriented in which each device is performed as a service requester or service provider else both activity. IoT has forwarded towards the model of things that focused on other things in providing combined services to the human being benefits. Understanding how any object's information can be effectively evaluated by any additional peer in the system is crucial when using a similar relationship model. The requester, who plays the role of the trustor in the IoT scenario, must have faith that the supplier, who then plays the role of the trustee, will deliver the requested service. However, malfunctioning devices are performed with different assaults while compared to benefits of other IoT nodes. Hence, fake suggestion or services have been provided to perform solely or organizing cooperating community devices for controlling hre service classes. Thus, the attacks as well as malfunctioning could completely negate any IoT advantages if they aren't get handled effectively [2] [3].

Depression is a mental illness characterized by pathological changes in mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration. It can be severe and sometimes fatal. Postpartum depression is a kind of mood disorder connected to childbirth which can act on both sexes.[1] [2] The arrival of PPD is generally in between One week and One month of successive childbirth.[1] Postpartum depression is also known as Postnatal depression. The drugs used to treat depression are known as Antidepressants. Neurosteroid antidepressants are well

known as neuroactive steroids which are endogenous or exogenous steroids that briskly change neuronal impulsive through association with ligand-gated ion channels and other cell surface receptors.[3][4] Neurosteroids based on structure and activity classified into Inhibitory neurosteroids, excitatory neurosteroids and pheromones.

Allopregnanolone is a naturally occurring neurosteroid which is synthesized in our body from progesterone hormone.[5][6] Brexanolone is synthetic form of allopregnanolone inhibitory neurosteroids, bought below the company brand name Zulresso,[7][8] and used to treat postpartum depression.[6][9][10] It is generally given by intravenous injection.

[6][7]Brexanolone is chemically 1-[(3R,5S,8R,9S,10S,13S,14S,17S)-3-hydroxy-10,13-dimethyl 2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl]ethanone with molecular weight C21H34O23 and molar mass of 18.501 g·mol−1.In March 2019, brexanolone was once as soon as authorised in the United States for the remedy of postpartum despair (PPD) in grownup women, the first drug generic by means of the U.S. Food and Drug Administration (FDA) particularly for PPD. The efficacy of Brexanolone was once as soon as verified in two scientific studies in individuals who obtained a 60-hour non-stop intravenous infusion of brexanolone or placebo and have been then accompanied for 4 weeks,[11][12].The FDA authorized allopregnanolone primarily based on proof from three scientific trials, performed in the United States, (Trial 1/NCT02942004, Trial 3/NCT02614541, Trial 2/ NCT02942017) of 247 female with real looking or severe postpartum depression. Brexonolone is an exogenous inhibitory pregnanolone neurosteroid. It is synthesized from progesterone hormone and it is positive allosteric modulator of the action of γ-aminobutyric acid (GABA) at GABAA receptor.

The present investigation aims to develop reverse phase HPLC method for the determination of Brexonolone in pharmaceutical dosage forms and to authenticate the method. The study also focusses on to find out chemical behaviour of brexonolone when revealed to different conditions and identify the effect of purity due to any degradation and hence, Brexanolone was subjected to many forced degradation parameters to establish a degradation pathway

MATERIALS AND METHODS

Chemicals:

Standard Brexanolone was procured from BMR chemicals Hyderabad with the purity not less than 99%. Reagents and chemicals used in the analysis are Acetonitrile, Methanol, water which were HPLC grade from Merck chemicals and reagents like Potassium dihydrogen ortho phosphate, tri ethyl amine, Ortho-phosphoric acid, sodium dihydrogen Ortho phosphate was of analytical grade procured from rankem chemicals.

PREPARATION OF REAGENTS: -

Preparation of Buffer: (0.1%Ortho phosphoric acid)

1ml of Ortho phosphoric acid was diluted to 1000ml with milli-Q ultrapure water.

Preparation of Mobile phase:

Mixture of 0.1%Ortho phosphoric acid buffer and acetonitrile taken as mobile phase in the ratio of 60:40

Preparation of diluent:

Mixture of 0.1%Ortho phosphoric acid buffer and acetonitrile taken as mobile phase in the ratio of 50:50

Preparation of Solutions:

Preparation of Standard stock solution:

50mg of brexanolone standard was accurately taken into 50 ml capacity of clean and dry volumetric flasks and dissolved by adding 10ml of diluent and sonicated for 10 minutes. Finally made up to the volume to 50ml by using diluents, which was 1000 µg/ml of brexanolone standard stock solution.

Preparation of Standard solution:

1ml of above stock solution was taken into 10ml volumetric flask and finally volume was made up to 10ml with diluent, whose concentration was 100 µg/ml of brexanolone.

Preparation of sample solution: -

In to 100ml of measuring flask, 20ml of brexanolone injection sample was pipetted and dissolved the drug in 50ml of diluents and ultrasonicated for 25 min and finally volume was then adjusted with diluents and filtered by using HPLC filters. (1000µg/ml brexanolone). 1ml of filtered sample solution was transferred to a 10ml volumetric flask and the final volume was made up with diluent (100µg/ml brexanolone).

Preparation of solutions for Forced Degradation studies:

Oxidation:

1 ml of 20% hydrogen peroxide (H2O2) was added separately to 1 ml of the brexanolone stock solution. The solutions were kept aside for 30 min at 600˚c temperature conditions. The HPLC study aims to dilute the resultant solution to obtain 100µg/ml. 10 µl were injected into the system and the chromatograms were put on file to evaluate the stability of the sample.

Acid Degradation Studies:

1ml of 2N Hydrochloric acid (HCL) was added to 1 ml of brexanolone stock solution of brexanolone and refluxed at 600°C for 30mins at 600˚c. The chromatograms were recorded to evaluate the strength of the sample f by diluting the resulting solution was diluted to acquire 100µg/ml and 10 µl solutions in to the system.

Alkali Degradation Studies:
To 1 ml of the stock solution (brexanolone) add 1 ml of 2N sodium hydroxide and refluxed at 600°C for 30mins at 600c. The resultant solution was subjected to chromatogram recordings by diluting it to obtain 100µg/ml solution and 10 µl were injected into the system to determine the firmness of the sample.

Dry Heat Degradation Studies:
To investigate degradation by dry heat, the standard solution was stored in an oven at 105°C for 12 hours. For the HPLC study, the resulting solution was diluted to 100µg/ml and 10µl were added to the system and the chromatograms were recorded to estimate the constancy of the sample.

Photo Stability studies:
The photochemical stability of the drug was also studied by exposing the 1000µg/ml solution to UV light by placing the beaker in the UV chamber for 7 days or at 200-watt hours/m2 in photo stability chamber. For the analytical HPLC studies, the resulting solution was diluted with solvent to obtain 100µg/ml solutions. 10 µl was delivered into the system and the chromatograms were recorded to assess the stability of the sample.

Neutral Degradation Studies:
In the stress test, the drug was analyzed by refluxing in water for 6 hours at a temperature of 60º under neutral conditions. For HPLC testing, the resulting solution was diluted to 100µg/ml and 10 µl were added to the system and the chromatograms were recorded to check the strength and safety of the drug

HPLC OPTIMIZED METHOD:

For the nature and solubility properties of brexanolone, reverse phase HPLC proved a preferable analytical method. Several trials were carried done varying the flow rate, proportion of mobile phase to composition, and solvent system. To attain the best drug separation under isocratic circumstances, brexanolone separated on Waters HPLC 2695 System with PDA detector integrated with Empower 2 software and Agilent C18, 150x 4.6mm, 5µm, 180Aº column using with isocratic elution of mobile phase of 0.1% ortho phosphoric acid and acetonitrile in the ratio of 60:40 at 30°C. The chromatographic detection was done using UV-VIS spectrophotometer at 245.0 nm. Details were projected in table 1 and optimized chromatogram in figure 1. The developed method was validated for accuracy, precision, selectivity, linearity, solution stability, LOD and LOQ as per ICH guidelines.

To determine the drug degradation routes that disclose the chemical behavior of brexanolone, forced degradation tests were also carried out, and samples derived from these investigations were examined. The drug's oxidation using 20% hydrogen peroxide (H2O2), acid and alkali degradation using 2N hydrochloric acid and sodium hydroxide, dry heat degradation, neutral degradation, and photo stability tests are among the factors for degradation.

RESULTS AND DISCUSSION:

The developed approach was validated in accordance with ICH Guidelines for all parameters. Five sample injections were performed, and the standard calibration curve method was used to determine the drug's purity.

Validation of method proposed:
Accuracy:
For accuracy, three concentration levels of 0%, 50%, 100%, and 150% were developed. Each concentration level was injected three times, and the standard deviation and percentage of recovery were computed. Table 2 displays the standard deviation and relative standard deviation together with the % recovery at each accuracy level.

system suitability:
The system suitability parameters were reached by administering six injections of the brexanolone working standard solution, which was prepared in accordance with the protocol. Table 3 displays the computed area of the peak, USP plate count, and tailing factor.

Linearity
Six sets of injections were made, and each set was eluted with 25, 50, 75, 100, 125, and 150 percent of the linearity levels. The table provided the average area found for each level. After constructing linearity, the slope was determined to be 30669, the y-intercept to be 22356.3, and the correlation coefficient to be 0.9999. Table 4 provided the percentage of the linearity level, the drug concentration, the area of the peak, and the linearity graph was displayed in Figure 2.

Method Precision or Reliability
Six injections of the working sample solution are made, and the solution is eluted at a 100 µg/ml concentration to ensure repeatability. Table 5 provides the average peak area of the solutions, standard deviation, and percentage RSD values.

Intermediate precision:

Operating Six injections of the sample solution are made the day after the solution is prepared, at a concentration of 100 µg/ml, for a 24-hour intermediate precision. Table 6 presents the average peak area of the solutions, standard deviation, and percentage RSD values. Figure 3 shows the chromatogram obtained after a 24-hour period of intermediate precision.

Limits of detection and limit of quantification:
Drug concentrations within the anticipated detection limit range of 3.3 to 10 µg/ml. These concentrations were used to generate the calibration curve. The regression line's Y-intercepts, the standard deviation was computed and entered the subsequent formulas to determine the drug's limit of quantification and detection. Values were provided in table 7.
Detection limit = σ × 3.3/S
Quantification limit = σ × 10/S
σ = Deviation from the mean
S, or slope

Robustness
Little adjustments to the chromatographic conditions were used to achieve robustness. The temperature, flowrate, and composition of the mobile phase were altered in accordance with ICH recommendations, and the percentage RSD was discovered to be within the bounds shown in Table 8 and 9.

FORCED DEGRADATION STUDIES:

studies on acid degradation
After testing for drug degradation in acid, it was discovered that the drug did not degrade and that there was good drug recovery as well as theoretical plates. For the acid research, a purity plot was also provided. Figure 4 show the chromatograms from the acid degradation research.

Studies on alkali degradation
Degradation in base was carried out, and further investigation revealed that the medication was not degrading. It was possible to obtain theoretical plates and good drug recovery. A purity plot was also provided for the investigation on alkalinity. Figure 5 show the chromatogram of base degradation analysis.

Studies on peroxide decomposition
When peroxide degradation was done, the medication did not show any signs of degradation. It was possible to obtain theoretical plates and good drug recovery. For the research of peroxide degradation, a purity plot was also created. Figure 6 gives the study's chromatograms.

Studies on photolytic deterioration
After testing for degradation under ultraviolet light, it was discovered that the medication has not degraded. It was possible to obtain theoretical plates and good drug recovery. For the study, a purity plot was also provided. Figure 7 show the chromatogram.

Studies on neutral degradation
After testing for degradation in water, it was discovered that the medication did not degrade. It was possible to obtain theoretical plates and good drug recovery. Figure 8 show the chromatogram.

CONCLUSION:

In summary, this procedure was designed to measure Brexanolone separated on an Agilent C_{18}150x 4.6mm, 5µ, column by isocratic elution using a mobile phase made up of 60:40 0.1% ortho phosphoric acid and acetonitrile at 30 °C. A UV-VIS spectrophotometric detection at 245.0 nm was used. The new method underwent complete validation in accordance with ICH requirements for accuracy, precision, linearity, stability of the solution, solubility at 24 hours, LOD, and LOQ.

To determine the drug degradation routes that reveal the chemical behavior of Brexanolone, forced degradation investigations were also carried out, and samples derived from this research were analyzed. The devised method's method precision was determined to be 100.52% with 0.69% RSD, 1.10 and 3.33 for LOD and LOQ, respectively. The drug was oxidized using 20% hydrogen peroxide (H2O2), the acid was broken down using 2 N hydrochloric acid, the alkali was broken down with 2 N sodium hydroxide, the degradation was done by dry heat, the degradation was neutral, and photo stability tests were carried out. The recovery rate was not less than 94.65%. This study used an explicit, unambiguous, and exact methodology that was validated for all parameters in accordance with ICH requirements, yielding a high percentage of recovery under a range of degradation situations. This approach is most appropriate.

Financial support and sponsorship: N/A
Conflicts of interest: No conflicts of interest exist.

REFERENCES: -

1. “Postpartum Depression Facts.” NIMH.
2. Paulson JF (2010). “Focusing on depression in expectant and new fathers: prenatal and postpartum depression not limited to mothers.” Psychiatric Times. 27 (2).

3. Paul SM, Purdy RH "Neuroactive steroids." FASEB Journal. 6 (6): 2311–22. Doi:10.1096/fasebj.6.6.1347506. PMID 1347506. S2CID 221753076.
4. Lan NC, Gee KW. "Neuroactive steroid actions at the GABAA receptor." Hormones and Behavior. 28 (4): 537–44. Doi:10.1006/hbeh.1994.1052. PMID 7729823. S2CID 40697424.
5. Reddy DS Neurosteroids: endogenous role in the human brain and therapeutic potentials. Prog. Brain Res. Progress in Brain Research. Vol. 186. Pp. 113–37. Doi:10.1016/B978-0-444-53630-3.00008-7. ISBN 9780444536303. PMC 3139029. PMID 21094889.
6. FDA approves first treatment for post-partum depression." U.S. Food and Drug Administration (FDA) (Press release).
7. "Zulresso- brexanolone injection, solution." DailyMed. 18 November 2019.
8. "ChemIDplus – 516-54-1 – AURFZBICLPNKBZ-SYBPFIFISA-N – Brexanolone [USAN] – Similar structures search, synonyms, formulas, resource links, and other chemical information". NIH Toxnet.
9. Frieder A, Fersh M, Hainline R, Deligiannidis KM "Pharmacotherapy of Postpartum Depression: Current Approaches and Novel Drug Development." CNS Drugs. 33 (3): 265–282. Doi:10.1007/s40263-019-00605-7. PMC 6424603. PMID 30790145.
10. Wilkinson ST, Sanacora G "A new generation of antidepressants: an update on the pharmaceutical pipeline for novel and rapid-acting therapeutics in mood disorders based on glutamate/GABA neurotransmitter systems". Drug Discovery Today. 24 (2): 606–615. Doi:10.1016/j.drudis.2018.11.007. PMC 6397075. PMID 30447328.
11. "FDA approves first treatment for post-partum depression." U.S. Food and Drug Administration (FDA)
12. "Drug Approval Package: Zulresso". U.S. Food and Drug Administration (FDA).
13. Bonagiri pavani, malothu Narender, Dintakurthi sree naga bala krishna Prashanth, chakravarthi Guntupalli, development and validation of a novel bioanalytical method for the simultaneous determination of glecaprevir and pibrentasvir in human plasma using reversed-phase high-performance liquid chromatography, . Egyptian pharmaceutical journal 2022, 21:424-431, July 12, 2023, IP: 203.202.232.10.
14. Linnet, K., Ejsing, T.B., 2008. A review on the impact of P-glycoprotein on the penetration of drugs into the brain. Focus on psychotropic drugs. (Review of how P-glycoprotein can limit the brain concentration of antidepressant and antipsychotic drugs), European Neuropsychopharmacology. 18, 157–169.
15. Laruelle, M., Abi-Dargham, A., Gil, R., et al., 1999. Increased dopamine transmission in schizophrenia: relationship to illness phases. The first direct evidence for increased dopamine function as a cause of symptoms in schizophrenia, Biological Psychiatry, 46, 56–72.
16. Gurdeep R.Chatwal , Sham K .Anand, Instrumental Methods of Chemical Analysis, Pg 2.566-2.638, 2007.
17. B.k Sharma, Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23rd Edition Goel publication, Meerut, 2007.
18. The United States Pharmacopoeia- the National Formulary, United States Pharmacopeial convention, Rockville, 2007.
19. ICH Harmonised Tripartite Guideline. (2005). Validation of analytical procedures: Text and methodology, Q2 (R1). International Conference on Harmonization.
20. Remington' s The Sciences and Practice of Pharmacy, 20th Edition, 2000.
21. Douglas A Skoog, F. James Holler, Timothy A. Niemen, Principles of Instrumental Analysis Pg 725-760.
22. Indian Pharmacopoeia, Ministry of Health & Family Welfare, Government of India, New Delhi, 1996.
23. David G. Watson. Pharmaceutical Analysis, A text book for Pharmacy students and Pharmaceutical Chemists. Harcourt Publishers Limited; 2nd Ed., Pg 221-232.
24. Connors Ka. A Textbook of Pharmaceutical Analysis, Wiley Inter sciences Inc; Delhi, 3rd Ed, Pg 373-421, 1994.
25. Kalyani G, Guddeti V, Kondeti Dp, Prasanthi G. cardioprotective effect of avena sativa linn against isoproterenol induced myocardial infarction in male sprague dawley rats. Advances in Pharmacology and Toxicology. 2015 Dec 1;16(3):19.
26. Krishnaswami V, Sugumaran A, Perumal V, Manavalan M, Kondeti DP, Basha SK, Ahmed MA, Kumar M, Vijayaraghavalu S. Nanoformulations-Insights Towards Characterization Techniques. Current Drug Targets. 2022 Oct 1;23(14):1330-44.

Optimized HPLC chromatographic conditions:

Table 1: chromatographic conditions of optimized method

Flow rate	1ml/min
Column	Agilent C18150x 4.6mm, 5µ.
Detector wave length	245.0 nm
Column temperature	30°C
Injection volume	30.0µL
Run time	6.0minutes

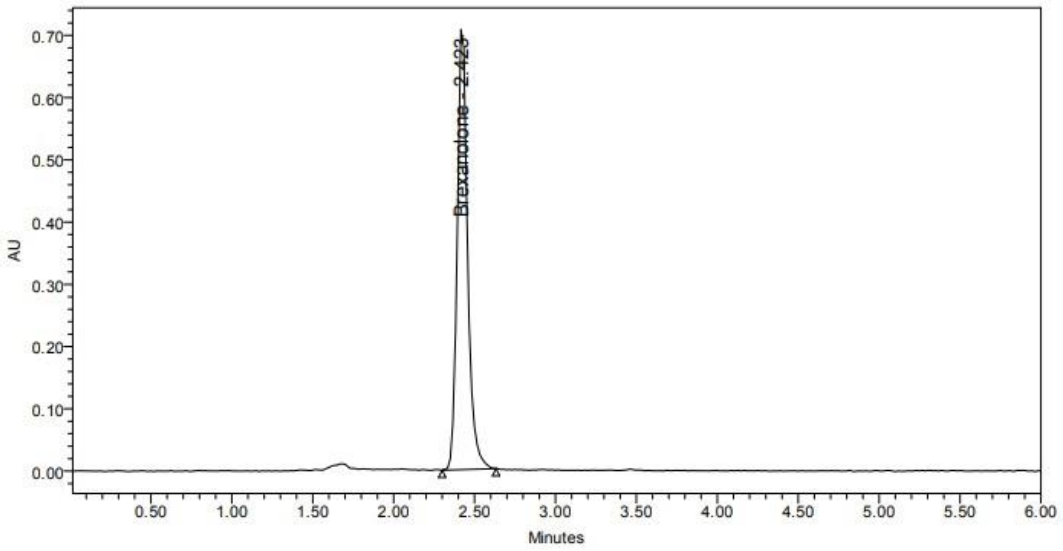

Figure 1: Chromatogram of Brexonolone

Accuracy:

Table 2: Accuracy data

% Level	Add ppm	% Recovery
ACCURACY_50%	50	99.30
	50	98.91
	50	100.88
ACCURACY_100%	100	99.50
	100	99.99
	100	98.62
ACCURACY_150%	150	99.29
	150	99.66
	150	100.06
	AVG	99.58
	STDEV	0.67
Average % recovery	%RSD	0.68

System Suitability:

Table 3: System suitability data

S No	RT in Min	Area of the peak	USP Plate count	USP Tailing
1	2.417	3378830	6923	1.23
2	2.423	3409879	7070	1.22
3	2.437	3396463	6895	1.23
4	2.448	3445514	7013	1.22
5	2.459	3457486	6967	1.23
6	2.482	3429557	7090	1.22

Linearity:

Table 4: Linearity results of Brexonolone

% of Linearity Level	Concentration of drug	Area of the peak
0	0	0
25	10	771299
50	10	1567430
75	10	2334920
100	10	3074612
125	10	3838110
150	10	4609055

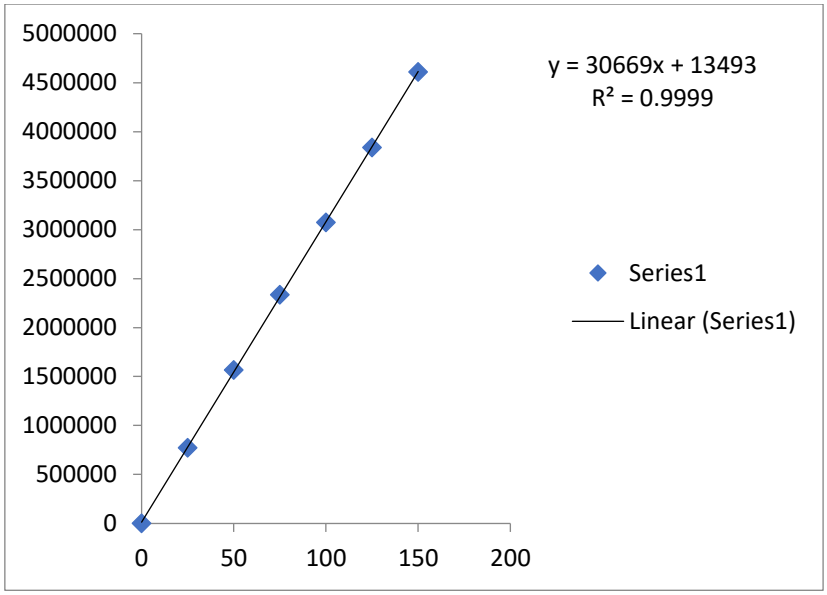

Figure 2: Linearity plot

Method Precision

Table 5: Method precision data

Serial number of injections	Area of the peak	% purity
1	3408019	99.46
2	3457089	100.89
3	3469543	101.26
4	3422220	99.88
5	3451854	100.74
6	3457486	100.91
AVG	3444369	100.52
SD	23813.9	0.69
% RSD	0.7	0.69

Intermediate Precision:

Table 6: Intermediate precision data

Serial number of injections	Area of the peak
1	3384882
2	3397691
3	3343404
4	3429093
5	3393916
6	3444319
AVG	3398884
SD	35431.9
% RSD	1.0

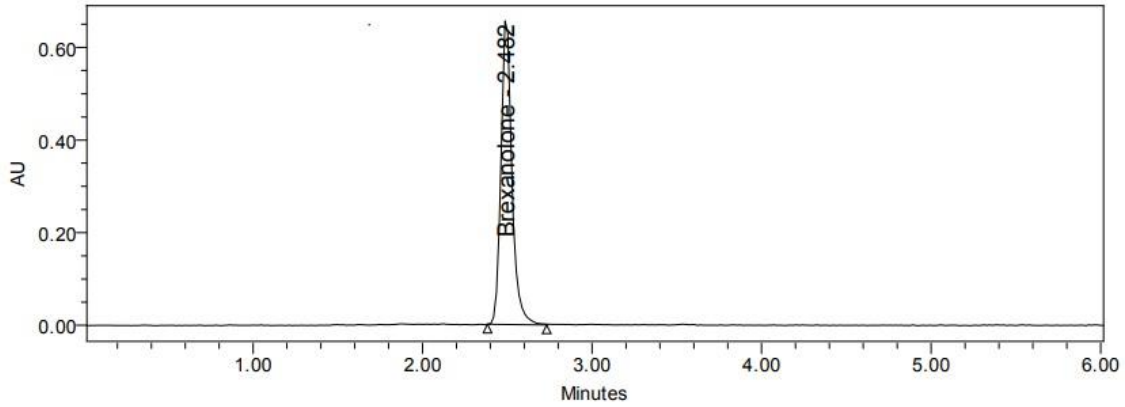

Figure 3: Brexonolone at 24 hrs of intermediate precision

Limit of detection and limit of Quantification:

Table 7: Data of LOD and LOQ

Test name	values
Limit of detection	1.10
Limit of quantification	3.33

Robustness:

Table 8: Robustness data

S.no	**Conditions**	**Composition values**	**%RSD of**
1	Flow-plus [Fp]	0.9ml/min	0.7
2	Flow-Minus [Fm]	1.1ml/min	1.1
3	Mobile Phase Plus [Mp+]	65%Buffer_35%OrganicSolvent	1.6
4	Mobile Phase Minus [Mp-]	55%Buffer_45%OrganicSolvent	1.3
5	Temperature-Plus [Tp]	28^{O}c	0.9
6	Temperature-Minus [Tm]	33^{O}c	0.7

Table 9: Robustness data

FM	FP	MM	MP	TM	TP
3364510	3265308	3286651	3337412	3432432	3259808
3402345	3225250	3254783	3297546	3420719	3283450
3398068	3314867	3392985	3327528	3410753	3244695
3385417	3286997	3324799	3409412	3396518	3287578
3429122	3309263	3293577	3319752	3357303	3296938
3395892	3280337	3310559	3338330	3403545	3274494
23691.6	36516.6	52363.6	42363.5	29021.8	21556.9
0.7	1.1	1.6	1.3	0.9	0.7

Forced degradation studies:
Acid degradation study:

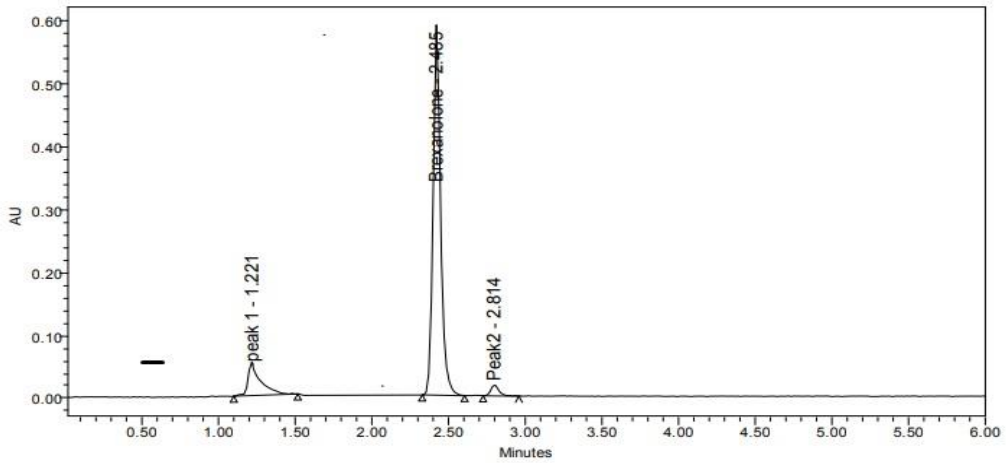

Figure 4: Acid degradation chromatogram

Alkali degradation study:

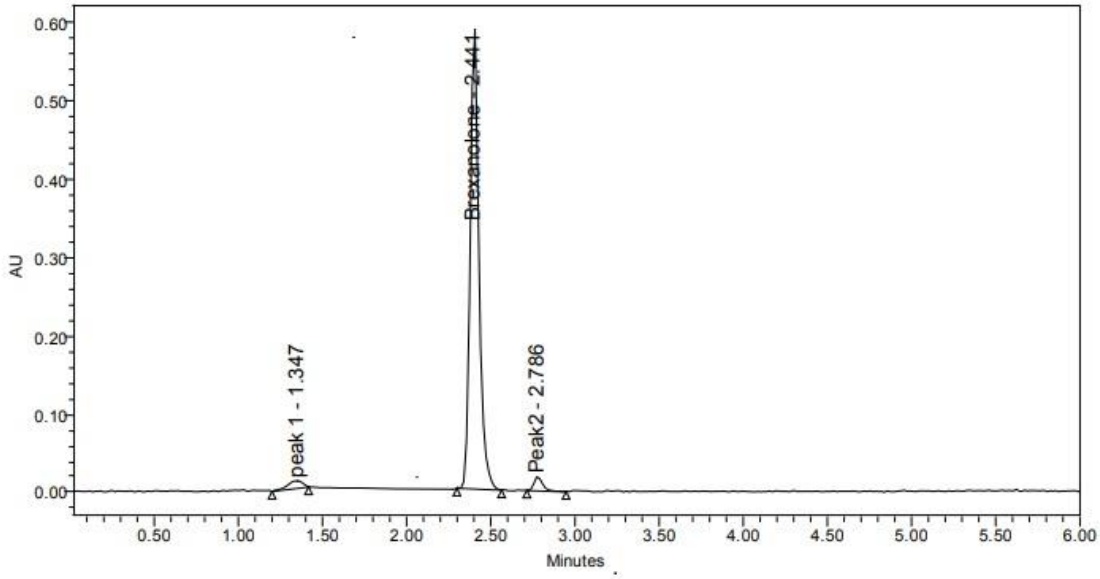

Figure 5: Alkali degradation chromatogram

Peroxide degradation study:

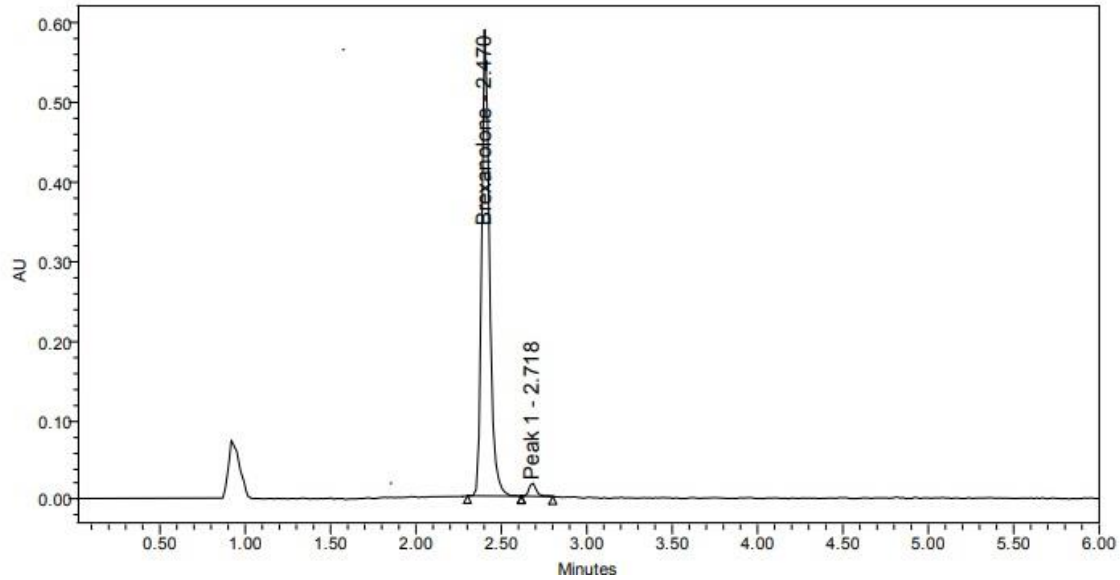

Figure 6: Peroxide degradation chromatogram

Photolytic degradation study:

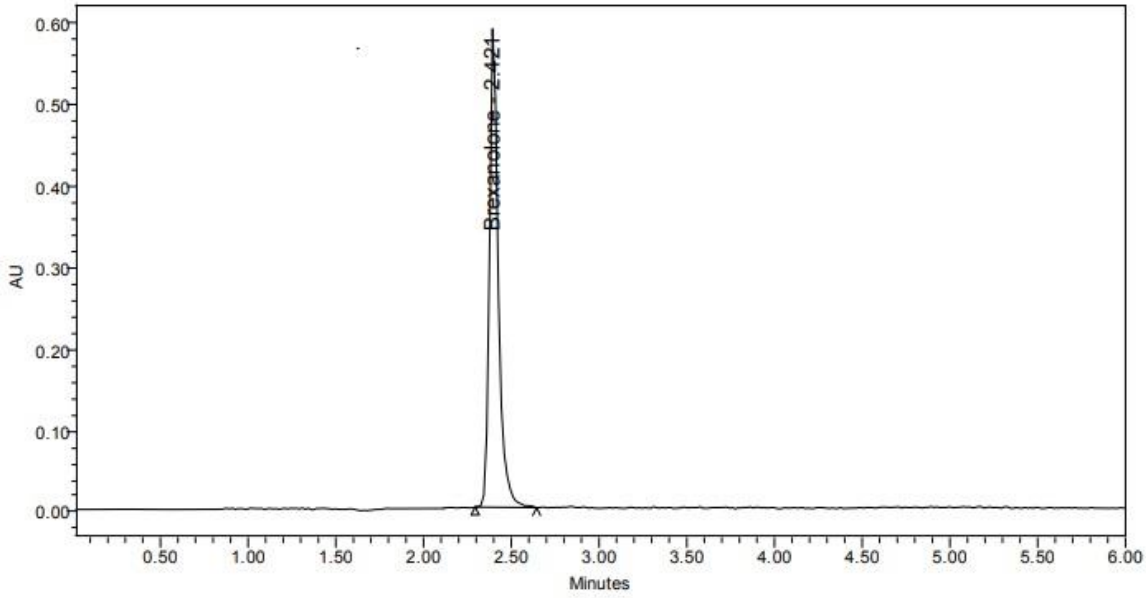

Figure 7: Photolytic degradation study chromatogram

Neutral degradation study:

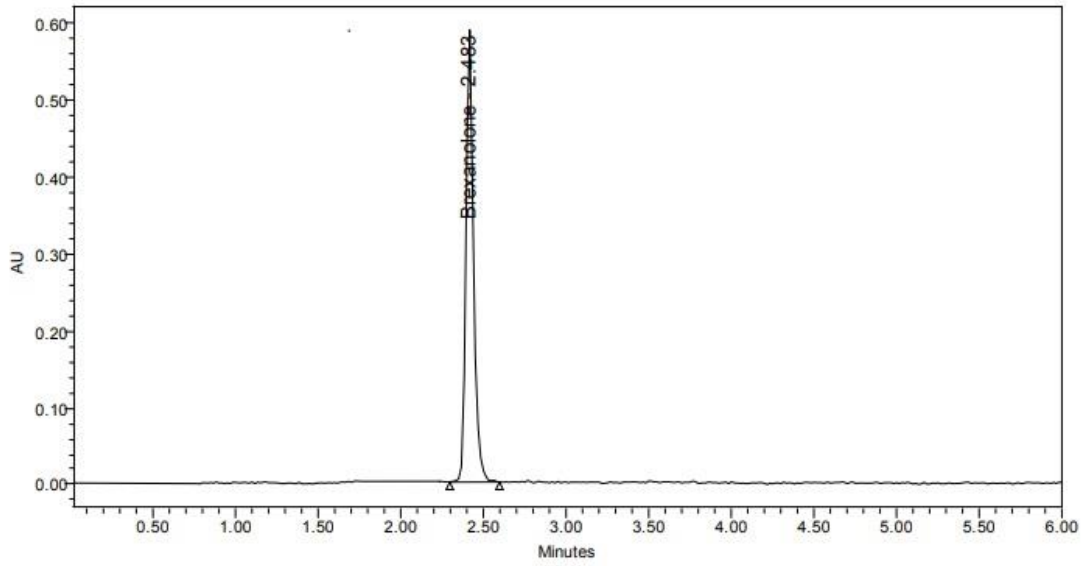

Figure 8: Neutral degradation study chromatogram