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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESKETAMINE AND BREXANOLONE IN ITS PURE & TABLET DOSAGE FORMS BY RP-HPLC

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ABSTRACT

Background: Estimation of Esketamine and Brexanolone are the drugs approved by US Food and Drug Administration as antidepressants. Esketamine is used to treat resistant depression and Brexanolone is used to treat Postpartum depression in adult females.

Objective: The main objective of the Simultaneous estimation of these drugs is to establish identity ,purity ,physical characteristics and potency of the drugs and to demonstrate the suitability of the assay method to provide useful data to ensure the technique gives satisfactory and consistent results.

Materials and methods: A HPLC (Alliance Water 2695) with UV/VIS Spectrophotometer, UV (lab India, UV 3000$^+$ series) and Inertsil ODS (4.6 x 250mm)5 µm column was used. new method was confirmed for simultaneous evaluation of Esketamine and Brexanolone by RP-HPLC method. The chromatographic environment was fortunately evolved for the partition of Esketamine and Brexanolone by using Inertsil ODS

(4.6 x 250mm)5 µm, flow rate was 1ml/min, mobile phase ratio was (20:80 v/v) OPA Buffer : ACN phosphate pH 3(pH was adjusted with orthophosphoric acid), wavelength was detected at 235nm.

Results: The results were in good agreement with those obtained with official HPLC with absorption maximum of 235 nm by preparing mobile phase 20:80 OPA Buffer : ACN phosphate with flow rate 1 ml/min and it run for 10 minutes by selecting column Inertsil ODS (4.6 x 250mm)5.0 µm of ambient temperature. All the results obtained with good precise, Inertsil ODS (4.6 x 250mm)5.0 µm accurate and robustness as per international conference on Harmonization (ICH) guidelines.

Conclusion: It can be concluded that the proposed RP-HPLC method is accurate, precise, sensitive, specific, robust and reproducible for the simultaneous analysis of Esketamine and Brexanolone with less tailing factor and is also economical. Inertsil ODS (4.6 x 250mm)5.0 µm, flow rate was 1ml/min. Both samples scan in the range of 200 to 400 nm and maximum wavelength was identified at 235 nm.

KEYWORDS: Inertsil ODS Column, Esketamine and Brexanolone, RP-HPLC

Introduction: Esketamine is (2S)-2-(2-chlorophenyl)-2-(methylamino)cyclohexan-1-one and belongs to category anti-depressant agents. It acts by blocking N-methyl D-asparate receptors. This reduces pain perception,induces sedation and produces dissociative anaesthesia. Brexanolone is 1-[(1S,3aS,3bR,5aS,7R,,9aS,9bS,11aS)-7-hydroxy-9a,11-a-dimethyl-hexadecahydro-1H cyclopenta[a]phenanthren-1-yl]ethan-1-one. and belongs to category neuroactive steroid. Brexanolone is a synthetic neuroactive steroid gamma-aminobutyric acid A (GABA (a)) receptor positive modulator indicated for the treatment of postpartum depression (PPD) in adult women.

Chemicals and Reagents: Esketamine and Brexanolone were supplied by Larus Labs Pvt. ltd. We used HPLC grade acetonitrile, water and methanol received from standard solutions Ltd , ortho phosphoric acid was supplied by FINAR Chemical Ltd.Hcl,H_20_2,NaoH received from MERCK.

Instrumentation:

HPLC (Alliance, Water2695) with UV/VIS Spectrophotometer, UV (lab India, UV 3000 series) and Inertsil ODS (4.6 x 250mm)5.0 µm column was used. The HPLC system was equipped with Empower software for data processing.

Chromatographic Condition:

The mobile phase includes mobile phase 20:80 OPA buffer : ACN was found to resolve Esketamine and Brexanolone. Ortho phosphoric acid was pre-owned for pH adjustment of bufferto3.0. The mobile phase was strained through 0.45 nylon filter and then ultrasonicated for 30 min. The flow rate was set to 1.0ml/min. The drug shows good absorbance at 235nm, which was selected as wavelength for further analysis.

Preparation of Mobile Phase: Accurately measured 200 ml (20%) of above buffer and 8100 ml of Acetonitrile HPLC (10%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Preparation of Buffer: 1 ml of OPA is taken in 1000 ml of HPLC water pH was adjusted with 0.1M NAOH up to 3.0.final solution was filtered through 0.45 µm Membrane filter and sonicate it for 10 mins.

Preparation of Sample solution: Accurately weigh and transfer 5 mg of Esketamine and 12.50 mg of Brexanolone working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent

Preparation of Standard stock solutions: Accurately weigh and transfer 10 mg of Esketamine and 25mg of Brexanolone working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

RESULTS AND DISCUSSIONS: Method Development

The identification wavelength was adopted by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in Ultraviolet (U.V) range from 200-400nm. The overlay spectrum of Esketamine and Brexanolone was obtained and the isobestic point of Esketamine and Brexanolone showed absorbance's maxima at 235nm. Chromatographic method development was optimized by various parameters both in Active Pharmaceutical Ingredient (API) and pharmaceutical dosage form.The Optimized Chromatographic conditions by preparing mobile phase 20:80 OPA buffer:ACN with flow rate 1 ml/min and it run for 10 minutes by selecting column Inertsil ODS (4.6 x 250mm)5.0 µm of ambient temperature. The retention time of Esketamine and Brexanolone are 2.131 mins and 2.816 mins respectively. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.1 and 0.7 for Esketamine and Brexanolone which shows that the method is precise.. The assay of Esketamine and Brexanolone was performed with tablets and the % assay was found to be 99.47and 100.02 which shows that the method is useful for routine analysis.

VALIDATION REPORT

Assay:

The percentage assay of Esketamine and Brexanolone was found to be 99.47 and 100.02 was shown in fig no:01 and the resuls are tabulated in table no 01.

SPECIFICITY:

The system suitability for specificity was carried out to evaluate whether there is any interference of any impurities in retention time of analytical peak the results was shown in fig no:02 and the results are tabulated in table no 02.

LINEARITY:

The linearity range was found to lie from 50µg/ml to 250µg/ml of Esketamine, 125 µg/ml to 625 µg/ml of Brexanolone and the results are tabulated in table no 03.

ACCURACY:

The accuracy study was performed for 50%, 100% and 150 % for Esketamine and Brexanolone.The percentage % retrieval was found to be 99.74% and 99.40% respectively and the results are tabulated in table no 6 and 7.

Precison (Repeatabilityi): The precision evaluation was performed for six injections of Esketamine and Brexanolone.Each standard injection was injected into chromatographic system. Precision study was performed for six injections was shown in Table no 4.

INTERMEDIATE PRECISION (ruggedness):

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variations.Resulta are tabulated in Table no 5.

LOD & LOQ:

The LOD was performed for Esketamine and Brexanolone and signal to noise ratio was estimated to be 2.98 and 3.0 respectively was shown in Fig No 3 and Table no: 08.The LOQ was performed for Sulfadiazine and Pyrimethamine was estimated to be 10.0 and 9.8 respectively was shown in Fig No 4 and Table no 9.

Robustness:

The standard and samples of Esketamine and Brexanolone were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.Resluts tablated in Table no 10,11,12,13.

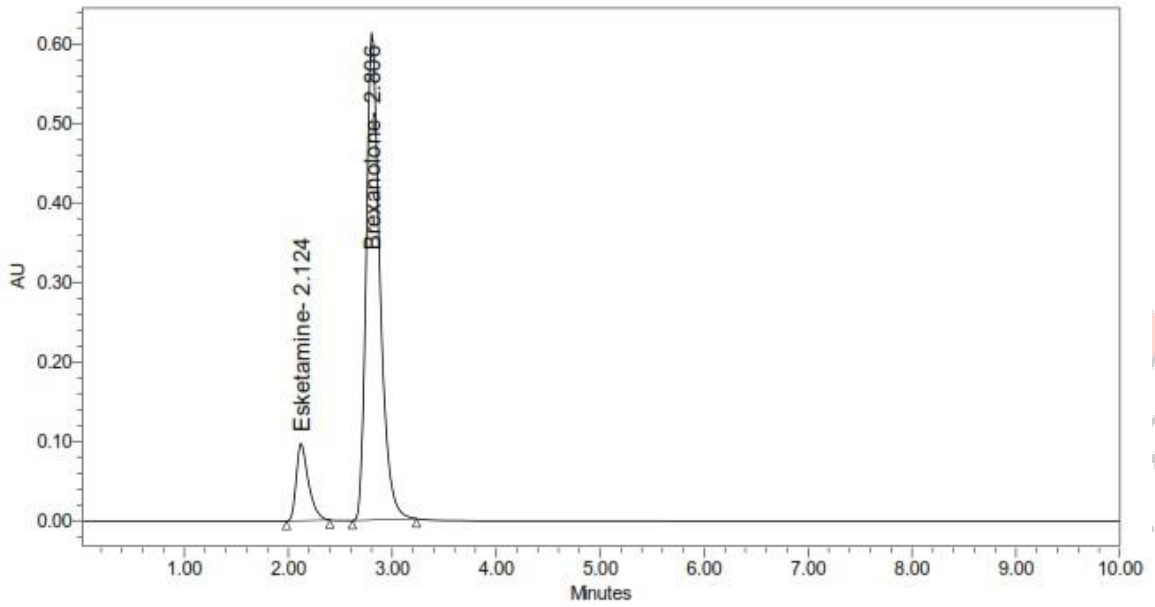

Fig No: 01 Chromatogram for Sample

	Label Claim (mg)	% Assay
Esketamine	28	99.47
Brexanolone	100	100.02

Table No: 01 Results of Assay for Esketamine and Brexanolone

S. No.	Name	RT(min)	Area (µV sec)	Height (µV)	USP plate count	USP tailing	USP Resolution
1	Esketamine	2.131	107339	48500	3009.99	1.14	--
2	Brexanolone	2.816	191234	238437	4609.37	1.48	3.43

Table No: 02 Results for system suitability parameters

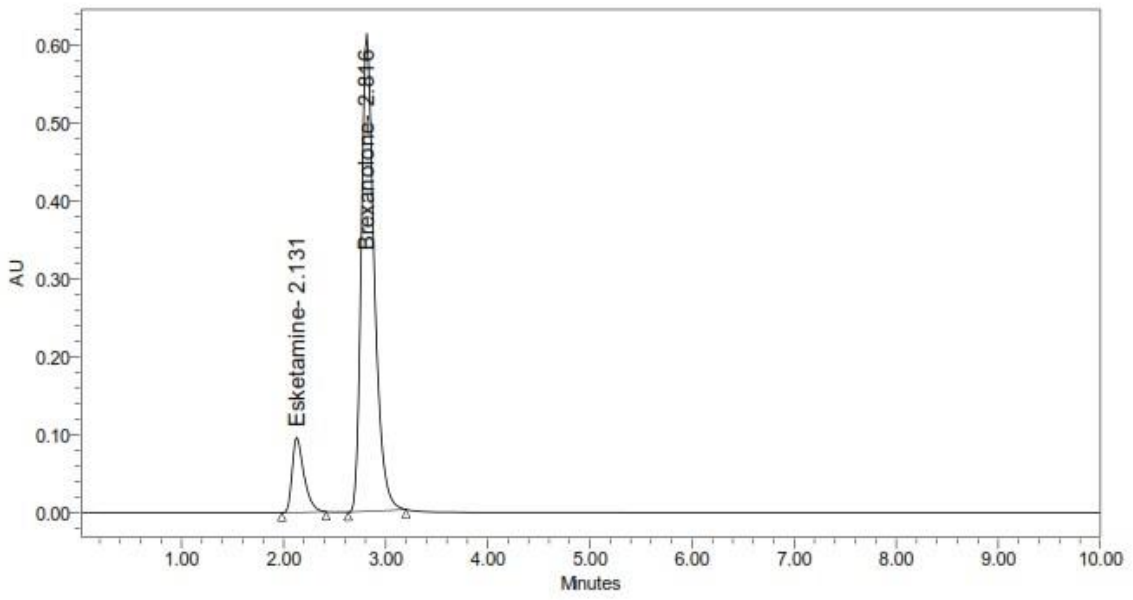

Fig No: 02 Chromatogram for system suitability

S. No	Esketamine		Brexanolone	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	50	32441	125	65787
2	100	67728	250	131783
3	150	100630	375	194311
4	200	134448	500	256245
5	250	172463	625	317748

Table No: 03 Area of different concentration of Esketamine and Brexanolone.

Injection	Area for Esketamine	Area for Brexanolone
Injection-1	107339	191345
Injection-2	107232	191232
Injection-3	107131	191671
Injection-4	107399	191999
Injection-5	107018	192898
Injection-6	107089	194679
Average	107201.3	192304.0
Standard Deviation	148.4	1308.1
%RSD	0.1	0.7

Table No: 04 Results of Precision for Esketamine & Brexanolone

Injection	Area for Esketamine	Area for Brexanolone
Injection-1	104533	192345
Injection-2	104232	192432
Injection-3	104531	192971
Injection-4	104399	192899
Injection-5	104018	192898
Injection-6	104689	192333
Average	104400.3	192646.3
Standard Deviation	241.9	305.8
%RSD	0.2	0.2

Table No: 05 Results of Intermediate precision for Esketamine & Brexanolone

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
10%	53846	5	5.01	100.24	99.74
100%	107344	10	9.99	99.91	
150%	159676	15	14.86	99.08	

Table No: 06 Accuracy (recovery) data for Esketamine

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	95105	12.5	12.43	99.47	99.40
100%	191399	25	24.92	99.67	
150%	285309	37.5	37.14	99.05	

Table No: 07 Accuracy (recovery) data for Brexanolone

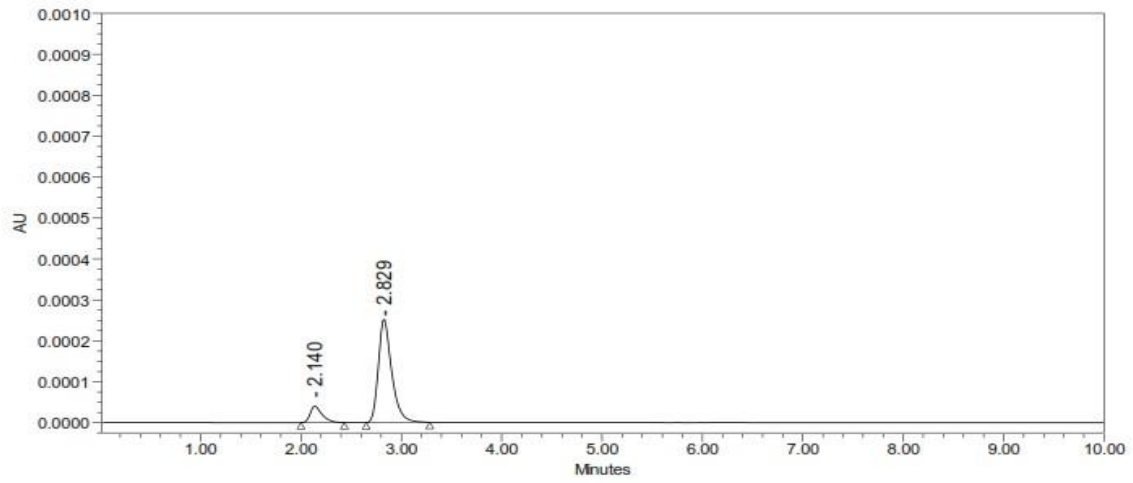

Fig No: 03 Chromatogram of Esketamine and Brexanolone showing LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Esketamine	58	173	2.98
Brexanolone	58	174	3.00

Table No: 08 Results of LOD

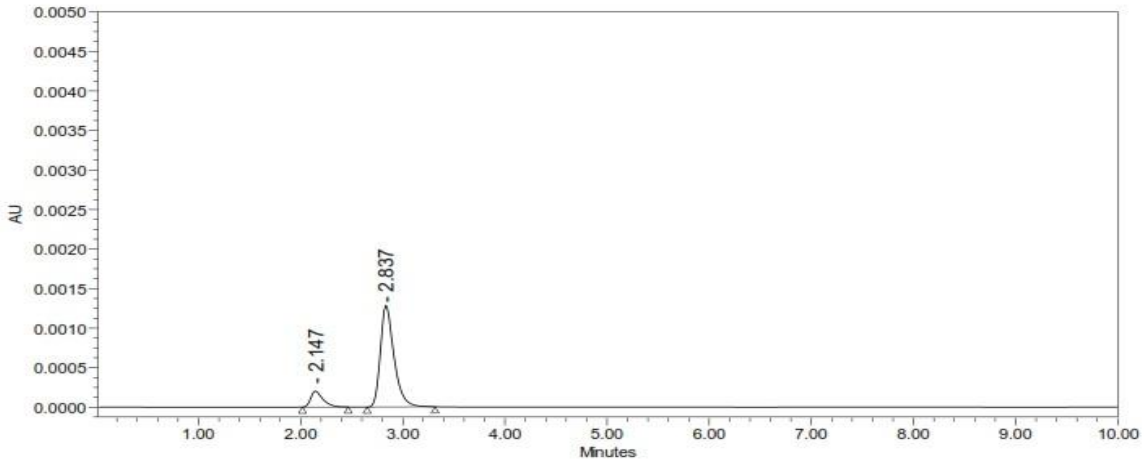

Fig No:04 Chromatogram of Esketamine and Brexanolone showing LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Esketamine	58	580	10.00
Brexanolone	58	579	9.98

Table No: 09 Results of LOQ

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	2950.01	1.25
2	1	3009.99	1.14
3	1.1	2694.74	1.47

Table No: 10 Results for variation in flow for Esketamine

S. No	Flow Rate (ml/min)	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	0.9	4411.3	1.44	3.27
2	1	4609.37	1.47	3.23
3	1.1	4033.25	1.42	3.07

Table No: 11 Results for variation in flow for Brexano

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2628.44	1.10
2	*Actual	3009.99	1.14
3	10% more	2694.74	1.47

Table No: 12 Results for variation in mobile phase composition for Esketamine

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	10% less	4478.77	1.43	5.63
2	*Actual	4609.37	1.47	3.23
3	10% more	4033.25	1.42	3.07

Table No: 13 Results for variation in mobile phase composition for Brexanolone

CONCLUSION:

The developed analytical method was validated as per ICH Q2 (R1) guidelines and it meets the acceptance criteria of each parameter. It is concluded that the developed method is specific, linear, precise accurate, robust and sensitive to analyze the simultaneous analysis of Esketamine and Brexanolone with less tailing factor and is also economical. Inertsil ODS (4.6 x 250mm)5.0 µm, flow rate was 1ml/min. Both samples scan in the range of 200 to 400 nm and maximum wavelength was identified at 235 nm. The main advantage of developed UV method over HPLC method is that it is less time consuming and also economical. Thus, the developed method can be used for routine analysis of Tacrolimus in pure form as well as in the pharmaceuticals.

CONSENT AND ETHICAL APPROVAL

It is not applicable

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COMPETING INTEREST

Authors have declared that no completing interests exists

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