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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF OXCARBAZEPINE IN FORMULATED PRODUCT BY USING RP-UPLC

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ABSTRACT

A simple, specific and accurate reverse phase ultra-performance liquid chromatographic (RP-UPLC) method was developed for the determination of Oxcarbazepine in the tablet dosage forms. The chromatographic separation was achieved on BEH C-18 (2.1 x 50mm) 1.7 um particle size and the mobile phase containing acetonitrile with 5mM ammonium acetate for Oxcarbazepine. The run time was 2.50 min and the retention time of Oxcarbazepine was 1.35. The detection was carried out 214nm using photo diode array detector (PDA) with a flow rate 0.6 ml/min. The linearity of Oxcarbazepine was in the range 50-250 ppm with correlation coefficient 0.9999. The recovery was found in the range (100±10%). The developed method was validated as per International Conference on Harmonization guidelines (ICH) with respect to specificity, linearity, accuracy, precision, ruggedness and robustness.

2.1 INTRODUCTION

2.1.1 Description

Oxcarbazepine is structurally a derivative of carbamazepine, adding an extra oxygen atom to the benzylcarboxamide group. This difference helps reduce the impact on the liver of metabolizing the drug, and also prevents the serious forms of anaemia occasionally associated with carbamazepine. Aside from this reduction in side effects, it is thought to have the same mechanism as carbamazepine - sodium channel inhibition - and is generally used to treat partial seizures in epileptic children and adults.

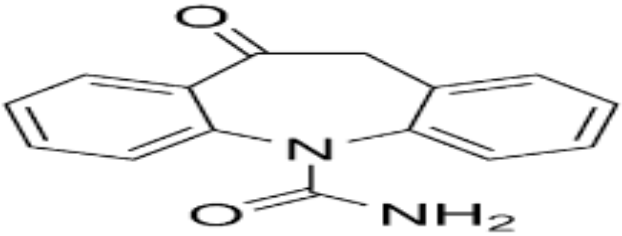

Fig. 1: Structure of Oxcarbazepine.

IUPAC name: 10,11-dihydro-10-oxo-5*H*-dibenz(b,f)azepine-5-carboxamide.

Molecular formula: $C_{15}H_{12}N_2O_2$

Molecular weight: 252.268 g/mol

Colour: Ox carbazepine is a white to yellowish powder.

Solubility: [308 mg/L at 25 °C (SRC PhysProp estimated -- MEYLAN,WM et al. (1996))]soluble in methanol, acetonitrile, formic acid and dimethylsulfoxide and insoluble in water

Melting point*:* 215.5 °C

Mechanisms of Action

The pharmacological activity of Trileptal is primarily exerted through the 10-monohydroxy metabolite (MHD) of oxcarbazepine. The precise mechanism by which oxcarbazepine and MHD exert their antiseizure effect is unknown; however, *in vitro* electrophysiological studies indicate that they produce blockade of voltage-sensitive sodium channels, resulting in stabilization of hyperexcited neural membranes, inhibition of repetitive neuronal firing, and diminution of propagation of synaptic impulses. These actions are thought to be important in the prevention of seizure spread in the intact brain. In addition, increased potassium conductance and modulation of high-voltage activated calcium channels may contribute to the anticonvulsant effects of the drug. No significant interactions of oxcarbazepine or MHD with brain neurotransmitter or modulator receptor sites have been demonstrated.

Purpose of Present Studies

Oxcarbazepine has been developed on high performance liquid chromatography to quantify the drug in multiple cases inscribing lengthy columns with high particle size having low surface area which requires more time for separation. The previous methods required large amount of mobile phases at high flow rate, peak obtained were not symmetrical and were less sensitive. The current study emphasize on developing a simple specific, precised, accurate time saving method for development of Oxcarbazepine in formulated product. For obtaining

the above mentioned condition we required a fast optimized and calibrated WATERS ACQUITY UPLC system which gives accurate and précised results in lesser time using less solvent thus becoming a relatively faster and economical method. The method is developed using a mass compatible volatile Ammonium Acetate buffer having 5 milli-Molar concentration and using a WATERS ACQUITY BEH column of dimension 2.1 x 50 mm having particle suface area of 1.7µm particle size. The method which is developed and further validated is performed as mentioned in ICH guidelines. The validation parameters which were studied are as followed i.e specificity, linearity, precision, accuracy, robustness, limit of quantitation, limit of detection and stability. The Active Pharmaceutical Ingredient used for this project was taken from Jubilant Generics Ltd. And it's tablet formulation was taken from a local pharmacy, the tablet name was Oxtellar-300 manufactured by Sun Pharma Ltd.

MATERIAL AND METHOD

Materials

Apparatus: Chemicals which were used in this study were Ammonium Acetate (Sigma Aldrich, USA) and Acetonitrile (J.T Baker), Formic Acid (Fluka), HPLC grade trifluoro acetic acid (TFA) (Sigma Aldrich, USA). Water used for UPLC analysis was purified using MilliporeMilli Q Plus water purification system (Millipore SAS, France).

Reagents and Chemicals: An approved well-characterized manufactured standard of Oxcarbazepine was procured from Jubilant LifeSciences Limited, India. Commercially available OXTELLAR-300 (Oxcarbazepine Tablet) purchased from local pharmacy (Noida, India) having manufactured by Sun Pharma Ltd.

Methods

Solubility: Solubility From the literature review, Oxcarbazepine is scarcely soluble in Acetonitrile and Water but comprehensively dissolves with 0.1 % Formic Acid.

Selection of chromatographic method

The selection criteria depends upon the nature of the sample (ionic / ionisable / neutral molecule), its molecular mass and solubility. The drug which is been worked in the present context is almost polar in nature and thus reversed phase or ion-pair or ion exchange chromatography method may be used. The reversed phase UPLC was selected for the separation because of its speed, simplicity, accuracy and suitability.

Selection of wavelength

The sensitivity of Oxcarbazepine was tried on many wavelength on the detector which was having deuterium lamp as the source. However during conditions optimization we found that 214 nm as the appropriate wavelength for this analysis.

Chromatographic conditions

METHOD

Chromatography separation studies were performed on Waters Acquity UPLC with photodiode array detector having deuterium lamp. The processing software for the compatibility of the WATERS Acquity UPLC system used was mass lynx. The chromatographic column is WATERS Acquity BEH C18 column (50 × 2.1 mm, 1.7µm). The mobile phase of 5Mm Ammonium Acetate in channel A1 and acetonitrile in channel B1 having a flow rate of 0.6 ml/m in under a gradient method. The injection volume was 1.0µL and the chromatographic runtime of 2.5 min was used. A linear gradient elution method was applied as follows:

Table. 1: Gradient Table.

Time (Min)	0.0	0.20	1.40	1.70	2.00	2.20	2.50
Flow	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Solvent A	90	90	65	10	10	90	90
Solvent B	10	10	35	90	90	10	10

Preparation of Solutions

Preparation of Buffer: Preparation of Buffer**:** weighed 386 mg ammonium acetate in 950 mL milli Q water, diluted with 50 mL ACN and sonicated it for 10 min.

Preparation of Diluents: Mixed well Milli-Q water: Acetonitrile in a ratio 50:50, with 0.1 % formic acid sonicated and degassed.

Preparation of Stock Solution

Weighed accurately and transferred about 25 mg of oxcarbazepine standard in a 25 ml volumetric flask. Added 12.5 mL water, 12.5 mL ACN with 0.1% formic acid and sonicated it for 10 min.

Preparation of sample: Weighed accurately and transferred about 25 mg of oxcarbazepine standard in a 25 ml volumetric flask. Pipette out 1.5 ml of this solution and volume made up to 10 mL, added 0.1 % formic acid sonicated and degassed it.

Preparation of Diluents

Mixed well Milli-Q water: Acetonitrile in a ratio 50:50, with 0.1 % formic acid sonicated and degassed.

Preparation of blank solution

Mixed well primary and secondary diluents in a ratio of (50:50), with 0.1 % formic acid sonicated and degassed.

Validation of developed UPLC method

Different chromatographic conditions such as mobile phase, wavelength, column and column temperature were experimented to achieve efficiency of the chromatographic system. Different gradients of buffer and solvents were checked in order to attain optimum retention of the API. Minimizations of run time and cost were the major tasks while developing the method. Based on International Conference on Harmonization(ICH) guidelines, the method was validated with regard to precision, specificity, reproducibility, accuracy, linearity, stability of solution, robustness, limit of detection and quantification.

5.7.1 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).This definition has the following implications.

Identification: to ensure the identity of an analyte. Purity Tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.

Preparation of sample

- From the stock solution of 1000 milli molar conc. A sample of 150 millimolar conc. Was made standard.
- 6 injections of 1 microlitre were injected.
- Retention time was observerd of about 1.35.

Table. 2: Specificity table of standard.

S.N.	Injection	RT	Area	Range
1	Std-1	1.35	19124.23	0.98
2	Std-2	1.35	19377.45	1.00
3	Std-3	1.35	19147.07	0.99
4	Std-4	1.35	19182.80	0.99
5	Std-5	1.35	19381.86	1.00
6	Std-6	1.35	18593.99	0.98
Mean	19194.6			
Std.Dev.	115.53			
%RSD	0.60			

Table. 3: Specificity table of test.

S.N.	Injection	RT	Range
1	Test-1	20494.84	0.95
2	Test-2	20604.04	0.97
3	Test-3	20666.91	0.96
4	Test-4	20369.23	0.94
5	Test-5	19975.00	0.96
6	Test-6	20350.89	0.95
Mean	20410.3		
Std.Dev.	130.82		
%RSD	0.64		

5.7.2 Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods for the establishment of linearity, a minimum of 5 concentrations is recommended. Other approaches should be justified.

- 5 concentrations of 50, 100, 150, 200 and 250 ppm were taken
- Area was observed of each 5 concentration
- Regression line of intercepy y following equation.
- Y=mx +c.
- 0.99 values for straight line were achieved.

Table. 4: Linearity Table.

Conc.	R.T	Area	Range
50	1.35	6305	0.3
100	1.35	13140	0.7
150	1.35	18622	1.0
200	1.35	25130	1.2
250	1.35	31409	1.4

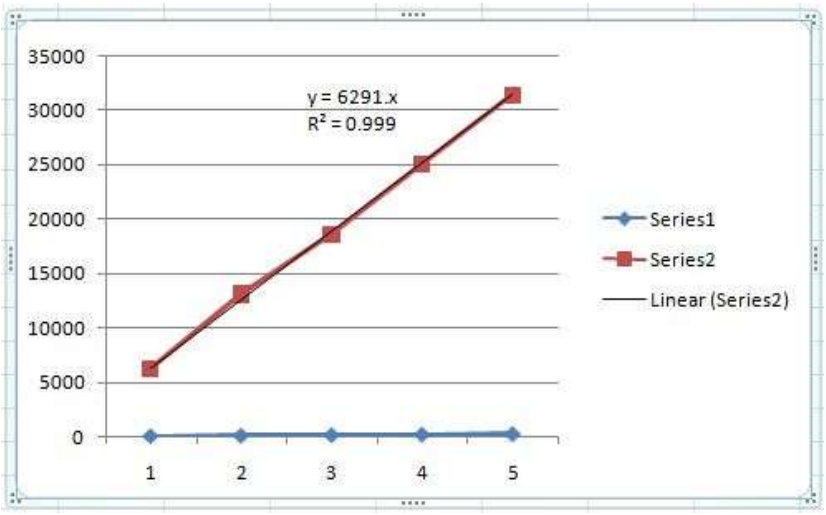

Fig. 2: Linearity Curve.

5.7.3 Precision

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. The applicant should establish the effects of random events on the precision of the analytical procedure. Typical variations to be studied include days, analysts, equipment, etc. It is not considered necessary to study these effects individually. The use of an experimental design (matrix) is encouraged

- STD. OF 150 was taken in 6 vial and injection of 1 uL were injected.
- Mean, Standard deviation and percentage RSD were calculated.
- Percentage rsd sould always be less than 2 percent.

5.7.4 Intermediate Precision: The intermediate precision will be evaluated by a different analyst on different day and/or different uplc probe and/or a different uplc spectrometer with a different column and photodetector. Integration of peaks as well as phase and baseline correction is the most subjective parts of the method. Six different sample preparations were prepared and analyzed on different day. The average of six analyses, standard deviation (SD) and RSD values are documented in table.

Table. 5: Intermediate precision of standard.

Sample	RT	AREA	MEAN	STD. DEV.	% RSD
STD-1	1.35	19269.37	19245.51	375.55	1.95
STD-2	1.35	18799.98			
STD-3	1.35	18944.98			
STD-4	1.35	19326.22			
STD-5	1.35	19247.22			
STD-6	1.35	19885.46			

Table. 6: Intermediate Precision of test.

S. No	Injection No.	Area	Area	Range
1	Sample-1	20070.22	20095	1.00
2	Sample-1-	20118.79		1.03
3	Sample-2	20094.98	20075	1.01
4	Sample-2-	20055.47		1.03
5	Sample-3	20148.51	20131	1.02
6	Sample-3-	20113.65		1.01
7	Sample-4	20111.07	20057	1.04
8	Sample-4-	20002.65		1.02
9	Sample-5	20113.65	19939	1.03
10	Sample-5-	19763.70		1.02
11	Sample-6	20003.29	20118	1.01
12	Sample-6-	20102.79		1.00
MEAN	20067.43			
STD. DEV	68.12			
.% RSD	0.34			

Table. 7: Intra-day precision of standard.

Sample	RT	AREA	MEAN	STD. DEV.	% RSD
STD-1	1.35	19228.39	19301.34	291.88	1.51
STD-2	1.35	19248.99			
STD-3	1.35	19141.23			
STD-4	1.35	19149.98			
STD-5	1.35	19149.63			
STD-6	1.35	19889.84			

Table. 8: Intra-day precision of test.

S. No	Injection No.	Area	Area	Range
1	Sample-1	20655.89	20549	1.00
2	Sample-1-	20432.25		1.03
3	Sample-2	20698.98	20710	1.01
4	Sample-2-	20722.00		1.03
5	Sample-3	20451	20574	1.02
6	Sample-3-	20696.96		1.01
7	Sample-4	20679.90	20705	1.04
8	Sample-4-	20729.84		1.02

9	Sample-5	20640.93	20637	1.03
10	Sample-5-	20633.32		1.02
11	Sample-6	20763.96	20690	1.01
12	Sample-6-	20616.00		1.00
MEAN	20644.25			
STD. DEV.	69.57			
% RSD	0.34			

5.7.5 Accuracy

the accuracy of an analytical method expresses the closeness of agreement between a formulation and the api. the accuracy of an analytical procedure should be established across its range. the ich documents recommend that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range**.**

Data from nine determinations over three concentration levels covering the specified range was determined. The accuracy was studied at 50 %, 100 % and 150% levels with respect to the sample by preparing the solutions in triplicate at each level. From the results as per Table, it was concluded that method for assay content was accurate between the ranges of 50 % to 150% level.% RSD at each level was level was found to be less than 2.00.

Table. 9: Accuracy of standard.

S. No.	Injection No.	RT	Area
1	Std-1	1.35	19036.96
2	Std-2	1.35	18957.98
3	Std-3	1.35	18987.19
4	Std-4	1.35	18108.88
5	Std-5	1.35	19157.10
6	Std-6	1.35	19179.66
Mean	18904.63		
Standard deviation	365.09		
% RSD	1.93 %		
Purity	100 %		

Table. 10: Accuracy of Test.

Conc. ppm	Area	Mean area	Sample wt (mg)	Amount added	Amount rec.	% rec.	Mean % rec.	Dev. Rec.	% RSD rec.
75	8871.42	8911	2.65	26.50	26.47	99.89	100.15	0.46	0.46
	8987.47				26.68	100.68			
	8874.70				26.47	99.89			
150	18391.25	18744	5.61	56.10	56.08	99.96	99.80	0.16	0.16
	19167.54				55.90	99.64			
	18672.07				55.98	99.79			
225	26953.79	26934	8.01	80.10	80.05	99.94	99.49	0.74	0.74
	26726.6				80.01	99.89			
	27121.56				79.01	98.64			

5.7.6 Robustness

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used.

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

• Influence of variations of flow in a mobile phase,

• Influence of variations in mobile phase concentration

Table. 11: Flow Variation of Standard.

S. No.	Flow	Injection No.	RT	Area	Absorbance	Parameter	Value
1	0.5	Std-1	1.39	11593.77	0.68	Mean	11583.03
		Std-2	1.39	11560.39	0.68		
		Std-3	1.39	11540.67	0.68	Std. Dev.	31.64
		Std-4	1.39	11606.18	0.68		
		Std-5	1.39	11578.77	0.68	% RSD	0.27
		Std-6	1.39	11618.40	0.68		
2	0.6	Std-1	1.28	9602.16	0.61	Mean	9678.36
		Std-2	1.28	9631.05	0.61		
		Std-3	1.28	9632.89	0.61	Std. Dev.	51.52
		Std-4	1.28	9630.11	0.61		
		Std-5	1.28	9632.50	0.61	% RSD	0.53
		Std-6	1.28	9641.50	0.61		

3	0.7	Std-1	1.19	8183.65	0.54	Mean	8238.23
		Std-2	1.19	8258.03	0.55		
		Std-3	1.19	8241.13	0.55	Std. Dev.	25.07
		Std-4	1.19	8242.92	0.55		
		Std-5	1.19	8251.61	0.56	% RSD	0.30
		Std-6	1.19	8252.08	0.56		

Buffer Conc. Change

Table. 12: Log H+ Conc.

10 mM buffer

Sample	RT	AREA	RANGE	MEAN	STD. DEV.	% RSD
STD-1	1.41	20100.13	1.02	20088.4	23.91	0.12
STD-2	1.41	20126.28	1.02			
STD-3	1.41	20086.69	1.02			
STD-4	1.41	20069.64	1.02			
STD-5	1.41	20102.28	1.02			
STD-6	1.41	20045.27	1.02			

5.7.8 The Limit of Detection.

The limit of detection (LOD or CCβ) is the lowest concentration of the measurand that can be detected at a specified level of confidence.

$$Limit\ of\ Detection = \frac{Standard \times 3.3}{Slope}$$

$$LOD = \frac{S.D. \times 3.3}{m}$$

Calculation

Table. 13: LOD Calculation.

S.No.	AREA
1	6305
2	13140
3	18622
4	25130
5	31409
Total	94606

Mean=18921.2

Standard Deviation=23513

Slope=6291

LOD=12

5.7.9 Limit of quantitation (LoQ)

The limit of quantification (LOQ) is "the lowest concentration at which the performance of a method or measurement system is acceptable for a specified use"

$$LOQ = \frac{Standard \times 10}{Slope}$$

LOQ = 37.37

5.7.10 Stability

The stability parameter was performed on the standard drug as well as the test drug. The initial injection was given at 0 hour and then the standard and test was kept at room temperature. After the time interval of 24 hour next injections was given of standard as well test. Six injection 0f test and six injections of standard were analysed using this method within the course of 6 days. After that there % cumulative RSD was calculated.

Table. 14 Stability Of Stanadard Sample.

Time	Injection	RT	Area
Day-1	Std-1	1.35	20096.43
Day-2	Std-2	1.35	19886.94
Day-3	Std-3	1.35	20188.18
Day-4	Std-4	1.35	20171.58
Day-5	Std-5	1.35	20184.26
Day-6	Std-6	1.35	20115.21
Mean	20107.1		
Std. dev.	138.44		
% RSD	0.69		

Table. 15: Stability Of Test Sample.

Time	Injection	RT	Area
Day-1	Test-1	1.35	20733.79
Day-2	Test-2	1.35	20843.64
Day-3	Test-3	1.35	20824.25
Day-4	Test-4	1.35	20900.76
Day-5	Test-5	1.35	20893.45
Day-6	Test-6	1.35	20917.05
Mean	20852.2		
Std. dev.	69.29		
% RSD	0.33		

Table. 16: Result Data of Validation Summary.

S. No	Parameter	Results in %RSD or Slope or	Acceptance limit
1	Specificity	Std-0.60	% RSD<2.00
		Test-0.64	
2	Linearity	Std- 0.999 (slope)	SLOPE=0.999
3	Precision (Intermediate)	Std-1.95	% RSD<2.00
		Test-0.34	
	Precision (Intra-day)	Std-1.51	% RSD<2.00
		Test-0.34	
4	Accuracy	Std-1.93	% RSD<2.00
	50% (75 ppm)	Test- 100.15	% recovery= 100±2
	100% (150ppm)	Test- 99.80	
	150% (225 ppm)	Test- 99.49	
5	Robustness		% RSD<2.00 RT should not shift by 0.10
	Flow 0.5	Std.-0.27	
	Flow 0.6	Std.-0.53	
	Flow 0.7	Std.-0.30	
	10 milli-Molar Conc.	Std.-0.12	
6	LOQ	37.37	
7	LOD	12.00	
8	Stability	Std.-0.69	% RSD<2.00 Peak shape should remain same
		Test-0.33	

CONCLUSION

During the study we were able to develop a new method on RP-UPLC to determine Oxcarbazepine efficiently in formulated product within a relatively short period. Precision was under % RSD 2.00 and Accuracy was found within the limit. The new method thus found to be simpler, faster, economical, specific, linear, précised, accurate, robust and stable. The elution rate was much faster possessing a good separation than conventional HPLC method.

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