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Research Article

Development and Validation of a RP- HPLC Method for Quantitation of Oxcarbazepine in Tablet Dosage Form

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ABSTRACT

A novel, accurate and precise HPLC method for determination of oxcarbazepine has been developed and validated. Separation was achieved on X Terra C_{18} column (50 x 4.6 mm internal diameter and 5µm particle size) using Ammonium Bicarbonate and Acetonitrile as a mobile phase at a flow rate of 1.0ml/min and photo diode array detection at 214nm. The developed method was applied for quantitative determination of above drugs in tablet dosage forms and the method was validated with respect to specificity, precision, linearity, accuracy, system suitability, robustness and solution stability. The method was linear over the range of 50-300ug/ml for Oxcarbazepine respectively. The mean recovery was found to be in the range of 99.01-99.28%. The percentage of relative standard deviation was found to be less than critical value. The method was found to be accurate, precise and selective for simultaneous estimation of Oxcarbazepine in tablets.

Key-words: Oxcarbazepine, Reverse-phase HPLC, Method development, Validation.

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Introduction

Oxcarbazepine is 10,11-Dihydro-10-oxo-5*H*-dibenz[*b,f*] azepine- 5-carboxamide; it is an anticonvulsant and mood stabilizing drug, used primarily in the treatment of epilepsy and bipolar disorder. Oxcarbazepine is structurally a derivative of carbamazepine, adding an extra oxygen atom on the dibenzazepine ring. This difference helps reduce the impact on the liver of metabolizing the drug, and also prevents the serious forms of anemia occasionally associated with carbamazepine. Aside from this reduction in side effects, it is assumed to have the same mechanism as carbamazepine - sodium channel inhibition - and is generally used to treat the same conditions. Oxcarbazepine has recently been found associated with a greater enhancement in mood and reduction in anxiety symptoms than other drugs employed to treat epilepsy.

Materials and Method

Material Used

Material Name	Manufacturer	Purity
1. Oxcarbazepine- standard	Jubilant Life Sciences,Noida	99.5 %
2. TRIOPTAL-300mg (sample)	NOVARTIS	NA

Chemicals and Reagents used

S. No.	Reagents	Manufacturer	Grade
1.	Acetonitrile	Sigma Aldrich	HPLC
2.	Milli Q water	Millipore (India) Ltd., Bangalore	HPLC
3.	Ammonium bicarbonate	Merck Ltd., Mumbai, India	AR

Instrument used

S. No.	Instrument	Manufacturer
1.	HPLC	Waters e2695 PDA Detector
2.	Balance	Mettler Toledo (XS 205 dual range)
3.	Sonicator	Ultrasonicator
4.	Column	Waters X-Terra C18 (4.6x 50 mm, 5µm)
7.	HPLC Detector	Photodiode Array Detector
8.	Software	Empower Pro
9.	Centrifuge machine	Spinwin

Procedure

Preparation of Buffer:

Weighed 395 mg Ammonium bicarbonate in 1000 ml Milli-Q water, mixed well, filter and degassed.

Mobile Phase Preparation:

Buffer and Acetonitrile were taken in separate bottles, filtered through 0.2µ Nylon membrane filter paper, sonicated and degassed and mixed automatically as per the method.

Preparation of Diluent:

Milli-Q water: Acetonitrile (ACN) in a ratio (30:70) were mixed well, sonicated and degassed.

Preparation of Standard Solution (200µg/ml) :
Accurately weigh 4 mg Oxcarbazepine standard was weighed and transferred to 10 ml volumetric flask. The sample was initially dissolved in diluent and sonicated .The volume was made up to the mark with diluent and mixed. 5 ml of this solution was diluted to 10 ml with diluent and mixed.

Preparation of Sample solution (200µg/ml) :
Accurately weigh 5.56 mg Oxcarbazepine sample was weighed and transferred to 10 ml volumetric flask. The sample was initially dissolved in diluent and sonicated .The volume was made up to the mark with diluent and mixed. 5 ml of this solution was diluted to 10 ml with diluent and mixed.

Table 1: Trial Taken For Optimize Condition **Trail No.**

	Column Used	**Mobile Phase**	**Mode**	**Injection Volume**	**Observation**	**Result**
1	X-Bridge C18 (2.1 × 50) mm, 5µm	Ammonium bicarbonate (5mM) : MeOH	Gradient	5µl	Peak shape was not good (broad peak observed) with tailing	Method rejected
2	X-Bridge C18 (2.1 × 50) mm, 5µm	Ammonium bicarbonate (5mM) : Acetonitirile	Gradient	5µl	Peak shape sharp but eluted in non polar	Method rejected
3	X-Terra C18 (4.6 × 250) mm, 5µm	Ammonium bicarbonate (neutral): Acetonitirile	Gradient	5µl	Peak shape good but large run time(20min) and low absorbance	Method rejected
4	X-Terra C18 (4.6 × 50) mm, 5µm	Ammonium bicarbonate (neutral): Acetonitirile	Gradient	5µl	Peak shape symmetric, good absorbance with short run time	Method accepted

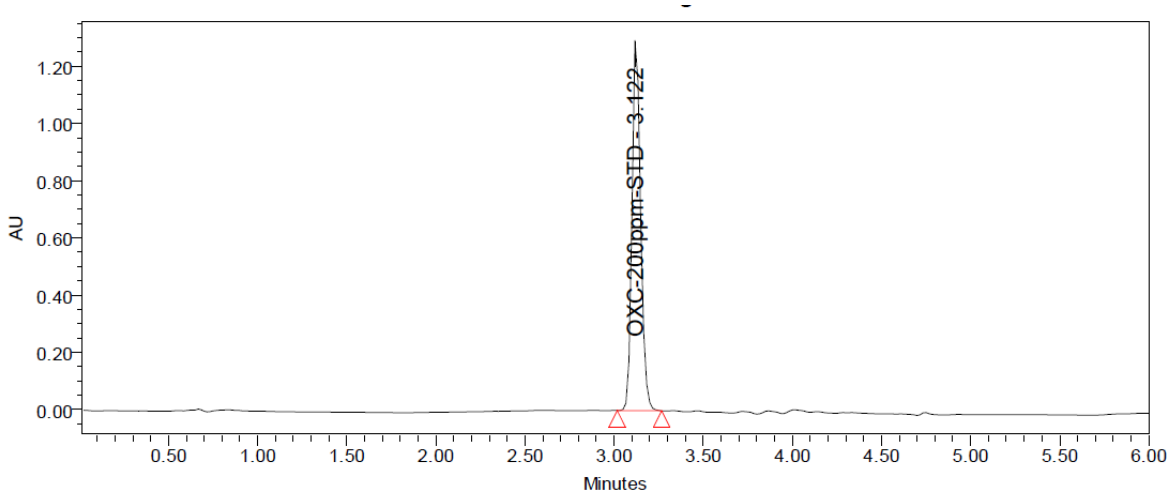

Figure: 1 Chromatogram of standard Oxcarbazepine

Table 2: Data for Optimize Chromatographic condition

NAME	RT(min)	Area (µV*sec)	Theoretical Plates
Oxcarbazepine	3.122	4403927	3.8369

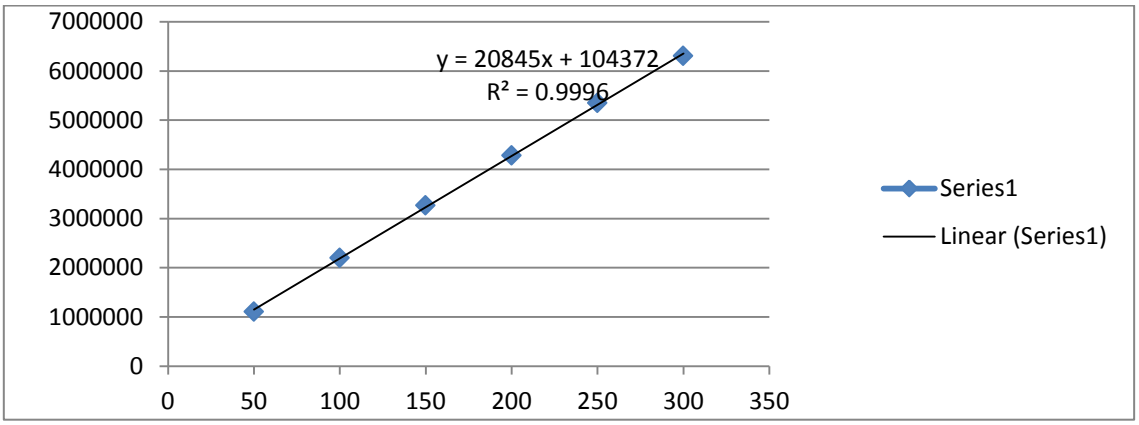

Figure: 2 Calibration curve of Oxcarbazepine

Results and Discussion

The method for anti epileptic drug by some trial for arising out the condition in which peak for Oxcarbazepine drug resolved in a single HPLC trial run. In gradient start with gradient of Buffer :(Ammonium Bicarbonate: Acetonitrile: 60:40) got a good & sharp peak at Retention time 3.12 min. The assay of Oxcarbazepine was done by this method and got 99.20% result.

A simple, precise, accurate, rapid, economica lanalytical method for estimation of Oxcarbazepine is developed by using RP-HPLC method. The developed method is validateas per ICH guidelines The developed method can be used for the analysis of routine quality control test. In the present work the RP-HPLC method for the estimation of Oxcarbazepine in API form has been developed. The proposed method is simple, precise and accurate and do not suffer from any interferences due to common excipients. The newly developed methods can be used in pharmaceutical industry for routine quantitative estimation of Oxcarbazepine in API form. The optimized chromatographic conditions and validation parameters are given below:

Selection of detection wavelength

Oxcarbazepine showed absorbance at 214 nm. So the wavelength selected for the determination of OXCARBAZEPINE was 214nm.

Selection of proper column

Waters X-Terra C18 (4.6 x 50 mm, 5µm)

Selection of chromatographic conditions

Optimized chromatographic conditions for estimation of Oxcarbazepine are finalized as shown in Table Figure shows the chromatogram of standard Oxcarbazepine at optimized method.

Table 3: Optimized chromatographic conditions

S.No.	Parameter Optimized	Optimized Condition
1	Instrument (HPLC)	Waters e26965 PDA detector
2	Column	Waters X-Terra C18 (4.6 x 50 mm, 5µm)
3	Mode	Gradient
4	Mobile phase	Ammonium bicarbonate : Acetonitrile
5	Auto sampler Temperature (^{o}C)	25^{0}C
6	Flow rate	1.00 mL/min
8	Detector	Photodiode array
9	Temperature	Ambient room temperature
10	Detection wavelength	214nm
11	Injection volume	5µl
12	Retention time (RT)	3.10 ± 0.400 min
13	Run time	6.0min

Table: 4. Percent Assay observed of Oxcarbazepine

S.No.	STD Area	Sample	Sample Area
1	4360752	INJ-01	4370683
2	4403927	INJ-02	4388928
3	4383780	**Mean**	**4379805.5**
4	4395893	**STD Dev**	**12901.16**
5	4397543	**% RSD**	**0.29**
6	4359924	**% Assay In Tablet Oxcarbazepine Content= 99.2%**	
Mean	**4383636.5**		
SD	**18811.41**		
% RSD	**0.43**		

Table: 5 Result of different parameter

PARAMETER		DRUG
Specificity		Specific
Linearity	Regression equation y=mx+c	20845x+10437
	Intercept	10437
	Correlation coefficient (r2)	0.999
Accuracy	Level 1	99.01%
	Level 2	99.16%
	Level 3	99.28%
Precision	Method precision (Repeatability) %RSD	0.36
	Intermediate Precision (Ruggedness) %RSD	0.54
Robustness (% RSD)		< 2
System Suitability		0.43

Conclusion

A simple, precise, accurate, rapid, economical analytical method for estimation of Oxcarbazepine form has been developed by using RP-HPLC method. The developed method it will be validate as per ICH guidelines. The developed method can be used for the analysis of routine quality control sample. The proposed method shows

good agreement with all validation parameters. The optimized method is precise, accurate and robust and so it can be applied as stability indicating for the estimation of Oxcarbazepine in API form. In the Specificity There should not be any interference from diluent and blank with main peak. In the Accuracy (recovery) % recovery is 99.01 and % RSD is 0.41 it meets criteria according to ICH Guideline. In the study linearity and range also observed, in which we observed the linear relation between the concentration and the result.

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