Research Article

A Study of Method Development, Validation and Forced Degradation for Quantification of Carbamazepine and Oxcarbazepine by RP-HPLC

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ABSTRACT

A simple, economic, new high performance liquid chromatographic method (RP-HPLC) was developed for quantification of Carbamazepine and Oxcarbazepine in bulk drug as well as in tablet dosage form. The mobile phase used was methanol: water in the proportion of 70:30 v/v at a flow rate of 0.8 ml/min. Over Grace C18 (250mm x 4.6ID, Particle size: 5 microns) column at ambient temperature. Carbamazepine was well retained at 244 nm while that of Oxcarbazepine at 253 nm. The method shows linear response with correlation coefficient (R2) value of 0.997 and 0.999 which were in the limit of the correlation coefficient. The % RSD values of Accuracy, intra-day and inter-day precision are in the allowable limit. LOD and LOQ were found to be 0.820µg/ml, 0.00010µg/ml and 2.48 µg/ml, 0.00031µg/ml of Carbamazepine and Oxcarbazepine respectively. Forced degradation of the drug product was carried out as per ICH and FDA guidelines. The Paracetamol is always used as an internal standard for the RP-HPLC method which does not show any interference in the Carbamazepine and Oxcarbazepine quantification is the major advantage of this method.

Keywords: Carbamazepine, Oxcarbazepine, Forced Degradation, HPLC.

INTRODUCTION

Carbamazepine (Fig.1) is a 5H-Dibenz [b.f] azepine-5-carboxamide, having Anticonvulsant, Antimimic category.[1] Oxcarbazepine is a 10, 11-dihydro-10-oxo-5H-dibenz [b,f] azepine-5-carboxamide[2] Carbamazepine is a white or almost white crystalline powder. It exhibits polymorphism. The drug is very slightly soluble in water, sparingly soluble in alcohol and in acetone, freely soluble in dichloromethane. Carbamazepine is a dibenzazepine derivative with antiepileptic and psychotropic properties.

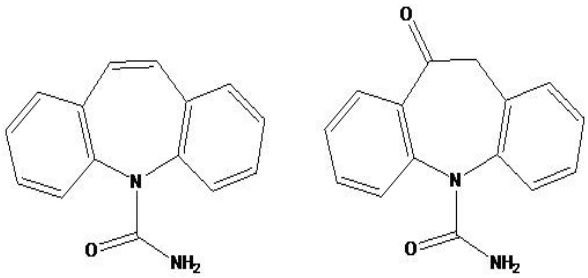

Fig.1 Structure of Carbamazepine and oxcarbazepine

It is used to control secondarily generalized tonic-clonic seizures and partial seizures and in some primary generalized seizures. Carbamazepine is also used in the treatment of trigeminal neuralgia and has been tried with variable success in glossopharyngeal neuralgia and other severe pain syndromes associated with neurological disorders such as tabbed or salis and multiple sclerosis. Another use of carbamazepine is in the prophylaxis of bipolar disorder unresponsive to lithium. Oxcarbazepine (Fig.1) is a derivative of carbamazepine with similar actions. It is used as monotherapy or adjunctive therapy in the treatment of partial seizures with or without secondarily generalized tonic-clonic seizures.[3]There are various methods are available for quantification of carbamazepine and Oxcarbazepine simultaneously[4-15] But no method is available by using paracetamol as internal standard and forced degradation study. The main objective for force degradation study is to check stability of API, to set stability indicating method, to study the chemical properties of drug substance.[16] Herein we put an effort to develop a cost-effective, rapid and robust reversed-phase (RP)-HPLC method with enough data of validation parameters. In proposed method for estimation of both drugs a common internal standard paracetamol is used which help for easy resolution of targeted drug.

MATERIALS AND METHODS

Materials

Working standards of Carbamazepine and Oxcarbazepine were procured from Amoli Organics Pvt. Ltd., Mumbai Maharashtra, India, as a gift sample for research purpose. All solvents including methanol, water were of HPLC grade.

HPLC System

HPLC Binary Gradient System (Model no.: HPLC 3000 Series) of Analytical Technologies Ltd. equipped with the UV-3000-M detector. P-3000-M Reciprocating (40MPa)

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Pump, Grace C18 (250mm x 4.6ID, Particle size: 5 microns) Column were used for analysis of standard and samples. The data were recorded using HPLC Workstation software.

Preparation of mobile phase

Various combinations of mobile phases including methanol, water, and acetonitrile were tried. Finally Methanol and water with 70:30 v/v was optimised and same was used for analysis of standard and sample. Both HPLC grade solvents were mixed with optimised proportion to make 1000 ml volume.

Preparation of standard solutions

The stock solution of carbamazepine, Oxcarbazepine and paracetamol internal standard were prepared in mobile phase separately by dissolving 0.01 gm of both API with 10 ml mobile phase to obtain 1000 µg/ml of stock solution. Both the stock solutions were sonicated for five minutes by using Wenser Ultra Sonicator (Model: WUC-4LCapacity: 4Liter). These stock solutions were further diluted with diluents as to get 10 µg/ml of Paracetamol as an internal standard in each of dilution made for various concentrations of Carbamazepine and Oxcarbazepine.

Chromatographic Conditions

All analysis was done at ambient temperature under isocratic condition. The mobile phase was run at a flow rate of 0.8 ml/min for 8.68 minutes run time. Before analysis, every standard and samples were filtered through 0.2 µm filter tips. The column Eluent was monitored with UV detection at 244nm.

Internal Standard

The internal standard paracetamol (10 ppm) was implemented throughout the quantification of Carbamazepine and Oxcarbazepine quantification.

Method Validation

The method was validated according to ICH guidelines with respect to accuracy, precision, linearity, robustness, ruggedness, system suitability.

Linearity

Linearity was checked on five different concentrations within 10-50 µg/ml and 20-100 µg/ml of nominal standard concentration. The linearity of proposed method was evaluated by using calibration curve to calculate the coefficient of correlation, slope and intercept values.

Accuracy

The accuracy of an analytical method expresses the nearness between the expected value and value found. In the present study, successive analysis (n=3) for three different concentrations of the standard mixture (20, 40, 60 µg/ml and 40, 60, 80 µg/ml) were carried out to determine the accuracy of the proposed method.

Precision

The precision of the assay was assessed with respect to repeatability and reproducibility. The precision of the proposed method was checked by Intra and inter-day repeatability of responses after replicate injections and expressed as %RSD.

Limit of detection (LOD) and Limit of Quantitation (LOQ)

LOD is lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. On the other hand, LOQ is the lowest amount of analyte in a sample that may be determined with acceptable accuracy and precision.

Robustness

Robustness is an indication of the reliability of the analytical method during normal usage. The effect of the deliberate change in chromatographic conditions was determined. In the proposed method by changing wavelength +2 nm the robustness of the method was determined.

Recovery

The recovery of the proposed method was obtained by 50,100,150 % level of standard by standard addition method.

System Suitability parameter

The purpose of system suitability test is to ensure that the complete testing system, including instruments, reagents, columns, analysts etc., is adequate for the intended analysis. The parameters like theoretical plate count, tailing factors, resolution and reproducibility are usually determined.

Forced degradation

Forced degradation studies were undertaken to degrade the active drug deliberately. These studies are used to evaluate analytical method's ability to measure an active ingredient and its degradation products without interference. Samples were exposed to acid, base, an oxidizing agent, light and thermal degradation. The degraded samples were then analyzed using the method to determine if there were interferences with the active ingredients.

RESULTS AND DISCUSSION

Table 1: Linearity parameters

Parameter	CAR	OXC
Linear Equation	y= 58537x-92979	y=50316x-1056
Correlation coefficient	R^2 =0.9973	R^2=0.9997
Linearity Range	10-50 µg/ml	20-100 µg/ml
LOD	0.820 µg/ml	0.00010 µg/ml
LOQ	2.48 µg/ml	0.00031 µg/ml

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Linearity, accuracy, Precision

The linearity of the standard mixture was examined on 10-50 µg/ml of nominal concentration. According to USP, the correlation coefficient (R^2) for a calibration curve must be >0.995.The correlation coefficients were found to be more than 0.995 for Carbamazepine and Oxcarbazepine indicating good linearity of the calibration curve. The linearity curves are shown in figure 2. The % RSD of accuracy and precision showed that proposed method provides acceptable accuracy, intra-and inter-day variations for Carbamazepine and Oxcarbazepine estimations. The results of linearity, accuracy and precision are summarized in table no.1, 2. The %RSD for all results was within the limit ≤2. The Paracetamol with concentration of 10µg/ml was used as an internal standard at each parameter.

Table 2: Accuracy and Precision

Accuracy (20,40,60µg/ml for CAR and 40,80,120 µg/ml)		**Precision** Inter-day(day 1,day 2) intra-day(morning, evening) for 30,60 µg/ml
	%RSD	**%RSD**
CAR	1.04	1.24
	0.18	
	0.18	1.01
OXC	0.22	1.01
	0.16	
	0.66	0.19

Table 3: Robustness and Recovery

Robustness		**Recovery**		
CAR	**%RSD**		**CAR**	**OXC**
	0.30	50%	99.65	100.27
OXC	0.34	100%	98.66	99.38
		150%	99.07	100.00

Table 4: System suitability parameter and stress degradation study

Parameter	**CAR**		**PARA**	**OXC**	**PARA**
% RSD t_R	0.693		0.47	0.26	0.15
%RSD of area	0.18		0.72	0.16	0.21
Peak asymmetr	1.12		1.09	1.10	1.07
Resolution	4.80		--	5.23	--
Theoretical Plate	5483		4084	7622	6884
Drug	**% Degradation against freshly prepared standard**				
CAR	Acid	Base	Oxidation	Photolytic	Thermal
	23.53	46.40	3.9	3.93	8.36
OXC	81.56	53.3	85.95	0.65	0.88

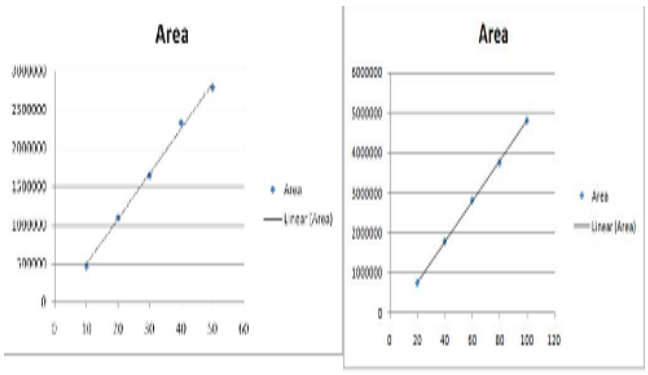

Figure 2: Linearity of CAR (10-50 µg/ml) and Linearity of CAR (20-120 µg/ml)

Robustness

Predetermined variation in wavelength was performed to access their robustness. The result of robustness was summarized in table no.3.

Recovery

For Carbamazepine, percent recoveries were found to be 99.65, 98.66, and 99.07%. For Oxcarbazepine, percent recoveries were found to be 100.27, 99.38, and 100.00%. The results are shown in table no.3.All results are within the acceptable limits for recovery.

System suitability Parameters

All system suitability parameters including peak area,

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theoretical plate, resolution, retention time, peak asymmetry met the compendium acceptance limits. Results are summarized in table 4.

Forced Degradation

Standard drugs were exposed to acid 0.1N HCL 0.1N NaOH at 60°C for 30min., an oxidizing agent (3% H_2O_2), at 24 hrs. Also photolytic degradation study for 24 hrs. and thermal degradation at 600° C for 24 hrs was carried out. The Results of degradation are summarized in table no.4. From the results it was found that carbamazepine was degraded strongly in acid and basic conditions. While it remains stable with oxidizing photolytic and thermal degradation. Forced degradation study will definitely helpful for stability-indication of both drugs. The Chromatograms of forced degradation study are shown in fig. 3 and 4.

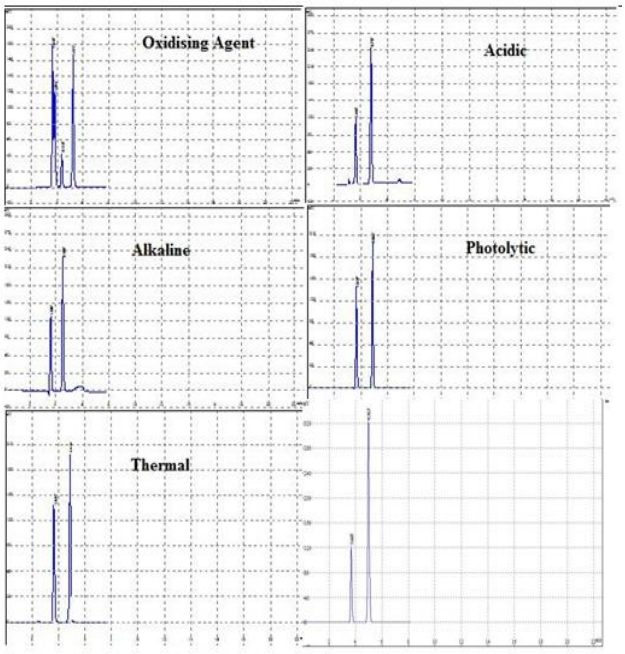

Figure 3: Chromatograms of Degradation study of CAR+PARA (1-5) and Plane CAR+PARA (6)

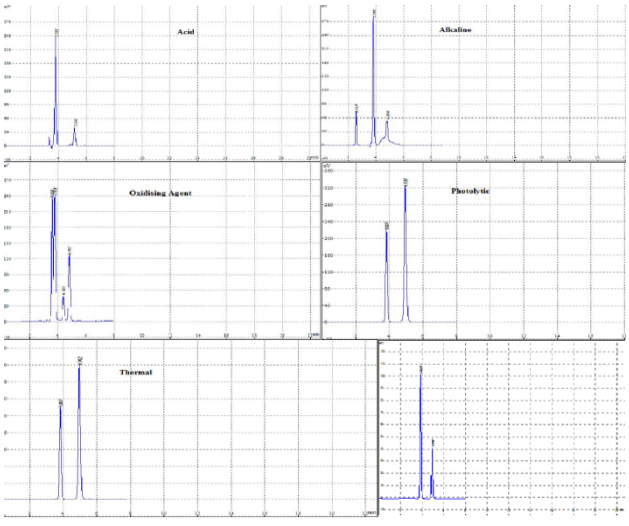

Figure 4: Chromatograms of Degradation study of OXC+PARA (1-5) and plane OXC+PARA (6)

CONCLUSION

The new, simple, economical and robust method was developed for quantification of Carbamazepine and Oxcarbazepine by using Paracetamol as an internal standard. The method was validated strictly according to guidelines of ICH, and FDA. The method was primarily designated for assay of Carbamazepine and Oxcarbazepine in tablet dosage form. Moreover, the content of degradation of Carbamazepine and Oxcarbazepine in various conditions such as acidic, alkaline, oxidation, photolytic, thermal were observed and quantitatively analyzed by this HPLC method. The method is found to be simple and economical for quantification of Carbamazepine and Oxcarbazepine from the bulk drug as well as formulation.

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Abbreviations

CAR-Carbamazepine

OXC-Oxcarbazepine

PARA-Paracetamol

ICH-International council for Harmonisation

FDA-Food and Drug Administration

API-Active pharmaceutical Ingredients

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