ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CARBAMAZEPINE BY HPLC IN REVERSE PHASE METHOD

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ABSTRACT

*Carbamazepine (CBZ), which was developed as an anticonvulsant drug, is also used as an anti-depressive, anti-analgesic, antiepileptic and antimanic drug. Carbamazepine method was developed and validated by RP-HPLC instrument. All parameters were determined with respect to ICH guidelines. Carbamazepine method developed and validated under isocratic conditions by using mobile phase consist of methanol and water ratio 80:20 v/v by using different columns. The assay was performed using Cogent C18 AR (150*4.6, 3µm, 100 A°) Pot no. 68318-15P, no peak interference was observed with run time of 10.00 min by 0.5 ml/min flow rate. System suitability reported as retention time is 3.64 min and peak area of 23,87,313.8 and linearity was obeyed in the range of 0.5 – 40 µg/1ml. The calibration curve was found to be linear with the equation y = 235438X, with correlation coefficient of 0.998098 (r) and Regression coefficient (R^2) of 0.9962 over a concentration range of 0.5-40 µg/ml. And limit of detection is 0.0852 µg/ml, limit of quantification is 0.0258 µg under 0.5 ml/min flow condition.*

Keywords: Carbamazepine, Isocratic elution, Development and Validation by RP-HPLC.

INTRODUCTION:

Carbamazepine belongs to "Anticonvulsant (prevent or reduce the severity of epileptic fits or others) family and medication works on Central Nervous System (CNS). Carbamazepine was discovered by the "Swiss Chemist Walter Schindler" in 1953 and release in market in 1962. It was sold under the brand "Tegretol".[1]

Chemically Carbamazepine is 5-H-dibense(b,f) azepine-5-carboxamide. It is a tricyclic lipophilic compound used to treatment of simple and complex partial seizures. And also used an antiepileptic, anti-depressive, anti-analgesic and anti-manic drug. It is almost completely metabolized in the body and only small traces are excreted unchanged in urine.[1][2]

After medication some side effects happen in body, increase the risk of suicide and effect on body's production of Red Blood Cells, White Blood Cells and Platelet counts but these don't progress more serios problems. Common adverse effects headache, migraine, nausea and dizziness and alcohol use while taking

carbamazepine to enhanced depression of the central Nervous System (CNS).[1][3]

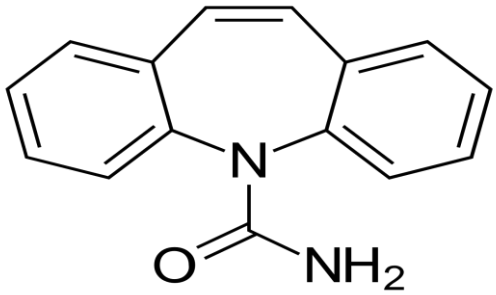


Figure 1. Chemical Structure of Carbamazepine

Instrument Details:

HPLC – Waters Model No.2695/5 compact system, Ultrasonic bath, PH Meter, Vacuum Pump and Column Washing Pump.

Chemicals:

Rankem water HPLC grade, Methanol, Ammonium Format (20 mM) maintain PH 3.0 with Ortho Phosphoric Acid, Formic Acid, Acetonitrile.

METHOD DEVELOPMENT IN HPLC

Method development is the process for new products there is no known methods are present. It is help reduce cost and time for better results like precision and ruggedness. Trial runs under different conditions, method is optimized and validated.

Steps involved in method development:

It is carried out through system; the whole process documented studies must be established in laboratory notebook or any electrical devices.

1. Analyte standard characterization:

- It is starts with collection of standards (100% pure) analyte.
- All information like physical and chemical properties must be studied
- Only those sample studied under HPLC is considered

2. Method requirements:

The analytical method consists goals or requirements to developed analytical figures are defined. In this include selectivity, linearity, range, accuracy, detection limits and precision are defined.

3. Literature search and prior methodology:

All types of information related to analyte is surveyed in literature search. They are analytical profiled (physico-chemical properties, Eg: pKa, melting point, degradation pathways, etc), solubility profile (solubility of drug in different solvents ad different pH conditions) and stability profile (heat, light, moisture etc) and different analytical methods, chemical manufactures and regulatory affairs such as NF/USP are checked.[5]

4. Choosing method:

a) The method is chosen based on the literature search and method is adopted wherever necessary. In this development process in case any changes occur it will be adjusted.

b) There is usually one compound for which analytical method already exist that is similar to the analyte of interest.[4][5]

5. Instrumental setup and initial studies:

In this setup standard operating procedures (SOP's), installation, operational and performance qualification are verified.[4]

6. Optimization:

It is technique used to reduce cost and time based on the requirements for HPLC analysis.

- Selection of column
- Selection of mobile phase
- Selection of detector
- Selection of method

7. Documentation of analytical figures of merit:

In this documentation Limit of Detection (LOD), Limit of Quantification (LOQ), linearity, cost, sample preparation, ruggedness, system precision and time per analysis are recorded.

8. Evaluation of method development with actual samples:

Analytical sample should give chromatogram containing the absolute identification with respect to analyte and not respond to other components in sample.

METHOD DEVELOPMENT OF CARBAMAZEPINE BY RP-HPLC

Method development was done by the changing mobile phase ratios, Diluents, columns and flow rate. So here the trials mentioned how the development was done.

TRIAL 1: Test for 1 milligram/1ml concentration of Carbamazepine

Preparation of Analytical Solutions:

Preparation of Mobile Phase: Weighing 0.15 grams of ammonium acetate and transferred into 100 mL beaker makeup with water, stirred and sonicate it 5 minutes and stored.

Preparation of Diluent:

Take 80:20 v/v ratio of methanol and water transferred into 100 mL beaker, stirred well and sonicate it 5 minutes and stored.

Chromatographic Conditions:

Mobile Phase : Ammonium Acetate(20 mM)

Diluent : Methanol: Water(80:20 v/v)

Column : Cogent C8 ((150 * 4.6)mm, 3µm, 100 A°)

Flow Rate : 1 ml/min

Column Temperature : Ambient

Injection Volume : 30 µL

Detector Wavelength : 240 nm

Pump Mode : Isocratic

Run Time : 05.00 min

Observation: In this trial Retention Time is void volume escaped and Plate count is less (2682.3). So, further trail was carried out.

Trail 2: Test for1 milligram/1ml of Carbamazepine

Preparation of Analytical solution:

Preparation of Mobile Phase:

Weigh the 0.46 grams of formic acid taken in a beaker makeup with water stirred well and sonicate it for 5 minutes until get clear solution.

Preparation of Diluent:

Take 70:30 (v/v) ratio of acetonitrile and water transferred into 100 mL beaker, stirred well and sonicate it for 5 minutes.

Chromatographic Conditions:

Mobile Phase : Formic Acid (0.1N)

Diluent : Acetonitrile: Water(70:30 v/v)

Column : Cogent C8 ((150 *4.6)mm, 3µm, 100 A°)

Flow Rate : 1 ml/min

Column Temperature : Ambient

Injection Volume : 30 µL

Detector Wavelength : 240 nm

Pump Mode : Isocratic

Run Time : 05.00 min

Observation: Some Peak Tailing was happened and to reduce cost of mobile phase further trail was chosen.

Trail 3: Test for 10 microgram/1ml of Carbamazepine

Chromatographic Conditions:

Mobile Phase : Methanol: Formic Acid(90:10 v/v)

Diluent : Methanol: Water(80:20 v/v)

Column : Cogent C18 RP((150*4.6)mm, 3µm, 100 A°)

Flow Rate : 0.5 ml/min

Column Temperature : Ambient

Injection Volume : 30 µL

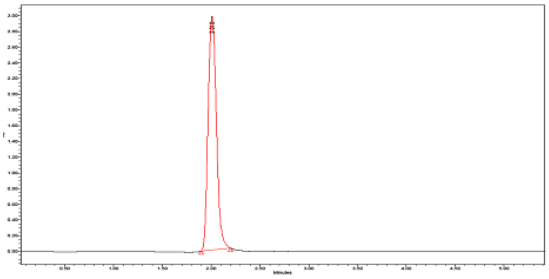
Detector Wavelength : 240 nm

Pump Mode : Isocratic

Run Time : 10.00 min

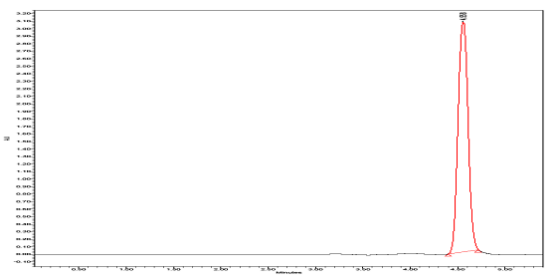
Observation: Carbamazepine peak has good resolution, tailing factor, plate count and eluted at 3.651 min Rt.

Fig 1: Chromatogram of trial 1:



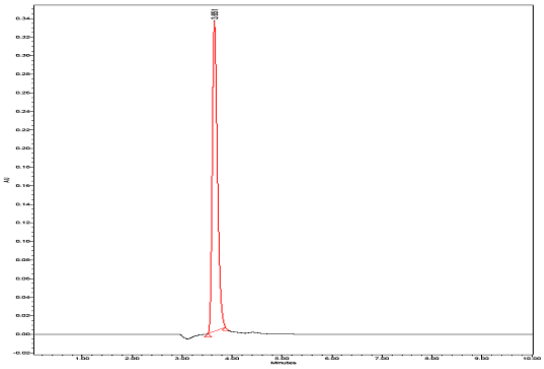
S.No	Compound Name	Column	RT	Area	USP Plate Count	USP Tailing Factor
1	Carbamazepine	Cogent C8(150 * 4.6, 3µm, 100 A°)	2.01	17514388	2682.3	1.15

Fig 2: Trial 2 of Carbamazepine



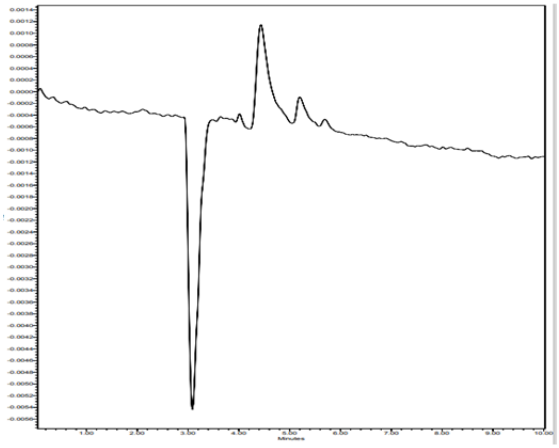
S. No	Compound Name	Column	RT	Area	USP Plate Count	USP Tailing Factor
1	Carbamazepine	Cogent C8 (150 *4.6, 3µm, 100 A°)	4.558	22285207	9348	1.02

Fig 3: Trial 3 of Carbamazepine



S. No.	Compound Name	Column	RT	Area	USP Plate Count	USP Tailing Factor
1	Carbamazepine	Cogent C18 AR (150 *4.6, 3µm, 100 A°)	3.651	2349828	62221.4	1.26

Blank:



METHOD VALIDATION OF CARBAMAZEPINE BY RP-HPLC

Method validation is a process to confirm the analytical procedure by doing various parameters based on USP (United States Pharmacopeial Convention) and ICH (International Conference on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) guidelines Q2 (R1), 2005.

METHOD VALIDATION PARAMETERS:

- **System Suitability**
- **System precision**
- **Linearity**
- **Range**
- **Robustness**
- **Limit of Detection (LOD)**
- **Limit of Quantification (LOQ)**

System Suitability:

System suitability are used to verify that the resolution and precision of the system are adequate for the analysis. System suitability test check for adequate performance before or during sample analysis, such as Plate Number(N), Tailing Factor (TF) and Retention Time based on ICH guidelines.

S. No.	Peak Area	Retention Time	Theoretical Plate Count	Tailing Factor
1.	Blank	-	-	-
2.	2405364	3.64	5957	1.31
3.	2388698	3.64	5903	1.29
4.	2366641	3.64	5929	1.30
5.	2389438	3.64	6298	1.30
6.	2376941	3.64	5904	1.28
7.	2396801	3.65	6157	1.28
Mean	2387313.8	3.641667	6024.667	1.293333
SD	13836.217	0.004082	164.5098	0.012111
%RSD	0.5795726	0.112105	3662.194	10679.35

Acceptance Criteria:

- The % RSD (Relative Standard Deviation) for peak area of carbamazepine is not more than 2%
- The % RSD for retention time of carbamazepine is not more than 0.5
- The Theoretical Plate Count of Carbamazepine must above 2000
- The Tailing Factor of Carbamazepine must be less than 2

Linearity:

The Linearity of analytical method was performed over the range of 0.5% to 40% of target standard concentration. It evaluates the analytical procedures and has the ability to obtain a response should be directly proportional to concentration of analyte in given sample.

Linearity generally is reported as the variance of the slope of the regression line. In order to evaluate the linearity in HPLC analysis first we take standard solutions containing different concentrations of analyte. Linearity explains about correlation coefficient®, regression coefficient, slope, intercept %Y-intercept at 100% bias and residual sum of squares.[7]

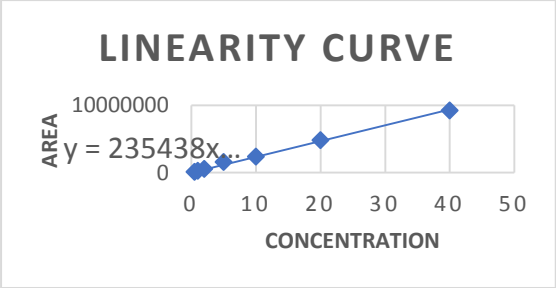
Preparation of Standard Linearity Solutions:

First weigh the 5 mg of carbamazepine compound and transferred into 10ml volumetric flask and make up with methanol and water (80:20 v/v), it gives the concentration of 1 mg/mL standard solution of analyte. From the solution take 1ml of standard solution and transfer into 10 ml of volumetric flask and make with methanol and water same ratio, it will give you 100 µg/mL solution. From 100 µg/1ml take 0.5, 1, 2, 5 ml and from the 1mg/mL solution take 0.1, 0.2, 0.4 ml transferred into various 10ml volumetric flask and make up with methanol and water, finally 0.5, 1, 2, 5, 10, 20, 40 µg linearity standard solution prepared.

S. No.	Compound Name	Sample Concentration	Level (%)	RT	Area
1.	Carbamazepine	0.5 µg	0.5%	3.65	142526
2.	Carbamazepine	1 µg	1%	3.65	301082
3.	Carbamazepine	2 µg	2%	3.64	580321
4.	Carbamazepine	5 µg	5%	3.65	1730443
5.	Carbamazepine	10 µg	10%	3.65	2404797
6.	Carbamazepine	20 µg	20%	3.65	4853535
7.	Carbamazepine	40 µg	40%	3.65	8470944

Correlation coefficient(r)	0.998098
Regression coefficient(R^2)	0.9962
Slope	229321.1
Intercept	165991.5
%Y-intercept at 100%	
Residual sum of squares	

Linearity Plot of Concentration in µg against Area of Carbamazepine:



Acceptance Criteria:

The correlation coefficient(r) should be not less than 0.99.

Number of Injections (Carbamazepine)	Peak Area
1	2405364
2	2388698
3	2366641
4	2389438
5	2376941
6	2396801
Average	2387313.8
Standard Deviation (SD)	13836.217
Relative Standard Deviation (RSD)	0.5795726

Conclusion:

Based on the above data and experimental results method is linear.

System Precision:

System Precision nothing but closeness of agreement among individual test results from repeated analysis of a homogenous sample. It is calculated by injection of standard solution many times and calculates the RSD (Relative Standard Deviation) of the peak area.[6]

Acceptance Criteria:

The % of RSD peak area of carbamazepine is not more than 2.

LIMIT OF DETECTION(LOD) AND LIMIT OF QUANTIFICATION(LOQ)

LOD: It is defined as lowest concentration of analyte (standard solution) that can be detected, but can't be quantified. It is the parameter of limit test.

LOD = 3.3*SD/Slope

Slope = from the calibration curve

SD = Standard Deviation

LOQ: It is defined as lowest concentration of an analyte in a sample that can be quantified with acceptable precision and accuracy by the test method.

LOD = 10*SD/Slope

Slope = from the calibration curve

SD = Standard Deviation

Detection of LOQ is two-step process, the limits should first be estimated from experimental data such as "Signal to Noise Ratio or slope of a calibration curve, latter value must be confirmed by results.

Parameter	RT	Linearity	Value
LOD	3.65	0.5-40	0.0852 µg/ml
LOQ	3.65	0.5-40	0.0258 µg

Robustness:

It is measure of capacity to obtain comparable and acceptable results when perturbed by small but deliberate variations. Robustness provides an indication of the test method's suitability and reliability during normal use.

Flow rate variation(-0.2ml) decreasing:

Parameter	Modified	Modified %RSD	Actual	Actual %RSD
Flow	0.3 ml/min	-	0.5 ml/min	-
RT	4.1 min	0.09	3.64 min	0.11
Tailing Factor	1.31	9856.33	1.29	10697.35

Conclusion:

This HPLC method is rapid, sensitive, accurate, precise and robust. In this validation process system suitability, linearity, system precision, limit of detection and limit of quantification was done based on the ICH guidelines Q2 (R1), 2005. The Carbamazepine method development and validation suitable for routine analysis.

Reference:

1. *Carbamazepine drug information Portal. U.S. National Library of Medicine.*
2. *Bradley MK, Rene H, Levy RR, Matson R, Meldrum B, Penry JK, Dreifuss FE (Eds.) Carbamazepine and antiepileptic drugs.*
3. *Franceschi L., Furlanut M. A simple method to monitor plasma concentration of oxcarbamazepine, carbamazepine, their main metabolites and lamotrignine in epileptic patients. Pharmacol. Res. 205; 51:297-302.*
4. *B.K Sharma, Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23rd Edition Goel publication, Meerut, (2007).*
5. *ICH Guideline Q2 (R1), 2005. Validation of analytical procedures: Text and methodology.*
6. *https://pubchem.ncbi.nlm.nih.gov/compound/Dasiglucagon.*
7. *Huang C, He Q, Chen H Flow injection photochemical spectrofluorimetry for the determination of carbamazepinein pharmaceutical prepartions, J Pharm Biomed Anal, 22 59-65, 2002.*
8. *Validation of Analytical procedures, proceedings of the International Conference on Hormonisation (ICH), Commission of the European Communities, 1996.*
9. *Walker ES Liquid chromatography determination of carbamazepine in tablets, J. Assoc Off Anal Chem., 71 523-525,1988.*
10. *Bertilsson, L. (1978). Clinical pharmacokinetics of carbamazepine. Clinical Pharmacokinetics, 3, 128-143.*
11. *Alrashood, S. T. (2016). Carbamazepine. Profiles of drug substances, excipients and related methodology, 41, 133-321.*
12. *CRILL, W. E. (1973). Carbamazepine. Annals of Internal Medicine, 79(6), 844-847.*
13. *Lertratanangkoon, K., & Horning, M. G. (1982). Metabolism of carbamazepine. Drug Metabolism and Disposition, 10(1), 1-10.*
14. *Lang, M., Kampf, J. W., & Matzger, A. J. (2002). Form IV of carbamazepine. Journal of pharmaceutical sciences, 91(4), 1186-1190.*
15. *Levy, R. H., Pitlick, W. H., Troupin, A. S., Green, J. R., & Neal, J. M. (1975). Pharmacokinetics of carbamazepine in normal man. Clinical Pharmacology & Therapeutics, 17(6), 657-668.*
16. *Ballenger, J. C., & Post, R. M. (1980). Carbamazepine in manic-depressive illness: a new treatment. The American journal of psychiatry, 137(7), 782-790.*
17. *Post, R. M., Uhde, T. W., Roy-Byrne, P. P., & Joffe, R. T. (1986). Antidepressant effects of carbamazepine. The American journal of psychiatry, 143(1), 29-34.*
18. *Clara, M., Strenn, B., & Kreuzinger, N. (2004). Carbamazepine as a possible anthropogenic marker in the aquatic environment: investigations on the behaviour of carbamazepine in wastewater treatment and during groundwater infiltration. Water research, 38(4), 947-954.*
19. *Meinders, A. E., Cejka, V., & Robertson, G. L. (1974). The antidiuretic action of carbamazepine in man. Clinical science and molecular medicine, 47(4), 289-299.*
20. *Post, R. M., Uhde, T. W., Roy-Byrne, P. P., & Joffe, R. T. (1987). Correlates of antimanic response to carbamazepine. Psychiatry Research, 21(1), 71-83.*