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Research

Analytical Method Validation for the Determination of Assay of Carbamazepine API by UPLC

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Abstract

A simple, rapid, reliable and precise reversed phase UPLC method has been developed and validated according to the regulatory guidelines for determination of carbamazepine API in bulk, which composed of isocratic mobile phase; Solution-A: 0.5mL of Triethyl amine and 0.5mL of Formic acid to 1000mL of water. Solution-B: 0.25mL of Formic acid to 1000mL of Methanol, with a flow rate of 0.3 ml/min, and column Acquity UPLC HSS CYANO 10cm x 2.1 mm, 1.8 µm, packing L10. The detection was carried out at 230 nm. The study showed that the proposed UPLC method can be used for the assessment of drug purity.

UPLC: It opened an innovative direction for liquid chromatography covering three major areas including speed, sensitivity and resolution of evaluation by means of the use of packing material with particles size less than 2 µm. The device is created to handle very high pressure experienced by the column.

Keywords: Validation, Carbamazepine API, UPLC, Assay

INTRODUCTION:

Instrument of UPLC:

Ultra performance liquid chromatography instrumentation is basically similar to that of HPLC. It is designed to work under much higher pressure without disturbance and increased maintenance.For UPLC detection, new electronics and firmware are used to support the UV/Visible detector at the high data rates. The UV/VIS detector comprises a 10 mm flow cell path length with a volume of only half a litre.

The instrumentation of UPLC includes:Sample injection, UPLC columns, Detectors

Sample injection:

The injector is used to add a small amount of solution containing the sample in the mobile phase that is precisely measured. The injection must be done consistently and precisely. Conventional injection valves can be manual or programmed, and the injection procedure must be somewhat pulse-free to protect the column from excessive pressure instabilities. To decrease the risk of band spreading, the device's swept volume should be kept to a minimum. To effectively benefit from the speed of UPLC, a short injection cycle time is required. Low volume injections with minimum carry over are required to increase sensitivity. In UPLC, the sample volume is usually 2-5 µl. For biological samples, direct injection techniques are now commonly used. Flow chart of UPLC shown below (Figure 3).

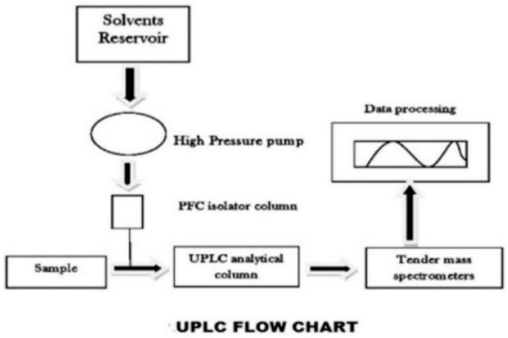


Figure 3: UPLC Flow Chart

Validation Parameters:

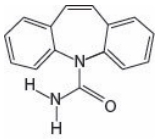
The system validation comprises all the procedures needed to prove the reliability for the intended application of a particular method for the quantitative determination of the analyte (or the sequence of analytes) concentration in a specific biological matrix. The method efficiency and reliability of the analytical results must be demonstrated by validation.

Applications of UPLC:

Natural product and herbal medicine, UPLC has the ability to provide high quality of separation and detection capability of active compound which is present in mixture[27].

DRUG PROFILE:

Carbamazepine: Molecular Formula:$C_{15}H_{12}N_2O$, **Molecular weight:** 236.27, **Solubility**-Insoluble in Water,



MATERIALS & METHOD:

Details of Instruments, Column, Chemicals, standards and Reagents, Instruments: UPLC system equipped with a UV detector / PDA detector Analytical balance

Column:

UPLC HSS CYANO 10cm x 2.1 mm, 1.8 µm, packing L10.Chemicals, Standards and Reagents Milli-Q-water or Higher grade Methanol, Triethyl amine, Formic acid, Carbamazepine RS, Carbamazepine related compound A, Carbamazepine related compound B

Description of Analytical Method (Methodology):

Method reference: As per USP-38

Procedure:

Solution-A: Add 0.5mL of Triethyl amine and 0.5mL of Formic acid to 1000mL of water.
Solution-B: Add 0.25mL of Formic acid to 1000mL of Methanol.

Table 1: Mobile phase Gradient Programme:

Time (min)	Solution A (%)	Solution B (%)
0.0	80	20
3.0	80	20
12.0	60	40
18.0	45	55
20.0	45	55
20.1	80	20
23.0	80	20

Diluent/Blank:

Methanol and water (50:50)

System suitability stock solution:

0.02mg/mL each of USP Carbamazepine RS and USP Carbamazepine related compound A RS prepared as follows. First dissolve the reference standard in 50% of the final flask volume of methanol, then dilute with water to volume.

System suitability solution:

0.002mg/mL each of USP Carbamazepine RS and USP Carbamazepine related compound A RS from system suitability stock solution in diluent.

Standard solution:

0.1 mg/mL of USP Carbamazepine RS prepared as follows. First dissolve the reference standard in 50% of the final flask volume of methanol, then dilute with water to volume.

Sample solution:

0.1 mg/mL of Carbamazepine prepared as follows. First dissolve the sample in 50% of the final flask volume of methanol, then dilute with water to volume. Pass through a suitable filter of 0.2µm pore size.

Chromatographic system:

UPLC Column	**2.1 mm X 10 cm; 1.8-µm packing L10**
Detector wave length	230 nm
Column Temperature	40°C
Flow rate	0.3 mL/min
Injection volume	2 µL
Run Time	23.0 min

Inject blank, System suitability solution and standard solution into the UPLC system and record the responses.

Validation Plan

Following parameters shall be verified.

S.No	**Verification Parameters**
1	System Suitability
2	Specificity
3	Precision i) System precision ii) Method precision iii) Intermediate precision
4	Linearity
5	Stability of Analytical solution

Note: More than one parameter can be performed at once with relevant sequence having common system suitability with bracketing preparations.

Analytical Method Validation:

System Suitability: To evaluate the system suitability, inject Blank, System suitability solution and five replicate injections of standard solution. Record resolution from system suitability solution, tailing factor from standard solution and calculate the % RSD from five replicate injections of standard solution.

Note: For preparation of blank, system suitability solution and standard solution; refer section Number: 5.0

First dissolve the reference standard in 50% of the final flask volume of methanol, then dilute with water to volume.

Preparation of Carbamazepine related compound B RSstandardsolution: 0.001mg/mL of USP Carbamazepine related compound B RS prepared as follows. First dissolve the reference standard in 50% of the final flask volume of methanol, then dilute with water to volume.

Preparation of Spiked Sample solution: 0.1 mg/mL of Carbamazepine, 0.001mg/mL of USP Carbamazepine related compound A RS and 0.001mg/mL of USP Carbamazepine related compound B RS prepared as follows. First dissolve the sample in 50% of the final flask volume of methanol, then dilute with water to volume. Pass through a suitable filter of 0.2µm pore size.

Procedure: Inject the solutions into the UPLC system as per the below mentioned sequence. Record the chromatogram and measure the area/response for all peaks.

System precision: The purpose of this study is to establish the precision of the instrument being used for the analysis or to check the ability of a measurement to be consistently reproduced by the instrument.

Note: For preparation of blank, system suitability solution and standard solution; refer section Number: 5.0

Table 4: Injection sequence:

S. No.	Name of the Solution	No. of Injections
1	Blank	1
2	System suitability solution	1
3	Standard solution	6

Acceptance criteria:

- The Resolution should be NLT 1.7 between Carbamazepine related compound A peak and Carbamazepine peak from the system suitability solution.
- The Tailing factor should be NMT 2.0 for Carbamazepine peak from the standard solution.
- The %RSD should be NMT 0.73% for Carbamazepine peak area from the replicate six standard injections.

Method Precision:

The precision is the degree of agreement among individual sample results when the procedure applied repeatedly to multiple sample portions of a homogeneous sample.

Note: For preparation of blank**, system suitability solution, standard solution andsample solution; refer section Number: 5.0**

Linearity:

To demonstrate the linearity of analytical method from 50 % to 150% of specification level concentration. A series of solutions shall be prepared at different concentrations from 50 % to 150 % of test concentration for Assay.

Note: For preparation of blank, system suitability solution and standard solution; refer section Number: 5.0

Linearity stock solution

Weigh and transfer about 100 mg of Carbamazepine reference standard into a 100 mL volumetric flask. Dissolve it in 50 mL of methanol and dilute to volume with water.

Stability of Analytical Solutions

Establish the stability of standard and sample solutions at room temperature (RT) and refrigerator conditions (2°C – 8°C) for two days.

Note: For preparation of blank, system suitability solution, standard solution and Sample solution; refer section Number: 5.0

Validation Results:System Suitability:As per methodology, injected blank and standard solutions five times intoUPLC system.

Results

Table 5: System suitability

System Suitability Parameters	Observed Value	Acceptance Criteria
% RSD for Carbamazepine peak from five replicate injections of standard solution.	0.18	NMT 0.73
Tailing factor for Carbamazepine peak in the first injection of standard solution.	1.3	NMT 2.0
The Resolution between Carbamazepine related compound A and Carbamazepine from the system	2.0	NLT 1.7

suitability solution should be not less than 1.7.

CONCLUSION

The above results reveal that the system meets the required system suitability criteria.

Specificity:

As per methodology, injected blank, System suitability solution, standard solution, Carbamazepine related compound A standard solution, Carbamazepine related compound B standard solution, sample solution and spiked solution and checked the peak interference of blank, Carbamazepine related compound A and Carbamazepine related compound B standard solution should not show any peak at the retention time of Carbamazepine. Prepared and injected each impurity at 1 % level individually and checked the interference at each impurity retention time.

Results

Table 6: System suitability

System Suitability Parameters	Observed Value	Acceptance Criteria
% RSD for Carbamazepine peakfrom five replicate injections of standard solution.	0.23	NMT 0.73
Tailing factor for Carbamazepine peak in the first injection of standard solution.	1.1	NMT 2.0
The Resolution between Carbamazepine related compound A and Carbamazepine from the system suitability solution should be not less than 1.7.	2.0	NLT 1.7

Table 7: Blank &Impurities Interference Data

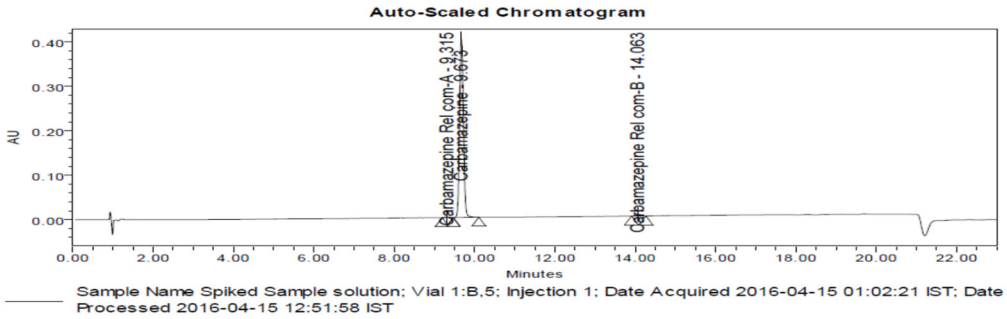
S.No	Name	Interference Due to Blank and Impurities (Yes/No)
1	Blank	No
2	Carbamazepine related compound A	No
3	Carbamazepine related compound B	No

Table 8:Retention time and peak purity of Carbamazepine in Sample solution

Peak Name	Retention time	Purity angle	Purity threshold	Peak Purity
Carbamazepine	9.673	0.061	0.373	Pass

Table 9:Retention time and peak purity of known Impurities and Carbamazepine in Spiked sample solution

Peak Name	Retention time	Purity angle	Purity threshold	Peak Purity
Carbamazepine	9.673	0.061	0.373	Pass
Carbamazepine related compound A	9.315	8.511	44.112	Pass
Carbamazepine related compound B	14.063	3.664	4.955	Pass



Precision: System Precision:

Injected six replicate injections of standard solution into UPLC system as per test method and evaluated the system precision and system suitability parameters.

System Suitability Parameters	Observed Value	Acceptance Criteria
% RSD for Carbamazepine peakfrom five replicate injections of standard solution.	0.09	NMT 0.73
Tailing factor for Carbamazepine peak in the first injection of standard solution.	1.3	NMT 2.0
The Resolution between Carbamazepine related compound A and Carbamazepine from the system suitability solution should be not less than 1.7.	2.0	NLT 1.7

Method Precision

Analyzed six test preparations of Carbamazepine as per the methodology and determined the % RSD of six sample preparations for Assay of Carbamazepine.

Results

Table 11: System suitability

System Suitability Parameters	Observed Value	Acceptance Criteria
% RSD for Carbamazepine peakfrom five replicate injections of standard solution.	0.18	NMT 0.73
Tailing factor for Carbamazepine peak in the first injection of standard solution.	1.3	NMT 2.0
The Resolution between Carbamazepine related compound A and Carbamazepine from the system suitability solution should be not less than 1.7.	2.0	NLT 1.7

Table 12: Method precision Results

Sample	% Assay
01	98.4
02	98.8
03	100.1
04	98.8
05	98.2
06	98.6
Average	98.8
S.D	0.6706
%RSD	0.7

Conclusion:

The above results reveal that the method is precise.

Intermediate Precision

Determined the Intermediate precision by preparing six test preparations of Carbamazepine as per the methodology and determined the % RSD of six sample preparations for Assay of Carbamazepine by different analyst on different day by using different system with same column.

Results

Table 13: System suitability

System Suitability Parameters	Observed Value	Acceptance Criteria
% RSD for Carbamazepine peaks from five replicate injections of standard solution.	0.14	NMT 0.73
Tailing factor for Carbamazepine peak in the first injection of standard solution.	1.2	NMT 2.0
The Resolution between Carbamazepine related compound A and carbamazepine from the system suitability solution should be not less than 1.7.	1.9	NLT 1.7

Table 14: Intermediate precision Assay results

Sample	% Assay
01	99.7
02	99.5
03	99.9
04	100.3
05	99.8
06	100.4
Average	99.9
S.D	0.3502
%RSD	0.4

Table 15: Method Precision and Intermediate precision Assay results

Preparation	Analyst –I /System-I	Analyst –II/System-II
1	98.4	99.7
2	98.8	99.5
3	100.1	99.9
4	98.8	100.3
5	98.2	99.8
6	98.6	100.4
Avg	98.8	99.9
SD	0.6706	0.3502
%RSD	0.7	0.4
%RSD (12 Prep)	0.8	

Acceptance criteria:

Overall % RSD for % assay of carbamazepine from twelve preparations of both method precision and intermediate precision solutions should be not more than 5.0

Conclusion: The above results reveal that the method is rugged.

Linearity:

Linearity for Carbamazepine was determined in the concentration range from 50 to 150 % levels of test concentration levels**.**

Results

Table 16: System suitability

System Suitability Parameters	Observed Value	Acceptance Criteria
% RSD for Carbamazepine peak from six replicate injections of standard solution.	0.10	NMT 0.73
Tailing factor for Carbamazepine peak in the first injection of standard solution.	1.3	NMT 2.0
The Resolution between carbamazepine related compound A and carbamazepine from the system suitability solution should be not less than 1.7.	2.0	NLT 1.7

Table 17: Linearity Results of Carbamazepine

Level (%)	Carbamazepine Concentration (in ppm)	Carbamazepine Peak Area
50 %	50.46	1337491
80 %	80.73	2141481
100 %	100.91	2679191
120%	121.09	3215527
150 %	151.37	4006513
Correlation Coefficient	1.000	
Slope	26471.8793	
Y-Intercept	4710.3155	

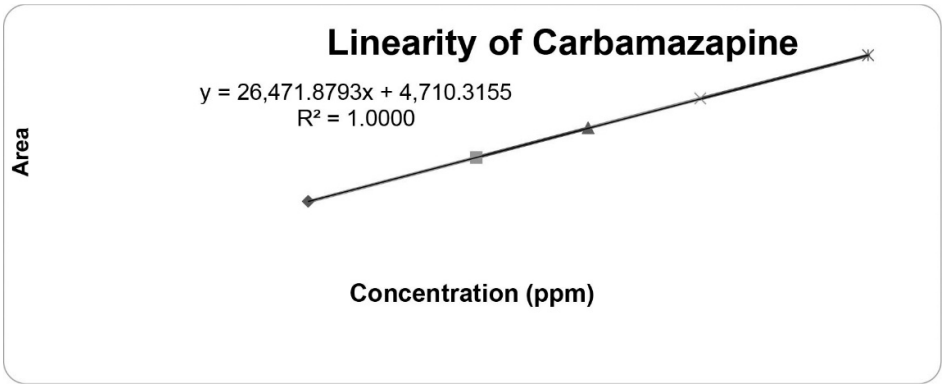


Figure 12: Carbamazepine Linearity graph

Acceptance criteria

- The Resolution should be NLT 1.7 between carbamazepine related compound A and carbamazepine from the system suitability solution.
- The Tailing factor should be NMT 2.0 for Carbamazepine Peak from the standard solution.
- The %RSD should be NMT 0.73% for Carbamazepine Peak from the replicate five standard injections.
- The Correlation coefficient should be not less than 0.99 for Carbamazepine.

Conclusion

The above results reveal that the method is linear over the range from 50 % to 150 % of test concentration level.

Stability of Analytical solution

Stability study of standard solution and sample preparation were performed at two conditions, one is at 2-8 °C, and second one at Room temprature.

Results

Table 18: System suitability

System Suitability Parameters	Observed Value			Acceptance Criteria
	Initial	Day1	Day2	
% RSD for Carbamazepine peak from five replicate injections of Standard solution.	0.14	0.23	0.22	NMT 0.73
Tailing factor for Carbamazepine peak in the first injection of Standard solution.	1.2	1.1	1.1	NMT 2.0
The Resolution between Carbamazepine related compound A and Carbamazepine from the System suitability solution should be not less than 1.7.	1.9	2.0	2.1	NLT 1.7

Table 19: Assay Standard solution stability results (2-8°C and RT)

Parameter		Similarity Factor
Day-1	Standard at 2-8°C	0.99
	Standard at RT	0.99
Day-2	Standard at 2-8°C	0.99
	Standard at RT	0.99

Table 20:Assay Sample solution stability results (2-8°C and RT)

Parameter		% Assay		% Difference from Initial
Day-1	Initial	Sample-1	99.7	NA
		Sample-2	99.5	NA
	Sample at 2-8°C	Sample-1	99.1	0.6
		Sample-2	99.1	0.4
	Sample at RT	Sample-1	99.2	0.5
		Sample-2	99.0	0.5
Day-2	Sample at 2-8°C	Sample-1	98.8	0.9
		Sample-2	98.6	0.9
	Sample at RT	Sample-1	98.8	0.9
		Sample-2	98.6	0.9

Acceptance criteria

The above results reveal that assay standard and sample solutions are stable up to 48 hours at both 2-8 °C and RT.

CONCLUSION:

The present analytical method was validated as per defined protocol and it meets the specified acceptance criteria. Hence, it was concluded that the analytical method is specific, precise, linear, accurate, rugged and robust. The standard and sample solutions were stable up to 48 hours. Hence, the present analytical method has been proved as stability indicating and as the results were within the acceptance criteria. Therefore the method can be used for regular analysis and its intended purpose. The current analytical method was validated according to the protocol, and it passes the acceptance criteria. Thus, it was determined that the analytical approach is particular, precise, linear, accurate, rugged, and robust. As a result, the current analytical approach is suitable for regular analysis and serves its intended function.

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