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Development and validation of RP-HPLC method for the analysis of carbofuran and in its formulations

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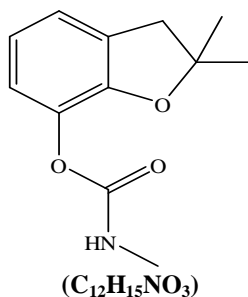
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ABSTRACT

A simple, selective, precise and accurate High Performance liquid Chromatographic method for the analysis of Carbofuran in its formulations was developed and validated in the present study. The mobile phase consist a mixture of Acetonitrile and Potassium Dihydrogen Orthophosphate in the proportion 60:40 and adjust the p^H to 5.8. This was found to give sharp peak of Carbofuran at a retention time of 4.052 min. HPLC analysis of Carbofuran was carried out at a wave length of 282 nm with a flow rate of 1.0 μ L/ min. the linear regression analysis data for the calibration curve showed a good linear relationship with a regression coefficient 0.999 in the concentration range of 7.5 μ g to 75 μ g. The linear regression equation was $y = 131065x + 16835$. The developed method was employed with a high degree of precision and accuracy for the analysis of Carbofuran. The method was validated for accuracy, precision, robustness, Ruggedness, Specificity. The Precision, accuracy, sensitivity, short retention time and composition of the mobile phase indicated that this method is better for the quantification of Carbofuran.

Keywords: Carbofuran, RP-HPLC Method development, Validation.

INTRODUCTION



IUPAC NAME: 2, 3-dihydro-1-benzofuran-7-yl methyl carbamate

Carbofuran is one of the most toxic carbamate pesticides. It is manufactured by the reaction of methyl isocyanate with 2, 3-dihydro-2, 2 - dimethyl-7-hydroxybenzofuran^[1-2]. It is used to control insects in a wide variety of field crops, including potatoes, corn and soybeans^[3-4]. Carbofuran is highly toxic by inhalation and ingestion and moderately toxic by dermal absorption. Carbofuran is soluble in water and has a moderately lengthy soil half-life

(3-60 days) ^[5]. Antonio et al. developed a LC method for analysis of environmental samples ^[6-7]. A. de Kok et al developed a HPLC method with fluorescence detection for analysis of multry residues ^[8]. GC-MS, micellar electro kinetic chromatography, SPE and HPLC-UV techniques were developed for analysis of Carbofuran but they are expens ^[9-12]. Young W Lee et al ^[13] analyzed Carbofuran and other pests in rape plants by using RP-HPLC method but recovered 81.3%. Nelsen et al ^[14] developed a HPLC method for the determination of Carbofuran in soil and water with recovery averaged 82.4% for soil and 82.2% for water and methanol/water used as mobile phase.

The RP-HPLC method described here is simple, sensitive, and reproducible for Carbofuran determination in Formulations with low background interference. An attempt has been made to develop and validate to ensure their accuracy, precision and other analytical method validation parameters as mentioned in various gradients. One method reported for the HPLC determination for developed based on the use of a C-18 column, with a suitable mobile phase, without the use of any internal standard. For pesticide formulation the proposed method is suitable for their analysis with virtually no interference of the usual additives presented in pesticide formulations.

MATERIALS AND METHODS

a. Instruments:

Waters HPLC 2-2695 series consisting pump, Auto sampler, UV-Visible detector, Thermostat column compartment connected with Waters (alliance) Empower software.

b. Methodology:

HPLC method is carried out by using the following conditions.

Chromatographic conditions:

Column	:	C18, 250 mm X 4.6 mm, 5 μ ,
Flow rate	:	1.0 mL /min
Wavelength	:	282nm
Column temperature	:	30°C
Injection volume	:	20 μ L
Run time	:	10 minutes
Diluent	:	Mobile phase
Elution	:	Isocratic
Needle wash	:	Water: Acetonitrile 90:10 (v/v)

c. Preparation of Mobile phase:

The content of the Mobile phase was prepared from filtered and degassed mixture of Phosphate buffer (1.6 gm of potassium hydrogen phosphate in 1 Lt Water and pH was adjusted to 5.8) and Acetonitrile in the ratio of 40:60 v/v.

d. Preparation of diluents (Solvent):

Measured accurately 100 ml of Acetonitrile (HPLC grade) by using volumetric or standard flask was used as a diluent.

e. Preparation of Carbofuran Standard Stock solution:

Weigh and transfer 7.5 mg of CARBOFURAN granules powder into 25 mL volumetric flask add 20 mL of diluent and Sonicate and Further filter the solution through 0.45 μ filter paper and make up with diluent.

f. Preparation of Sample solution:

The sample is extracted after 10 minutes sonication from the fortified soil (The soil is collected from Tirumala hills) for that Acetonitrile is used as a solvent. Dilute the 10 ml of Carbofuran solution to 100 ml and make up to volume with diluent.

g. Procedure:

Inject 20 μ L of blank solution, placebo solution, six times of Standard solution, Disregard peaks due to blank and placebo.

System suitability requirements from SST solution:

- Tailing factor : NMT 2.0
- Theoretical Plates : NLT 2000

h. Precision (Repeatability): Preparation of precision solution:

Dilute the 10 ml of standard stock solution to 100 ml and make-up to volume with diluent. The same procedure is repeated to remaining six preparations. % RSD for the RT and Area are tabulated as below in Table 1.1 and 1.2.

Acceptance criteria: The %RSD of areas from six preparations precision level should not be more than 2.0%.

i. Accuracy:

The accuracy of the test method was demonstrated by preparing recovery samples (i.e. test sample with known quantities of at the level of 50%, 100% and 150 % of target concentration)

The observations of Area are tabulated as below in Table 1.3.

j. Linearity:

The Linearity of detector response for was demonstrated by prepared solutions of over the range of 25 to 150% level of the target. Observations are tabulated in Table 1.4.

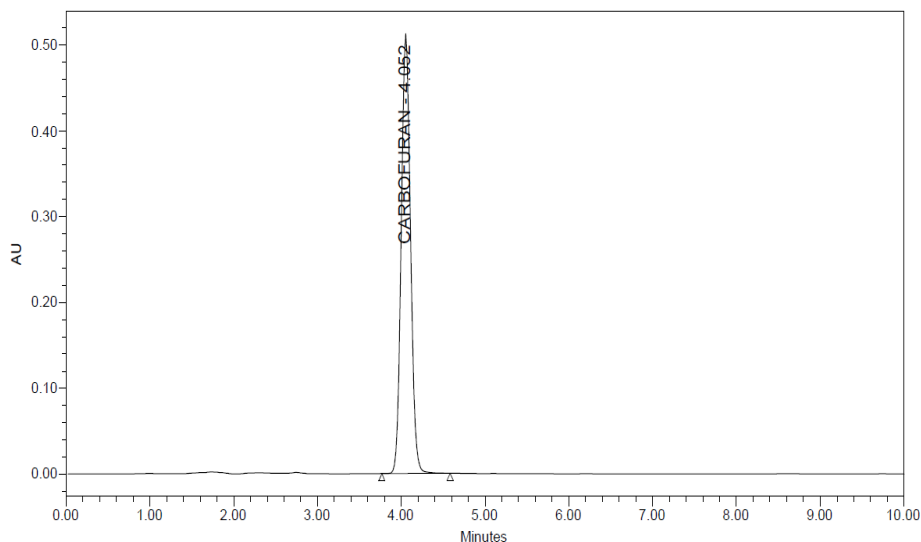


Fig.1.1: Chromatogram of Carbofuran

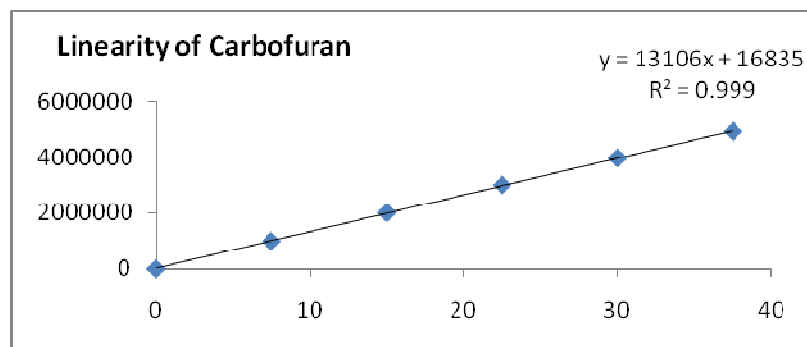


Fig.1.2: Linearity of Carbofuran

$$y = 131065 x 3955352 + 16835 \quad r^2 = 0.9999$$

The correlation coefficient shall not be less than 0.998

k. Assay :

10 ml of standard stock solution dilute to 100 mL volume with diluent.

Repeat the same procedure for remaining three preparations and observations are tabulated in Table 1.5.

l. Ruggedness :

The ruggedness of test method was demonstrated by carrying out precision study in six preparations of sample on a single batch sample by different analysts. The results of the intermediate precision study are tabulated as below in Table 1.6.

m. Robustness :

The robustness of test method was demonstrated by carrying out flow variation $\pm 20\%$, i.e. (0.8ml to 1.2 ml/min), buffer variation $\pm 10\%$ i.e. (35:65 - 45:55) and the results are tabulated as below in Table 1.7.

n. Limit Of Detection (LOD):

0.

$$\text{LOD} = 3 \times \text{STDEV} / \text{SLOPE}$$

7.1751

p. Limit Of Quantization (LOQ) :

$$\text{LOQ} = 10 \times \text{STDEV} / \text{SLOPE}$$

21.7427

p. Specificity studies:

The sample was analyzed in Specific conditions i.e. in 0.1M HCl Acidic Medium, 0.1M NaOH Basic Medium and Heat variation, and UV -light range Variation. Then RT and Area tabulated below in Table: 1.8.

Table 1.1 System precision

S. No.	RT	Area
1	4.008	3978154
2	4.01	3965428
3	4.008	3941256
4	4.009	3982153
5	4.01	3961124
6	4.01	3905465
Avg.	4.009167	3955597
St. dev.	0.000983	28496.03
%RSD	0.02	0.72

Table 1.2 Method Precision

S. No.	RT	Area
1	4.052	3961576
2	4.051	3952475
3	4.051	3924576
4	4.052	3985287
5	4.051	3906495
6	4.053	3942485
Avg.	4.051667	3945482
St dev.	0.000816	27779.08
%RSD	0.02	0.70

Table 1.3 Accuracy Study

Accuracy	Std.	50%Spike	100%Spike	150%Spike
Trial-1	3939073	1942146	3922352	5876481
Trial-2	3940245	1961545	3912147	5824584
Trial-3	3940157	1951104	3954624	5869855
Avg.	3939825	1951598	3929708	5856973
amt. Recovered	100	49.53515	99.7432	148.6607
% of Recovery	100	99.07031	99.7432	99.10717

Table 1.4 Linearity Study (Preparation at 25% to 150% Level)

S. No:	Linear solutions (%)	Stock solution taken in (mL)	Diluted to volume (mL) with diluent	Area
1	25%	2.5	100	988622
2	50%	5	100	2005686
3	75%	7.5	100	2992671
4	100%	10	100	3955352
5	125%	12.5	100	4903467
6	150%	15	100	5876483

Table 1.5 Assay Study of Carbofuran (3%)

Std-1	3942548
Std-2	3941989
Std-3	3942245
Average weight.	3942261
Spl-1	3943102
Spl-2	3942261
Average	3942682
LC	30mg
Standard weight	7.5
Sample weight	25ml
Standard factor	0.004
Sample factor	0.004
Standard purity	99.2
Average weight	100
Amount in mg.	29.76mg
%assay	99.21%

Table 1.6 Ruggedness Study

S. No.	RT	Area
1	4.018	3943102
2	4.01	3943124
Avg.	4.014	3943113
St dev.	0.005657	15.55635
%RSD	0.14	0.00

Table 1.7 Robustness Study

S. No:	RT	Area
Buffer-1	4.968	3930160
Buffer-2	3.889	3614565
flow-1	4.81	4387323
Flow-2	3.645	3628419

Table 1.8 Specificity Study

Specificity	RT	Area
Acid	4.018	2721003
Base	4.021	1099013
Heat	4.023	1441069
UV-light	4.0104	1156151

Table 1.9 Performance calculations

Parameter	RP-HPLC Method
Retention time (t) min	4.010
Theoretical plates (n)	7732
Plates per meter (N)	30928
Height equivalent to theoretical plate (HETP)	0.0323
Linearity Range ($\mu\text{g/ml}$)	0-40
Peak asymmetry	1.05
LOD ($\mu\text{g/ml}$)	7.1751
LOQ ($\mu\text{g/ml}$)	21.7427
Regression equation ($y^* =bc+a$)	
Slope (b)	131065
Intercept (a)	16835
Correlation coefficient (r^2)	0.9999
Method Precision Relative Standard Deviation (%RSD)	0.70
System Precision Relative Standard Deviation (%RSD)	0.72

RESULTS AND DISCUSSION

The appropriate wavelength in UV region has been selected for the measurement of active ingredient in the proposed method. This method was validated by linear fit curve and all the other parameters were calculated.

Parameters Fixation

In developing methods, systematic study of the effects of various parameters was undertaken by varying one parameter at a time and controlling all other parameters. The following studies were conducted for this purpose.

a). Mobile phase Characteristics

In order to get sharp peaks and base line separation of the components, carried out number of experiments by varying different components like percentage of Organic phase in the mobile phase, p^H of the aqueous phase, total p^H of the selected mobile phase and flow rate by changing one at a time and keeping all other parameters constant. The optimum conditions obtained were included in the procedure proposed.

b). Detection Characteristics

To test whether Carbofuran has been linearly eluted from the column, different amounts of Carbofuran were taken and analyzed by the above mentioned procedures. The peak area ratios of component areas were calculated and the values are graphically represented in Fig.1.2. The linear fit of the system was illustrated graphically. Least square regression analysis for the method was carried out for the slope, intercepts and correlation coefficient. The results are presented in table-1.4.

c). Performance Calculations

To ascertain the system suitability for the proposed method, a number of statistical values have been calculated with the observed readings and the results are recorded in Table-1.9.

d). Method Validations

The UV absorption maximum for Carbofuran was fixed at 282 nm respectively. As the final detection was made by the UV absorption spectrum, each method was validated by linear fit curve.

e). Precision

The Precision of the method and system was ascertained separately from the peak area ratios obtained by actual determination of a fixed amount of pesticide. The percentage of relative standard deviation is calculated for Carbofuran and readings presented in Table-1.1 and Table-1.2 The Precision of the assays was also determined in terms of dilution variation in the peak areas for a set of pesticide solution was calculated in terms of %RSD and the results are presented in Table-1.5.

f). Accuracy

To determine the accuracy of the proposed methods, different amount of formulation grade (STARFURON 3% CG) samples of Carbofuran within the linearity limits were taken and analyzed by the proposed method. The results (%RSD error) are recorded in Table-1.3.

g). Ruggedness:

The ruggedness of test method was demonstrated by carrying out precision study in six preparations of sample on a single batch sample by different analysts. The results of the precision study are recorded in Table-1.6.

h)Robustness :

The robustness of test method was demonstrated by carrying out flow variation $\pm 20\%$, i.e. (0.8ml and 1.2 ml/min), buffer variation $\pm 10\%$ i.e. (35:65 and 45:55). Results of this study are recorded in Table-1.7.

i) Specificity Studies

The Specificity Studies are carried out by varying specific conditions they are Heat, UV-light range and in Acidic, Basic medium. The results are recorded in Table-1.8.

CONCLUSION

The method was found to be accurate and precise, as indicated by recovery studies close to 100 and % RSD is not more than 2. The summary of validation parameters of proposed HPLC method is given in tables.

The simple, accurate and precise RP-HPLC method for the determination of Carbofuran as Technical and formulation has been developed. The method may be recommended for routine and environmental analysis the investigated drug in formulations. The analytical solution hence, it is concluded that the analytical method is validated and can be used for routine analysis.

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