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Der Pharmacia Lettre, 2015, 7 (10):163-171
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Development and validation of RP-HPLC method for the determination of glufosinate in its formulations

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ABSTRACT

A simple, selective, precise and accurate High Performance liquid Chromatographic method for the analysis of Glufosinate in its formulations was developed and validated in the present study. The mobile phase consist a mixture of 5 ml/L ammonium acetate aqueous solution (containing 0.2% (v/v) formic acid) and acetonitrile in the proportion 50: 50 (v/v). This was found to give sharp peak of Glufosinate at a run time of 15 min. HPLC analysis of Glufosinate was carried out at a wave length of 195 nm with a flow rate of 1.3mL/ 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 50% to 150%. The linear regression equation was y =3650.1 x -217.1 (y = mx+c). The developed method was employed with a high degree of precision and accuracy for the analysis of Glufosinate. The method was validated for accuracy, precision, robustness, ruggedness and specificity. The Precision, accuracy, sensitivity, short retention time and composition of the mobile phase indicated that this method is useful for the quantification of Glufosinate.

Keywords: Glufosinate, HPLC Method, Development and Validation.

INTRODUCTION

Farmers are attempting to suicides in India particularly in Andhra Pradesh and Telangana states because of loss in cultivation. One of the reasons for loss in cultivation is using of inefficient pesticides. Determination of Pesticide persistence in Formulations this method is suggestible. Organophosphate insecticides are commonly used Worldwide among them Glufosinate is used to control a wide range of weeds or for total vegetation control on land which is not used for cultivation. Glufosinate was also used to desiccate (dry of) crops before harvesting the crops. It is a fine crystalline solid and broad-spectrum herbicide that is used to control weeds. It is applied to young plants during early development for full effectiveness. FMC 200 HERBICIDE is a blue liquid soluble in water formulation containing 200 g/l. FMC 200 is metabolised (broken down) by microorganisms in the soil to become inactive.

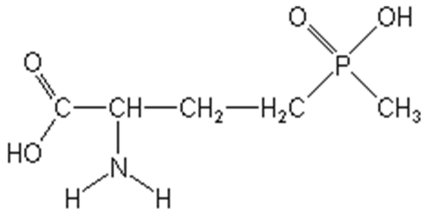


Figure-1 Chemical structure of Glufosinate

Several methods of analysis have been developed by Baki *et al.*, 2004[1], Hiroyuki *et al.*, 1996[2], Sancho *et al.*, 1994[3], Maria *et al.*, 2005[4], Tsunoda *et al.*, 1993[5], Yashushi *et al.*, 2001[6], Vreeken *et al.*, 1998[7] for the determination of dissociated organo phosphorus pesticides in various matrices such as fatty food, soil, water,

vegetables and human serum. Most of these methods are complicated, tedious, used large amounts of solvent and time-consuming.

The extraction procedure for determining Glufosinate residues in vegetables oil crops was published by Sochor in 1991[8]. The author reported that the Glufosinate residues were extracted from plant and animal material with water and then cleaned-up by de fatting with dichloromethane. After evaporation of the solvent, the residues were treated with tri methyl ortho acetate to form derivatives prior to gas chromatographic analysis with phosphorus-specific flame-photometric detection. However, this method is very tedious, solvents and time- consuming during the sample preparation steps.

Constantine *et al*. (2001)[9] reported that the determination of Glufosinate at low levels of concentration was difficult mainly because of their high polarity and solubility in water. It often requires an extensive sample treatment including enrichment steps and laborious derivatisation to reach the low levels of the target compounds. The extraction procedures are also tedious and time-consuming. Therefore, there is a need for better methods of analysis and this article describes the novel application of a modified QuEChERS method for Glufosinate determination in its formulations.

The HPLC method described here is simple, sensitive, and reproducible for 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

Instruments Required
High performance liquid chromatography, with UV / PDA detector, HPLC Analytical column of Waters Atlantis dC18 3.5 µm 2.1 x 50 mm, Analytical weighing balance - Mettler Toledo B204S, Millipore Nylon 0.2µm.

Chemicals Required
Working Standard, FMC 200 Herbicide, Analytical grade solvents which are Acetonitrile, Ammonium Acetate, Formic Acid, Hydrochloric Acid, Sodium Hydroxide and Millipore Water were used.

Chromatographic Conditions:
Column **:** A Waters Atlantis dC18 3.5 µm 2.1 x 50 mm
Mobile Phase **:** For isocratic system, prepared a mixed buffer of ammonium acetate aqueous solution (containing 0.2% (v/v) formic acid) and acetonitrile in the proportion 50: 50 v/v. Filtered through 0.2 µ Nylon membrane filter paper and degassed prior to use.

Wave length **:** 195 nm
Flow rate **:** 1.3ml/minute
Injection volume **:** 20 µl
Run time **:** 15 minutes
Blank solution **:** Acetonitrile
Diluent **:** Acetonitrile

Preparation of Standard Solution
Weighed accurately 50 mg of working Standard compound and transferred to a 25 ml volumetric flask. Added 10 ml of diluent and sonicated to dissolve. Diluted to volume with diluent and mixed. Transfered 1.0 ml of solution into a 10 ml of volumetric flask and diluted to volume with the diluent and mixed.

Preparation of Test Solution
1ml of sample solution transferred to a 100 ml volumetric flask. Added 50 ml of diluent and sonicated to dissolve. Diluted to volume with diluent and mixed. Transfered 1.0 ml of solution into a 10 ml of volumetric flask and diluted to volume with the diluent and mixed.

System Suitability Solution:
Used Standard working solution as system suitability solution.

Procedure
Equal volumes of blank and five replicate injections of system suitability solution separately injected. Then injected two injections of test solution and recorded the chromatograms. Disregarded any peak due to blank in the test solution and Calculated % RSD of five replicate injections of system suitability solution. Checked tailing factor and theoretical plates of the peak in the chromatogram obtained with 5th injection of system suitability solution (Standard working solution). The results are given in table-1.

The limits are as below,
1) Theoretical plates should be not less than 2000.
2) Tailing factor should be less than 2.0.
3) % RSD should be not more than 2.0%.

Table-1 System suitability - Selectivity

Sr. No.	Area of Glufosinate
1	2667.24
2	2655.15
3	2686.09
4	2668.82
5	2665.99
Mean	**2668.66**
Standard Deviation (±)	**11.13**
(%) Relative Standard Deviation	**0.42**

Specificity / Selectivity
Selectivity was performed by injecting the diluent blank solution, excipient blend, system suitability solution, test solution

Acceptance criteria
The peak should be well resolved from any other peak and from each other.

The diluents blank solution, excipients blend solution should not show any peak at the retention time of the Glufosinate.

Forced Degradation
The forced degradation studies are performed to establish the stability indicating nature of the assay Method and to observe any degraded compounds. WS and Sample (FMC 200 Herbicide) are subjected to stress with 5N HCl, 5N NaOH, Thermal degradation and UV degradation. All the above solutions are chromatographed and recorded the chromatograms. The results are recorded in table-2.

Conditions – Forced Degradation

Sample stress condition	Description of stress condition
Acid degradation	5N HCl heated at about 60°C for 10 min on a water bath.
Alkali degradation	5N NaOH heated at about 60°C for 10 min on a water bath.
Thermal degradation	105ºC for 12 hours
UV degradation	expose to UV-radiation for 7 days

Table-2 System suitability – Forced Degradation

Sr. No.	Area of Glufosinate
1	2740.91
2	2710.02
3	2741.24
4	2777.48
5	2739.22
Mean	**2741.77**
Standard Deviation (±)	**23.93**
(%)Relative Standard Deviation	**0.87**

Acceptance Criteria:
The degradation peaks should be well separated from each other. The peak purity for peak should pass.

Linearity

Linearity and Range for standard:

For the linearity study five standard solutions were prepared from the range starting from 50% to 150% of the theoretical concentration for assay preparation.

The system suitability solution and the linearity solutions were injected as per the protocol. The linearity graph of concentration against peak response was plotted and the correlation coefficient was determined. The results are given in tables -3 & 4.

Table-3 System suitability - Linearity of standard

Sr. No.	Area of Glufosinate
1	2816.72
2	2803.28
3	2810.12
4	2821.01
5	2828.41
Mean	**2815.91**
Standard Deviation (±)	**9.69**
(%) Relative Standard Deviation	**0.34**

Table-4 Results of linearity of standard

Linearity Level	Sample Concentration (in %)	Sample Concentration (in ppm)	Peak Area	Correlation Coefficient
Level – 1	50	100	1575.51	**0.999**
Level – 2	75	150	2567.20	
Level – 3	100	200	3403.68	
Level – 4	125	250	4394.04	
Level – 5	150	300	5224.76	

Acceptance criteria:

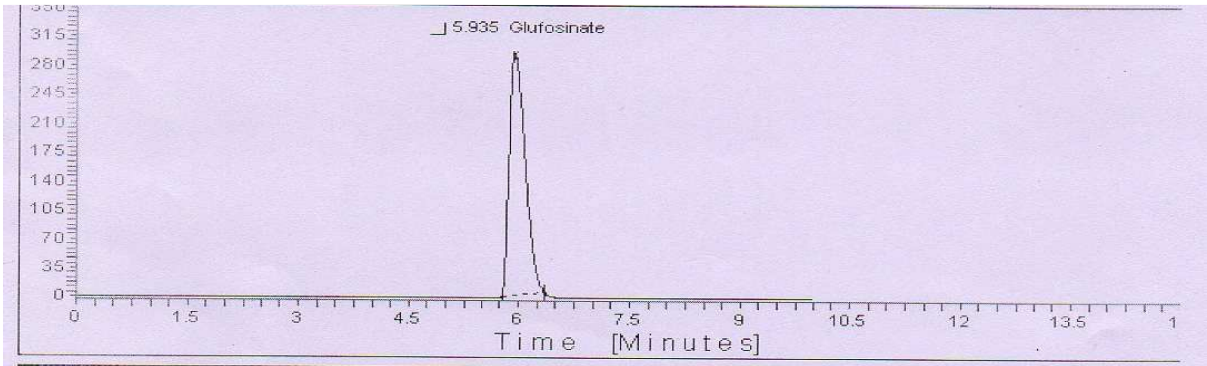
Correlation coefficient should be greater than or equal to 0.999.

Precision:

System Precision:

Procedure:

The system precision was performed by injecting 10 replicate injections of system suitability solution and the chromatograms are reviewed for the system suitability criteria. The results are presented in table -5.



Result-A Table

Peak No	Retn.Time	Area	Height	Area %	Height %
1	5.935	4394.043	293.523	100	100
Total		**4394.043**	**293.523**	**100**	**100**

Figure-2: Sample chromatogram of Glufosinate

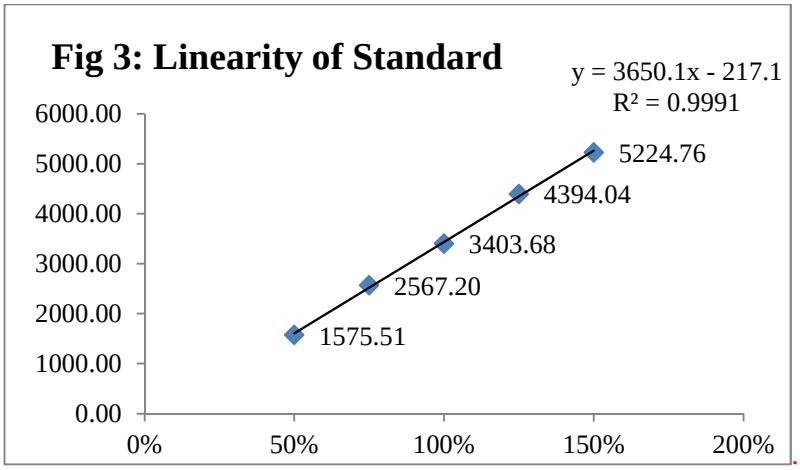


Figure -3: Linearity graph of standard

Table- 5 System precision

Sr. No.	Area of Glufosinate
1	2742.08
2	2714.02
3	2743.28
4	2781.84
5	2741.08
6	2751.68
7	2722.22
8	2750.26
9	2709.88
10	2724.48
Mean	**2738.08**
Standard Deviation (±)	**21.37**
(%) Relative Standard Deviation	**0.78**

Acceptance criteria:
% RSD of peak areas of ten replicate injections of system suitability solution should not be more than 2.0% and system suitability criteria should pass as per analytical Method.

Method Precision:
Procedure:
Six test solutions of in FMC 200 Herbicide were prepared as per the analytical Method. The % RSD of % assay of six test solutions was calculated. The results are presented in table -6.

Table-6 Results of Method precision

Test Solution	% Assay of
1	100.87
2	98.15
3	100.03
4	98.62
5	99.95
6	99.69
Mean	**99.55**
Standard Deviation (±)	**1.00**
(%) Relative Standard Deviation	**1.00**

Acceptance criteria:
% RSD of the results of six test solutions should not be more than 2.0%.

Intermediate Precision:
Procedure:
Six test solutions of FMC 200 Herbicide was prepared as per the analytical Method on different day. These test solutions were analyzed by a different analyst using different HPLC column of same make but having different serial number and different HPLC system. The % RSD of % assay results of twelve test solutions (six samples from Method precision and six samples from intermediate precision) was calculated. Results of twelve test solutions of in

FMC 200 Herbicide. The results of six of Method precision & six of intermediate precision are presented in table - 7.

Table -7 Results of Ruggedness

Analysis performed during Method precision study By Analyst 1 on system 1 and on column 1 on day 1	
Same column	**% Assay of Glufosinate**
1	100.87
2	98.15
3	100.03
4	98.62
5	99.95
6	99.69
Analysis performed during intermediate precision study By Analyst 2 on system 2 and on column 2 on day 2	
Test Solution	**% Assay of Glufosinate**
7	99.75
8	99.96
9	99.05
10	101.11
11	98.76
12	98.29
Mean of twelve samples	**99.52**
Standard Deviation (±)	**0.96**
(%) Relative Standard Deviation	**0.96**

Acceptance criteria:
% RSD of the results of twelve test solutions (six of Method precision and six of intermediate precision) should not be more than 2.0%.

Robustness:
Experiment:
Prepare two test solutions of the same lot (as used in 7.0.a and 7.0.b) of in FMC 200 Herbicide as per analytical Method. Inject this solution along with diluent blank solution and system suitability solution along different chromatographic conditions as shown below:

Change in column lot (same make, different serial no.)
Change in flow rate (± 0.2 ml/minute)
Change in wavelength (± 2 nm)
Change in composition of mobile phase (± 20ml).
The results are recorded in tables-8 to11.

Change in Column Lot:
[Normal Experimental Condition: Waters Atlantis dC18 3.5 µm 2.1 x 50 mm**)**
The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical Method.

Table-8 Results for Change in Column Lot

Flow rate →	**Same column**	**Diff column**
Sample	**% Assay**	
Test solution	100.98	100.69
Average assay result from Method precision	100.03	100.38
Mean	**100.51**	**100.54**
Standard Deviation (±)	**0.67**	**0.22**
(%) Relative Standard Deviation	**0.67**	**0.22**

Change in Flow Rate (± 0.2 mL/minute):
(Normal Experimental Condition: 1.3ml/minute)
The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical Method.

Table- 9 Results for change in flow rate

Flow rate →	1.1mL/minute	1.5 mL/minute
Sample	**% Assay**	
Test solution	99.2	97.27
Average assay result from Method precision	99.93	97.23
Mean	**99.57**	**97.25**
Standard Deviation (±)	**0.52**	**0.03**
(%) Relative Standard Deviation	**0.52**	**0.03**

Change in Wavelength (± 2 nm):
(Normal Experimental Condition: 195nm)
The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical Method.

Table-10 Results for change in wavelength

Wavelength →	193 nm	197 nm
Sample	**% Assay**	
Test solution	99.7	99.74
Average assay result from Method precision	100.31	100.31
Mean	**100.01**	**100.03**
Standard Deviation (±)	**0.43**	**0.40**
(%) Relative Standard Deviation	**0.43**	**0.40**

Change in composition of Mobile Phase:
(Normal Experimental Condition: Buffer : Acetonitrile = 50ml:50ml)
The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical Method.

Table-11 Results for change in composition of mobile phase

Composition of Buffer : Acetonitrile	60ml:40ml	40ml:60ml
Sample	**% Assay**	
Test solution	98.92	98.77
Average assay result from Method precision	99.71	98.6
Mean	**99.32**	**98.69**
Standard Deviation (±)	**0.56**	**0.12**
(%) Relative Standard Deviation	**0.56**	**0.12**

Stability of Analytical Solution:
Procedure:
System suitability solution and test solution of FMC 200 Herbicide were prepared on 0^{th}, 12^{th}, 24^{th}, 36^{th} and 48^{th} hour of experiment and stored these solutions at room temperature for every time interval up to 48 hrs and analyzed these solutions on 48 hrs with freshly prepared test solution. The system suitability solution was prepared freshly at the time of analysis. The assay of FMC 200 Herbicide in the sample was calculated. The results are given in table-12.

Table -12 Results of Stability

% Assay results calculated against the freshly prepared system suitability standard	
Sample	**% Assay of Glufosinate**
0^{th} hr	99.80
12^{th} hr	99.11
24^{th} hr	100.08
36^{th} hr	100.98
48^{th} hr	102.47
Mean	**100.49**
Standard Deviation (±)	**1.30**
(%) Relative Standard Deviation	**1.29**

Acceptance criteria:
The analyte is considered stable if there is no significant change in assay.

Table -13: Performance calculations, detection characteristics precision and accuracy of the proposed method for Glufosinate

Parameter	HPLC Method
Wavelength (nm)	195
Retention time (t) min	5.935
Linearity range (in **%**)	50-150
LOD	0.009
LOQ	0.026
Regression equation (y=mx+c)	y= 3650.1 x -217.1
Slope (b)	3650.1
Intercept (a)	-217.1
Correlation coefficient(r^2)	0.999
Standard deviation	9.69
Relative Standard deviation (%RSD	0.34

%RSD of six independent determinations

RESULTS AND DISCUSSION

Selectivity:
The peak in test solution was found to be well resolved from peaks due to diluent blank solution. The diluent blank do not show any peak at the retention time of the Glufosinate.

Forced Degradation:
Standard
The peaks due to degradation products are found to be well separated from the peak. The peak purity criteria of was found to pass at each condition of degradation.

Sample
The peaks due to degradation products are found to be well separated from the peak. The peak purity criteria of was found to pass at each condition of degradation.

Linearity and Range of Standard
Correlation coefficient = 0.999
Range = 100 ppm to 200 ppm
System precision = % RSD = 0.78
Method precision = % RSD = 1.00
Intermediate precision = % RSD = 1.01

Robustness
1] System suitability criteria are found to meet the pre-established acceptance criteria.
2] % RSD between results obtained with changed condition and average result of Method precision, are found less than 2.0%.

Stability of analytical solution
No significant change is observed in the % assay upto 48 Hrs. Hence the solution is found to be stable up to 48 hours at room temperature.

CONCLUSION

The above summary and the validation data summarized in this document shows that the analytical Method of assay of FMC 200 Herbicide by HPLC is found to be suitable, selective, specific, precise, linear, accurate and robust. The analytical solution is found to be stable up to 48 hrs at room temperature.

Hence, it is concluded that the analytical Method is validated and can be used for routine analysis and for stability study.

Acknowledgements
The author thanks to Analog labs Hyderabad, India and Department of Chemistry, Sri Venkateswara University, Tirupati, India for providing laboratory facilities.

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