IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
www.iosrphr.org Volume 5, Issue 1 (January 2015), PP.24-36

Development and Validation of the HPLC Method for the Analysis of Ametridione in Bulk and Comercial Dosage Forms

P.Padmavathi, P.Supriya, P.Suguna & N.V.S.Naidu
Department of Chemistry, S.V.University, Tirupati-517502, A.P., India.

Abstract: *A simple, economic, selective, precise, and accurate High Performance liquid Chromatographic method for the analysis of Ametridione in bulk and commercial formulations was developed and validated in the present study. The mobile phase consists of a mixture of Methanol and water in the proportion 70:30 and adjust the pH to 6.0 ± 0.05 with sodium hydroxide solution. This was found to give a sharp peak of Ametridione at a retention time of 4.421min. HPLC analysis of Ametridione was carried out at a wavelength of 254nm with a flow rate of 1.0 mL/min. The linear regression analysis data for the calibration curve showed a good linear relationship with a regression coefficient of 0.999 in the concentration range of 50 µg ml^{-1} to 150 µg ml^{-1}. The linear regression equation was y =2188x-465.3. The developed method was employed with a high degree of precision and degradation for the analysis of Ametridione. The developed method was validated for precision, robustness, detection and quantification limits as per the ICH guidelines. The wide linearity range, sensitivity, short retention time and composition of the mobile phase indicated that this method is better for the quantification of Ametridione.*

Keywords: *Ametridione, HPLC, Validation*

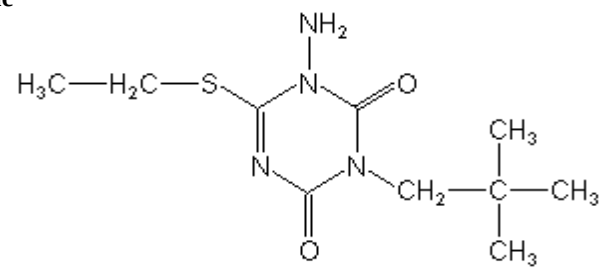
I. Introduction:

A survey of the literature revealed that different analytical techniques for the assay of MTM have been reported. Voltametric detection of the herbicide Ametridione at a bismuth film electrode in nondeaerated solution[1]Electroanalisis of Ametridione and metribuzen on lignin By Adsorption[2] ,Electrochemical reduction of Ametridione[3] , Identification of different products obtained by electrochemical and photochemical reduction of the Ametridione[4]Votametric determination of Ametridione with an elctrogeneratedmolecularity imprinted polymer microsencer[5] Electrochemical determination of the effect of lead(II) on the photochemical degradation of the pesticide Ametridione[6] Votametricdetermination of Herbicide Ametridione using Mercury and silver solid amalgam electrode[7]Preconcentration and votametric determination of the herbicide Ametridione with a silica modified carbon paste electrode[8] Determination and method validation of Ametridione in soil by RP-HPLC[9] Electrochemical determination of the effect of Copper (II) on the photochemical degradation of the pesticide Ametridione[10] .

Early, analysis of Ametridione in Human plasma by HPLC with fluorescence detection , HPLC determination of Ametridionepolyglutamates after Low-Dose Ametridione therapy in patients with Rheumatoid arthritis ,Quality control of Ametridione by HPLC and Polarographic and voltammetric methods for the quantitation of MTM in pharmaceuticals and plasma samples have been published.

There is however no reported HPLC method for the analysis of Ametridione in its technical grade and formulations. This is describes a validated HPLC method for the quantitative determination of Ametridione.

1. **Structure: Ametridione**



2. **Chemical name:**1. 1-amino-6-ethylthio-3-neopentyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione
3. **Empirical formula:** $C_{10}H_{18}N_4O_2S$
4. **Molecular weight:** 258.3

The HPLC method described here is simple, sensitive, and reproducible for Ametridione determination in Formulations with low background interference. An attempt has been made to develop and validate to ensure their , 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-18column, with a suitable mobile phase, without the use of any internal standard. For formulation the proposed method is suitable for their analysis with virtually no interference of the usual additives presented in formulations.

1. Instrumentation

HPLC Analytical column ChromolithRP - C18, 300mm x 4.0mm x 5µ Chromatographic conditions for Ametridione.

Stationary phase	**Mobile phase**	**Flow rate (ml min^{-1})**	**Run time (min)**	**Column Temp (0^c)**	**Volume of injection loop (µl)**	**Detection wavelength (nm)**	**Retention time (min)**
RP - C18, 300mm x 4.0mm x 5µ	Methanol and water 70:30	1..0	10	25	20	254	4.421

II. Analytical Methodology

1. Preparation of Mobile phase

For isocratic system, prepare a mixture of Methanol and water in the proportion 70:30 respectively. Mix well, adjust the pH to 6.0 ± 0.05 with sodium hydroxide pellets. Filter through 0.2 µ Nylon membrane filter paper and degas prior to use.

2. Chromatographic conditions

Separation was performed on C -18, 100mm x 6mm x 5µ Column. DimethyleSulfoxide used as a Diluent and Mobile phase consists of mixture of Methanol and water in the proportion 70:30. Injection volume of 20 µl was used. Mobile phase was filtered before use through 0.5 µm Nylon membrane filter paper and degassed with helium purge for 10 min. The components of the mobile phase were pumped from solvent reservoir to the column at flow rate 1.0 ml $min^{-1}$and wavelength was set to 294 nm. The column temperature was set at 25^oC.

III. Analytical methodology:

❖ **Preparation of Ametridione Standard Solution:**

Weigh accurately about 25 mg of Ametridioneworking Standard and transfer to a 25 ml volumetric flask. Add 10 ml of diluent and sonicate to dissolve. Dilute to volume with diluent and mix. Transfer 1.0 ml of solution into a 10 ml of volumetric flask and dilute to volume with the diluent and mix.(Dilution scheme: 25mg $\rightarrow$ 25.0 ml $\rightarrow$ 1 ml /10.0 ml)

❖ **Preparation of Test Solution:**

Weigh accurately about 60 mg of sample and transfer to a 25 ml volumetric flask. Add 10 ml of diluent and sonicate to dissolve. Dilute to volume with diluent and mix. Transfer 1.0 ml of solution into a 10 ml of volumetric flask and dilute to volume with the diluent and mix.(Dilution scheme: 60mg $\rightarrow$ 25.0 ml $\rightarrow$ 1 ml /10.0 ml)

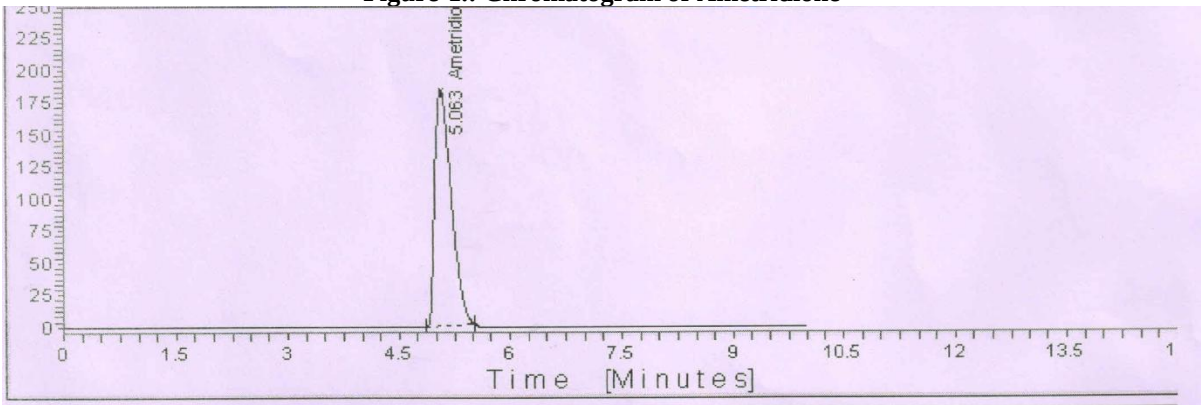
❖ **System Suitability Solution:**

Use AmetridioneStandard working solution as system suitability solution.

❖ **Procedure:**

Separately inject equal volumes of blank, five replicate injections of system suitability solution (AmetridioneStandard working solution). Then inject two injections of test solution and record the chromatograms. Disregard any peak due to blank in the test solution. Calculate % RSD of five replicate injections of system suitability solution (AmetridioneStandard working solution). Check tailing factor and theoretical plates of the peak in the chromatogram obtained with 5^{th} injection of system suitability solution (AmetridioneStandard working solution).

Figure-1.: Chromatogram of Ametridione



Result-A Table					
Peak No	**Retn.Time**	**Area**	**Height**	**Area %**	**Height %**
1	5.063	2894.956	185.932	100	100
Total		**2894.956**	**185.932**	**100**	**100**

Figure-2: Linearity graph of Ametridione standard

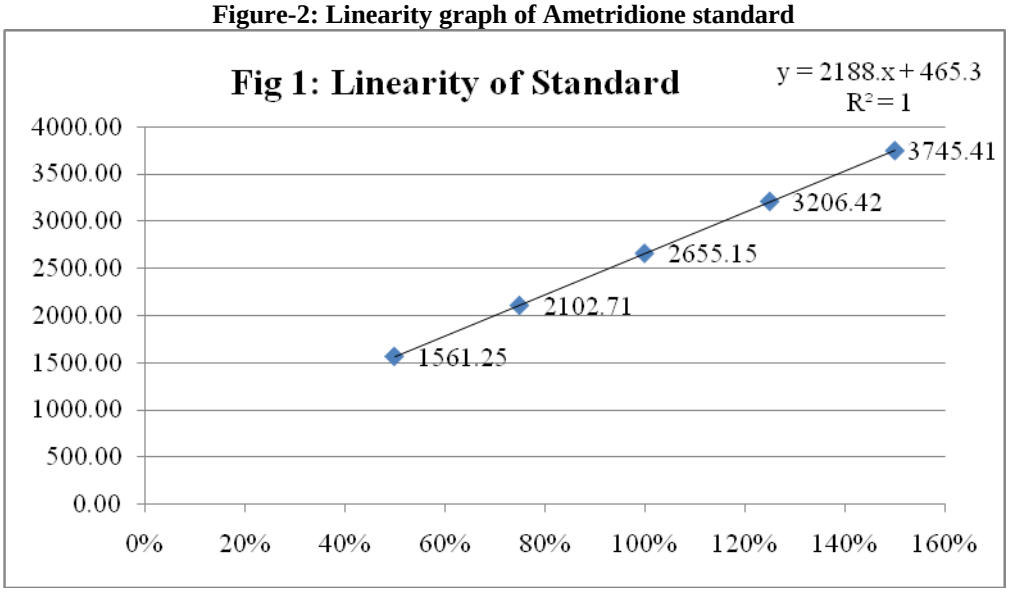
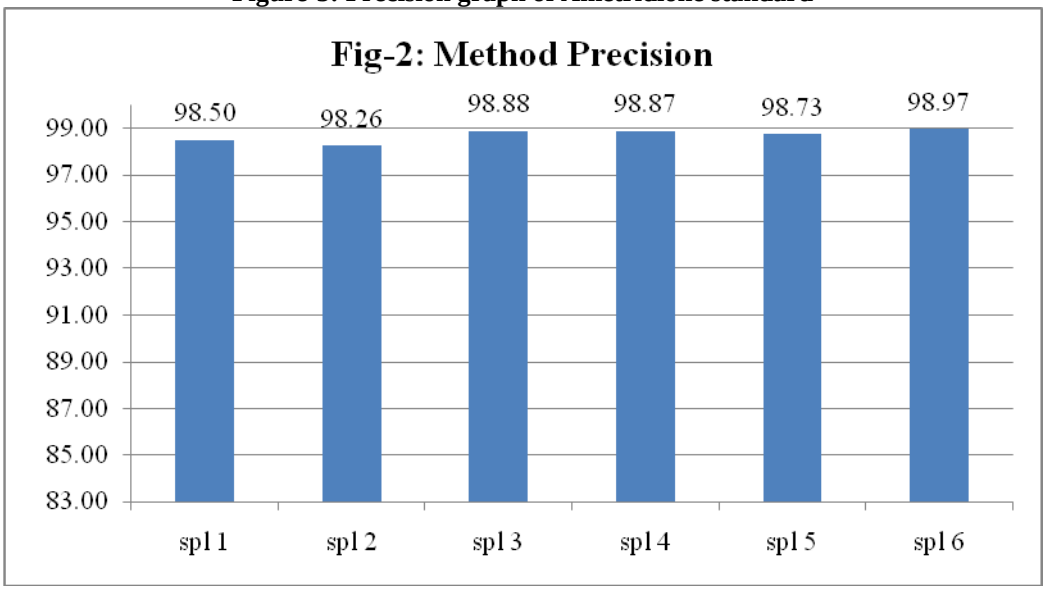


Figure-3: Precision graph of Ametridione standard



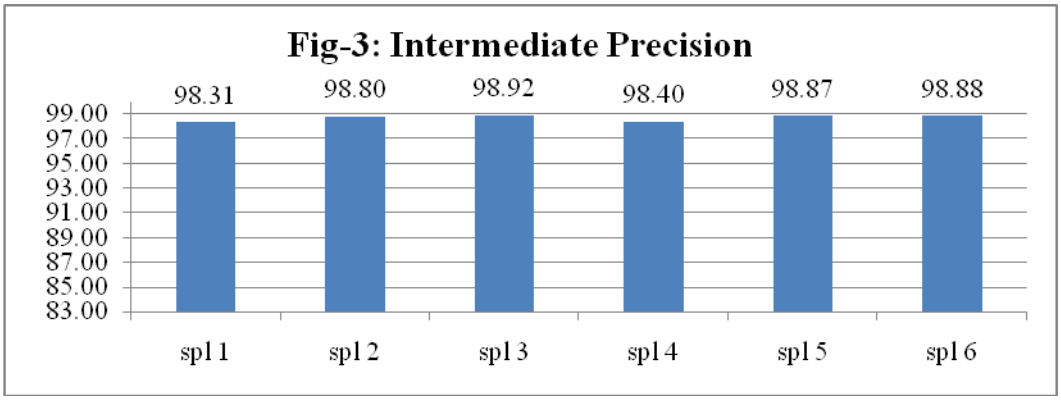


Table -1.: Performance calculations, detection characteristics precision and accuracy of the proposed method for Ametridione

Parameter	**HPLC Method**
Wavelength (nm)	254
Retention time (t) min	5.063
Linearity range (µg ml^{-1})	50-150
LOD(µg ml^{-1})	0.002
LOQ(µg ml^{-1})	0.00669
Regression equation (y=bc+a)	
Slope (b)	2188
Intercept (a)	465.3
Standard deviation (SD)	1.464
Correlation coefficient(r^2)	0.9999
Relative Standard deviation (%RSD)*	0.055
Intermediate Precision (%RSD)	0.56
Range of errors	
Confidence limits with 0.05 level	2.869
Confidence limits with 0.01 level	3.771

***RSD of five independent determinations**

Table - 2:System suitability - Selectivity

Sr. No.	**Area of Ametridione**
1	2866.31
2	2878.06
3	2896.05
4	2898.76
5	2872.99
Mean	**2882.43**
Standard Deviation (±)	**14.32**
(%) Relative Standard Deviation	**0.50**

1.0 FORCED DEGRADATION

Table – 3: System suitability – Forced Degradation

Sr. No.	**Area of Ametridione**
1	2814.90
2	2830.64
3	2803.28
4	2814.67
5	2791.88
Mean	**2811.07**
Standard Deviation (±)	**14.49**
(%) Relative Standard Deviation	**0.52**

Table – 4: 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

Acid Stress	**% Degradation**
Standard	0.105
Sample	0.015
Alkali Stress	**% Degradation**
Standard	0.015
Sample	0.024
Thermal Stress	**% Degradation**
Standard	0.001
Sample	0.003
UV Stress	**% Degradation**
Standard	0.003
Sample	0.219

2.0 Linearity

Table 5: System suitability - Linearity of standard

Sr. No.	**Area of Ametridione**
1	2894.95
2	2896.87
3	2896.16
4	2877.96
5	2832.47
Mean	**2879.68**
Standard Deviation (±)	**27.53**
(%) Relative Standard Deviation	**0.96**

Table 6: Results of linearity of standard

Linearity Level	Sample Concentration (in %)	Sample Concentration (in ppm)	Peak Area	Correlation Coefficient
Level – 1	50	50	1561.25	**0.999**
Level – 2	75	75	2102.71	
Level – 3	100	100	2655.15	
Level – 4	125	125	3206.42	
Level – 5	150	150	3745.41	

The linearity plot of peak area of **Ametridione** Vs. standard concentration in percentage is presented in figure-1.

3.0 Precision:

3.1 System Precision:

.**Table 7: System precision**

Sr. No.	Area of Ametridione
1	2415.59
2	2402.53
3	2366.80
4	2376.49
5	2466.64
6	2459.74
7	2408.17
8	2446.17
9	2363.85
10	2331.63
Mean	**2403.76**
Standard Deviation (±)	**44.66**
(%) Relative Standard Deviation	**1.86**

3.2 Method Precision:

Table - 8:System suitability - Method precision

Analyst – 1 **HPLC No.: EH/R&D/HPLC-024**

Sr. No.	Area of Ametridione
1	2817.12
2	2820.10
3	2793.15
4	2844.15
5	2833.75
Mean	**2821.65**
Standard Deviation (±)	**19.29**
(%) Relative Standard Deviation	**0.68**

Table - 9: Results of method precision

Test Solution	% Assay of Ametridione
1	98.50
2	98.26
3	98.88
4	98.87
5	98.73
6	98.97
Mean	**98.70**
Standard Deviation (±)	**0.27**
(%) Relative Standard Deviation	**0.27**

3.2 Intermediate Precision:

Table - 10: System suitability - Intermediate precision

Analyst – 2 **HPLC No.: EH/R&D/HPLC-023**

Sr. No.	Area of Ametridione
1	2698.02
2	2727.37
3	2742.85
4	2752.29
5	2762.60
Mean	**2736.63**
Standard Deviation (±)	**25.17**
(%) Relative Standard Deviation	**0.92**

Table - 11: Results of Intermediate precision

Test Solution	% Assay of Ametridione
1	98.31
2	98.80
3	98.92
4	98.40
5	98.87
6	98.88
Mean	**98.70**
Standard Deviation (±)	**0.27**
(%) Relative Standard Deviation	**0.27**

Table - 12: Results of twelve test solutions of Ametridione in Ametridione BS 400 g/l(six of method precision & six of intermediate precision)

Analysis performed during method precision study By Analyst 1 on system 1 and on column 1 on day 1	
Same column	**% Assay of Ametridione**
1	98.50
2	98.26
3	98.88
4	98.87
5	98.73
6	98.97
Analysis performed during intermediate precision study By Analyst 2 on system 2 and on column 2 on day 2	
Column sr. no.	**015337030136 01**
Test Solution	**% Assay of Ametridione**
7	98.31
8	98.80
9	98.92
10	98.40
11	98.87
12	98.88
Mean of twelve samples	**98.70**
Standard Deviation (±)	**0.26**
(%) Relative Standard Deviation	**0.26**

.5.0 Robustness:

5.1

5.1.1 Change in Column Lot:

[Normal Experimental Condition:RP - C18, 300mm x 4.0mm x 5µ**)**

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table - 13 for system suitability results).

Table - 13: System suitability - Robustness withChange in Column Lot

Sr. No.	Area of Ametridione	
	Same column	**Diff column**
1	2518.46	2672.11
2	2519.21	2677.38
Mean	**2518.84**	**2674.75**
Standard Deviation (±)	**0.53**	**3.73**
(%) Relative Standard Deviation	**0.02**	**0.14**

The assay results obtained with different flow rate conditions are as given in Table - 14.

Table - 14: Results for Change in Column Lot

Flow rate →	**Same column**	**Diff column**
Sample	**% Assay**	
Test solution	98.49	98.93
Average assay result from method precision	98.70	98.7
Mean	**98.60**	**98.82**
Standard Deviation (±)	**0.15**	**0.16**
(%) Relative Standard Deviation	**0.15**	**0.16**

5.1.2 Change in Flow Rate (± 0.2 mL/minute):
(Normal Experimental Condition: 1.0ml/minute)

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table - 15 for system suitability results).

Table - 15: System suitability - Robustness with change in flow rate

Sr. No.	Area of Ametridione	
	0.8mL/minute	**1.2 mL/minute**
1	2377.31	2589.95
2	2372.32	2599.70
Mean	**2374.82**	**2594.83**
Standard Deviation (±)	**3.53**	**6.89**
(%) Relative Standard Deviation	**0.15**	**0.27**

The assay results obtained with different flow rate conditions are as given in Table 16.

Table - 16: Results for change in flow rate

Flow rate →	**0.8mL/minute**	**1.2 mL/minute**
Sample	**% Assay**	
Test solution	98.71	98.9
Average assay result from method precision	98.7	98.7
Mean	**98.71**	**98.80**
Standard Deviation (±)	**0.01**	**0.14**
(%) Relative Standard Deviation	**0.01**	**0.14**

5.1.3 Change in Wavelength (± 2 nm):
(Normal Experimental Condition: 254nm)

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table - 17 for system suitability results).

Table - 17: System suitability - Robustness with change in wavelength

Sr. No.	Area of Ametridione	
	252 nm	**256 nm**
1	2808.14	2826.46
2	2825.05	2829.90
Mean	**2816.60**	**2828.18**
Standard Deviation (±)	**11.96**	**2.43**
(%) Relative Standard Deviation	**0.42**	**0.09**

The assay results obtained with different wavelength conditions are as given in Table - 18.

Table - 18: Results for change in wavelength

Wavelength →	**252 nm**	**256 nm**
Sample	**% Assay**	
Test solution	98.87	98.89
Average assay result from method precision	98.70	98.70
Mean	**98.79**	**98.80**
Standard Deviation (±)	**0.12**	**0.13**
(%) Relative Standard Deviation	**0.12**	**0.14**

5.1.4 Change in composition of Mobile Phase (± 20ml):
(Normal Experimental Condition: methanol: water = 700ml: 300ml)

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method (Refer to Table - 19 for system suitability results).

Table - 19: System suitability - Robustness with change in composition of mobile phase

Sr. No.	Area of Ametridione	
	720ml:280ml	**680ml:320ml**
1	2575.27	3426.60
2	2596.31	3403.68
Mean	**2585.79**	**3415.14**
Standard Deviation (±)	**14.88**	**16.21**
(%) Relative Standard Deviation	**0.58**	**0.47**

The assay results obtained with change in composition of mobile phase are as given in Table - 20.

Table - 20: Results for change in composition of mobile phase

Composition of methanol & water	**720:280**	**680:320**
Sample	**% Assay**	
Test solution	98.82	98.85
Average assay result from method precision	98.7	98.7
Mean	**98.76**	**98.78**
Standard Deviation (±)	**0.08**	**0.11**
(%) Relative Standard Deviation	**0.09**	**0.11**

5.2 Stability of Analytical Solution:

TIME	**Std Area**	**Avgstd area**	**Spl area**	**AvgSpl area**
0^{th}hr	2733.45	2741.855	2672.41	2673.46
	2750.26		2674.51	
12^{th}hr	2794.31	2772.34	2698.22	2683.52
	2750.37		2668.82	
24 hr	2753.89	2757.8	2706.3	2701.275
	2761.71		2696.25	
36 hr	2765.81	2770.03	2662.76	2665.135
	2774.25		2667.51	
48 hr	2722.22	2733.24	2680.82	2680.17
	2744.27		2679.53	
Mean	**2755.05**	**2755.05**	**2680.71**	**2680.71**
Standard Deviation (±)	**20.49**	**17.18**	**14.72**	**13.47**
(%) Relative Standard Deviation	**0.74**	**0.62**	**0.55**	**0.50**

Table - 21: Results for solution stability

% Assay results calculated against the freshly prepared system suitability standard	
Sample	**% Assay of Ametridione**
0^{th}hr	98.94
12^{th}hr	98.38
24 hr	98.60
36 hr	98.58
48 hr	98.55
Mean	**98.61**
Standard Deviation (±)	**0.20**
(%) Relative Standard Deviation	**0.21**

IV. Results and Discussion:

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

Parameters fixation:

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

a. Mobile phase characteristics

In order to get sharp peaks and baseline separation of the components, carried out number of experiments by varying different components like percentage of organic phase in the mobile 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.

1. Detection Characteristics

To test whether Ametridione had been linearly eluted from the column, different amounts of Ametridione 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 Fig1.1, 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.

2. . 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.

3. Method validations

The UV absorption maximum for Ametridione was fixed at 310 nm respectively. As the final detection was made by the UV - absorption spectrum, each method was validated by linear fit curve.

4. Precision

The precision of the method was ascertained separately from the peak area ratios obtained by actual determination of a fixed amount of sample. The percent of Relative Standard deviation calculated for Ametridione and are presented in Tables-7,8,9,10,11&12. The precision of the assays was also determined in terms of intra and inter-day variation in the peak areas for a set of sample solution was calculated in terms of coefficient of variation (CV)

5. Interference Studies

The effect of wide range of excipients and other additives usually present in the formulations of Ametridione in the determinations under optimum conditions were investigated. The common excipients such as colloidal Silicon dioxide, ethyl cellulose, hydroxyl propyl methyl cellulose, magnesium state, microcrystalline cellulose provide have been added to the sample solutions and injected. They have not disturbed the elution or quantification of Drug. In fact many have no absorption at this λ_{max}

6. Analysis of Formulation

To find out the stability of the proposed methods for the assay of formulations containing Ametridione was analyzed by the proposed and reference methods. The proposed method does not differ significantly in precision from reference method. The results are recorded in Table-3.

7. Forced degradation:

There is no interference between the peaks obtained for the chromatograms of degradation preparations. The degradation peaks under forced degradation are well separated from each other. The peak purity for Ametridionepeak is passing.

Hence, the method is very precise, selective and specific to the estimation of Assay of Ametridionein in Ametridione SG 700 g/l by HPLC and the same method is stability indicating, as the degraded products are well separated from Ametridioneand as well from each adjacent peaks.

8. Ruggedness and Robustness

Ruggedness of the proposed method was determined by carrying out the analysis by two different analysts using similar operational i.e. Robustness with Change in Column Lot, Change in Flow rate, Change in wavelength and Change in p^H of the Mobile phase . The results were indicated by % CV in Tables – 13,14,15,16,17,18,19,20. Robustness of the method was determined by carrying out the analysis at two different wavelengths i.e. at 308 nm and 312 nm and the results were indicated by % CV in Table -18..

9. Solution Stability

The stability of the solutions under study was established by keeping the solution at room temperature for 48 Hours. The results indicate no significant change in assay values indicating stability of Drug in the solvent used during analysis. The results are recorded in Table -21.

V. 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 summery of validation parameters of proposed HPLC method is given.

The simple, accurate and precise HPLC method for the determination of Ametridione as bulk and form has been developed. The method may be recommended for routine and quality control analysis of the investigated drug in bulk and formulations. 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.

References:

[1] N Goudarzi, M Goodarzi, MCU Araujo… - Journal of agricultural …, 2009 - ACS Publications
[2] A Laganà, G Fago, A Marino, VM Penazzi - AnalyticaChimicaActa, 2000 – Elsevier
[3] Z Jie, Y Binghua, H Jiangrui… - Water Resource and …, 2011 - ieeexplore.ieee.org
[4] J Huuskonen - Journal of chemical information and computer …, 2003 - ACS Publications
[5] R Cazorla-Reyes, JL Fernández-Moreno… - Talanta, 2011 – Elsevier
[6] Y Hirahara, M Kimura, T Inoue, S Uchikawa… - Journal of health …, 2005 - jlc.jst.go.jp
[7] MR Hadjmohammadi… - Journal of …, 2007 - chromsci.oxfordjournals.org
[8] I Kahn, D Fara, M Karelson, U Maran… - Journal of chemical …, 2005 - ACS Publications
[9] As.Arribas,E.Berimizo,2006 (Willey online librury)
[10] J.Ludivik,P.Zuman 2000,Elsevier (Microchemical journal)
[11] ,J.Ludivik ,F.Riedi Journal of electroanalyticalchemisry 1998 Elsevier
[12] C.Olmedo,L.debanElectrochemica 1994 Esevier
[13] A.Gomez-caballero 2007 (Willey online librury)
[14] D.Sancho,M.Vega 1999,Taylor &Francis
[15] R,Selesovska,L.Bandzuchova 2004
[16] A.Arranz,SF De Betono 1997,Spriger
[17] S.Kumar,S.Tandon 2014 Spriger
[18] D.Sancho,M.Vega 1999,Taylor &Francis
[19] M.MansourChemospear 1996
[20] AM Vinggaard, J Niemelä, EB Wedebye… - Chemical research in …, 2008 - ACS Publications