New Validated Rp-HPLC Method For The Estimation of Afloqualone In Pharmaceutical Formulation

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Abstract

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Afloqualone in Bulk and Pharmaceutical tablet Formulation. Isocratic elution at a flow rate of 1ml/min was employed on symmetry Shimadzu LC-20 AT$_{VP}$ Kromasil C-18 column Column at ambient temperature. The mobile phase consisted of Acetonitrile:1% Acetic acid: Water(40:40:20 v/ v/v). The UV detection wavelength was 285nm and 20 µl sample was injected. The run time for Afloqualone is 6 min. The flow rate was found to be 1ml/min. The percentage recovery of the method was found to be 100.59%. The LOD and LOQ for Afloqualone was found to be 10µg/ml and 30µg/ml respectively.The method was validated as per the ICH guidelines. The method was successfully applied for routine quality control analysis of pharmaceutical formulation. The HPLC method can be successfully applied for the routine quality control analysis of Afloqualone formulations, which could be the better choices compared to the reported methods of literature

Keyword: *Afloqualone, Rp- HPLC, UV detection, Recovery, Precise.*
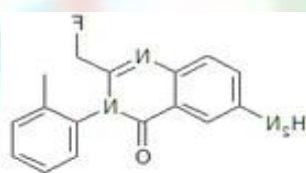
1. Introduction

Fig:1 Structure of Afloqualone

Afloqualone (Arofuto)[1] is an analogue of methaqualone developed in the 1980s and has sedative and muscle relaxant effects,[2] and has clinical use, although it causes photosensitization[3] as a side effect which can cause skin problems such as dermatitis.[4] Pharmacological properties[5] of afloqualone, differ from anti-anxiety drugs, hypnotics, and stimulants as related to behavior. Afloqualone (AFQ), 6-amino-2-fluoromethyl-3-(o-tolyl)-4-(3H)-quinazolinone[6,7], is one of the centrally acting muscle relaxants. It has been reported that AFQ is extensively metabolized in humans[8], rats[5] dogs, and monkeys[9] and there are species differences in the composition of urinary metabolites. It has been reported that when AFQ is administered orally to humans, rats, dogs, and monkeys, the parent AFQ and its metabolites are excreted into urine (human, 20% of dose; experimental animals, 50-70% of dose), in which AFQ N-glucuronide is the most abundant metabolite (8% of dose) in human urine[8,9]. The N-glucuronidation of AFQ plays an important role in the metabolism of AFQ in humans[10,11].

Yun HY etal[12], proposed two methods for determining the central-acting muscle relaxant afloqualone in human plasma were developed and compared using API2000 and API4000 liquid chromatography tandem mass spectrometry (LC/MS/MS) systems. After drying the organic layer, the residue was reconstituted in a mobile phase (0.1% formic acid-acetonitrile:0.1% formic acid buffer, 80:20 v/v) and injected onto a reversed-phase C(18) column. The isocratic mobile phase was eluted at 0.2ml/min. The ion transitions monitored in multiple reaction-monitoring mode were m/z 284-->146 and 251-->117 for afloqualone and methaqualone, respectively. In both assays, the coefficient of variation of the precision was less than 11.8%, the accuracy exceeded 91.5%, the limit of quantification was 0.5ng/ml, and the limit of detection was 0.1ng/ml for afloqualone.

Nagendrakumar A. V. D et.al.[13] proposed a simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Carvedilol in Bulk and Pharmaceutical tablet Formulation. Isocratic elution at a flow rate of 1.0ml/min was employed on symmetry C18 (250 mm x 4.6 mm, 5μm) Column at ambient temperature. The mobile phase consisted of Methanol: Acetonitrile: 1% OPA in the ratio of 80:18:2 v/v/v. The UV detection wavelength was 240nm and 20μl sample was injected. The retention time for Carvedilol was 2.1 min. The percentage RSD for precision and accuracy of the method was found to be 0.179%.

2. Experimental

2.1. Instrumentation

Peak HPLC containing LC 20AT pump and variable wavelength programmable ***PDA detector*** and Rheodyne injector was employed for investigation. The chromatographic analysis was performed on a Kromasil C_{18} column 250 x 4.6 mm ID with 5 μ particle size and the column were maintained at ambient temperature. Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. A Denwar analytical balance was used for weighing the materials.

2.2. Chemicals and solvents

The reference sample of Arofuto was obtained from Cipla, Mumbai. The Formulation was procured from the local market. Water, Acetonitrile and Acetic acid used were of HPLC grade and purchased from the chemicals were procured from E-Merck, India, Limited.

2.3. The mobile phase

The mobile phase was prepared by mixing Acetonitrile:1% Acetic acid: Water(40:40:20 v/v/v). Prepared mobile phase was filtered through 0.45μ membrane filter and sonicated. Sample solution was prepared by dissolving the drug in mobile phase and sonicated for 30 minutes.

2.4. Preparation of solutions

2.4.1. Preparation of Mobile Phase Solution

The mobile phase was prepared by mixing Acetonitrile and 1% Acetic acid and Water (40:40:20 v/v) by ultra bath sonicator for 30 min.

2.4.2. Preparation of standard

Stock solution of Afloqualone was prepared by dissolving accurately weighed 10mg of drugs in 10ml Methanol (final concentration, 1mg/ml). The prepared stock solutions were stored away from light. From the stock, standard solutions was freshly prepared during the day of analysis.

2.4.3. Preparation of working standard solution (a.p.i)

From the stock solution 20 µg/ml solution was prepared.

2.4.4. Preparation of working standards for linearity

Solutions in the concentration range of 1-5 µg/ml were prepared from the standard working solution.

2.4.5. Preparation of formulation sample solution

5mg of formulation powder was taken from AROFUTO (100 mg formulation) and dissolved in 5ml of mobile phase and injected into HPLC and chromatogram was recorded. The amount of drug present in the formulation was calculated from linearity graph.

3. Method Development

For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choosing stationary and mobile phases. The following studies were conducted for this purpose:

3.1. Detection of wavelength

The spectrum of 10ppm solution of afloqalone was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength 285nm was observed.

3.2. Choice of stationary phase and mobile phase

Finally the expected separation and peak shapes were obtained on Kromasil C_{18} column 250 x 4.6 mm ID with 5 µ particle size.

3.3. Flow rate

Flow rates of the mobile phase were changed from 0.1-1.5 ml/min for optimum separation. It was found from experiments that 1.0 ml/min flow rate was ideal for elution of analyte.

4. Validation Procedure and Requirements

The analytical performance of the method of analysis was checked for specificity, System suitability, detection limit, and method precision.

4.1. Linearity And Calibration

Linearity was assessed by performing single measurement at several analyte concentration varying quantities of stock standard solution diluted with the mobile phase to give a concentration of 1, 2, 3, 4, 5 µg/ml Injection was made at intervals of 6 min. The linearity was tested for the concentration ranging from 1µg/ml to5 µg/ml. The peak area ratio of the drug was plotted against concentration. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

4.2. Precision

Reproducibility was performed by injecting three replicates concentrations of standard and sample solutions which were prepared and analyzed by same analyst on same day. Inter-day variations in the peak area of drug solutions and the amount of drug were calculated in terms of Percentage Relative Standard Deviation. The sample concentration is 20µg/ml.

4.3. Accuracy

Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 80%, 100% and 120% to the pre analyzed sample formulation.

4.4. Assay

The estimation of drug in pharmaceutical dosage forms AROFUTO 100mg strength was evaluated for the amount of Afloqualone present in the formulation. Each sample was analyzed in triplicate after extracting the drug. The amount of drug present in formulation was calculated by comparing the mean peak area from standard.

4.5. Intermediate Precision or Ruggedness

Inter-day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation.

4.6. Robustness

Robustness was carried out by varying two parameters from the optimized chromatographic conditions.

4.7. Specificity

The method was determined as specific by comparing test results obtained from analyses of sample solution containing excuse ingredients with that of test results those obtained from standard drug.

4.8. System Suitability Parameter

System suitability tests were carried out on freshly prepared standard stock solutions of Afloqualone and it was calculated by determining the standard deviation of Afloqualone standards by injecting standards in five replicates at 6 minutes interval and the values were recorded.

5. Formulation Analysis

5.1. Preparation Of Serum Sample Solution

From a local hospital blood was collected and serum was separated. 5ml of this serum was taken in a test tube and added 100µl of diltizem hydrochloride (1µg/ml) and 0.1ml of 1M NaOH and 5ml of dichloromethane and mixed about 20min in vortex mixer and centrifuged at 3000 rpm for 10min.

5.2. Analysis

From this centrifuged solution 4ml of organic layer was separated and evaporated to dryness to get residue. To this residue 100µl of 1M acetic acid and 3ml of n-Hexane and mixed for 5 min by vortex mixer and evaporated the organic layer and finally the remaining sample was injected into HPLC and chromatogram was recorded. The amount of drug present in the blood sample was calculated from linearity graph.

5.3. Serum data of afloqualone

Drug estimation in human serum by developed protocol:-From linearity graph we can estimate amount of drug present in the sample.

Amount of AFLOQUALONE present in serum = 4.576mg/ 5ml.

6. Conclusion

The RP-high performance liquid chromatographic method developed and validated for the analysis of Afloqualone from their formulations was found to be accurate and precise. This method is easy and more practicable compared to the methods reported in the literature. Thus, the proposed HPLC method can be successfully applied for the routine quality control analysis of Afloqualone formulations.

7. Result And Discussion

The Reverse Phase High Performance Liquid Chromatography method was developed a stability indicating assay method. Pure drugs chromatogram was run in different mobile phases containing methanol, acetonitrile, THF, and different buffers like potassium dihydrogen phosphate, sodium dihydrogen phosphate,Ortho phosphoric acid in different volumes ratios. Different columns like C_8, C_{18}, phenyl, cyano with different dimensions were used. Then retention time and tailing factor were calculated. Finally Acetonitrile and 1% Acetic acid and Water in the volume of ratio 40:40:20 v/v (P^H: 4.82) and Kromosil C_{18} analytical column was selected which gave a sharp and symmetrical peak with 1.84 tailing. Calibration graph was found to be linear at range 1µg/ml to 5 µg/ml. five different concentrations of Afloqualone in range given above were prepared and 20µl of each concentration injected in HPLC as shown in

the Figure no: 2. The slope (m) and intercept (c) obtained were found to be 60073.59 and 0.072465428.The correlation of coefficient (r^2) obtained was found to be 0.9996 as shown in the Table no: 1. It was observed that the concentration range showed a good relationship. The limit of detection for Afloqualone was found to be 10µg/ml and the limit of quantification was found to be 30µg/ml. It proves the sensitivity of the method. The Percentage assay of Afloqualone in formulation was found to be 100.59%.as shown in the Table no: 1 and figure no: 4. The relative standard deviation value obtained was below 1 which indicates the precession of the method. The validation of the proposed method was further verified by recovery studies. The data was presented by in the Table no: 2 and figure no: 3. The percentage recovery was found to be 102.92% which shows a good index of accuracy of the developed method. The amount of drug present in the human serum sample was calculated from the linearity graph was found to be 4.576mg/ 5ml as shown in Figure no: 5.

Parameters	Afloqualone
Linearity range(µg/ml)	1 – 5
Correlation coefficient (r)	0.9996
Slope (m)	60073.59
Intercept (c)	0.072465428
Limit of detection(LOD; µg/ml)	10
Limit of Quantification (LOQ; µg/ml)	30
Tailing factor	1.21
Retention time (min)	1.671
Theoretical plates	2110
(%) R.S.D	0.044
(%) Accuracy	102.92
FORMULATION ASSAY(%)	100.59
SERUM (mg/5ml)	4.576

Table:1 Optical characterization of Afloqualone

Pharmaceutical Formulation (Brand Name)	Labeled amount (mg)	Percentage Assay	Percentage recovery
AROFUTO	100 mg	100.59	102.92

Table : 2 Recovery Data of Afloqualone
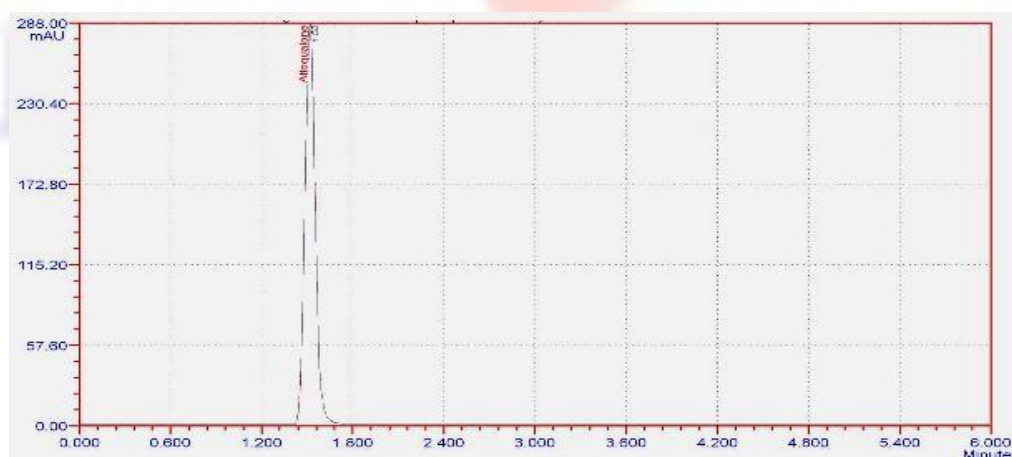
S.No	Name	Rt.	Height	Area	Conc.	Tailing factor	Theoretical plates
1	Afaloqualone	1.520	16145	52824.4	100.000	0.85	4299
			16145	52824.4	100.0000		

Fig:2 Chromatogram Of Afloqualone (Standard) and Their Results
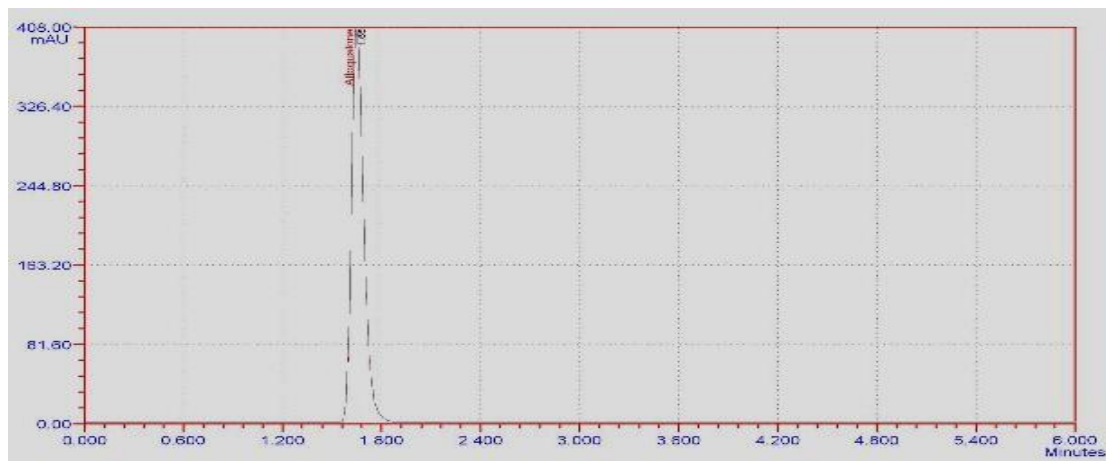
S.No	Name	Rt.	Height	Area	Conc.	Tailing factor	Theoretical plates
1	Afaloqualone	1.647	37689	175902.0	100.000	1.31	2482
		Sum	37689	175902.0	100.0000		

Fig:3 Chromatogram Of Afloqualone (Accuracy) and Their Results
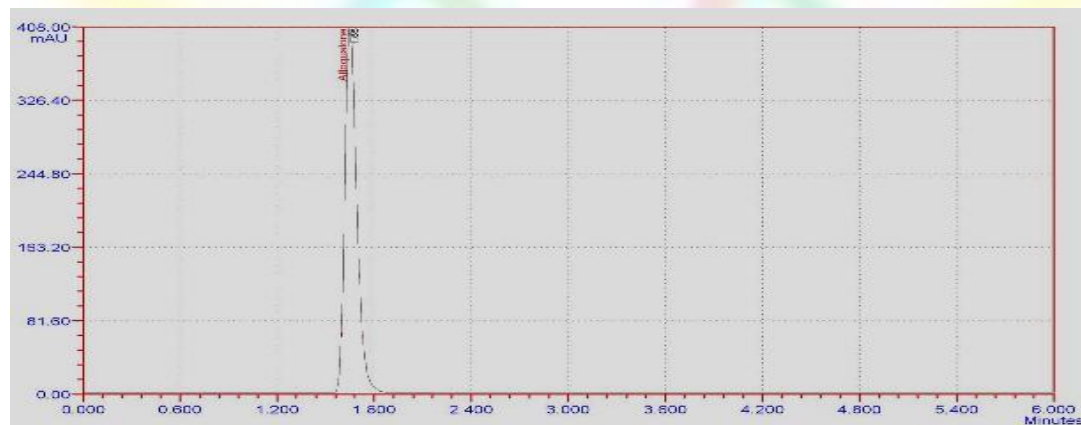
S.No	Name	Rt.	Height	Area	Conc.	Tailing factor	Theoretical plates
1	Afaloqualone	1.647	37689	175902.0	100.000	1.31	2482
		Sum	37689	175902.0	100.0000		

Fig:4 Chromatogram of Afloqualone (Formulation Assay) and their results
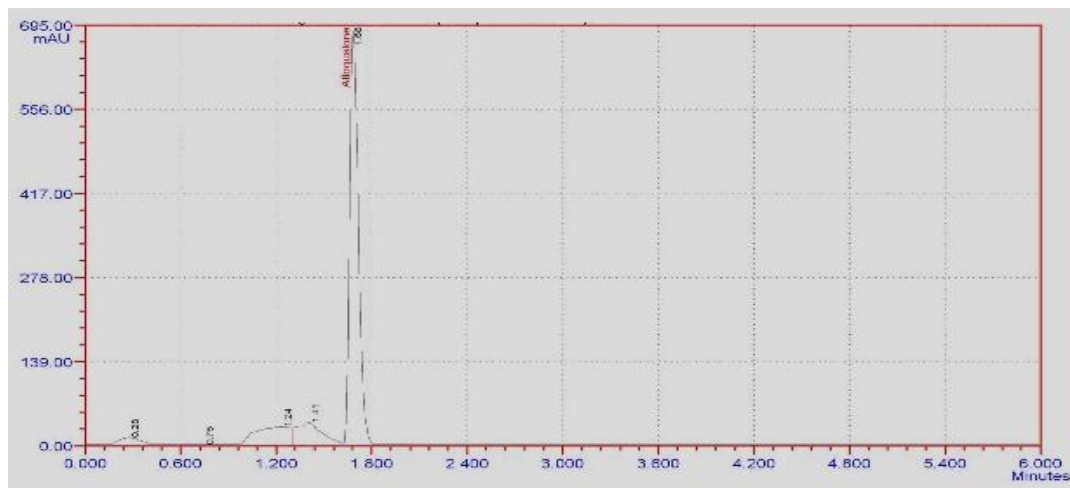
S.No	Name	Rt.	Height	Area	Conc.	Tailing factor	Theoretical plates
1	Afaloqualone	0.278	1740	24497.6	5.596	1.20	8
		0.748	1055	10279.1	2.348	1.03	118
		1.240	4075	69889.8	15.965	0.61	104
		1.412	4741	58208.0	13.296	1.46	264
		1.682	69863	274903.9	62.795	1.79	3640
	Sum		81474	437778.5	100.000		

Fig:4 Chromatogram of Afloqualone (Serum) and their results

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