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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR THE SIMULTANEOUS ESTIMATION OF ATOVAQUONE AND ACECLOFENAC

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ABSTRACT

A basic, specific, fast, exact and prudent converse stage HPLC technique has been produced for the concurrent assessment of Aceclofenac and Atavoquone from mass. The LC20AD Conspicuousness Fluid Chromatography SPD20-A Shimadzu, Japan with UV-Vis identifier and C18 section with measurement on 250×4.6 mm had been utilized for the strategy improvement with stream rate 1.0 ml/min at a 274 nm. The created strategy was approved as far as exactness, accuracy, linearity, breaking point of recognition, cutoff of quantitation. The proposed strategy can be utilized for the assessment of these medications in joined measurement structures in future. The present work emphasis on the novel techniques used till date and also guides the path for the further studies in which the work is undone. The proposed method can be used for the estimation of these drugs in biological fluids.

Key-words: HPLC, Validation, Aceclofenac, Atavoquone**.**

INTRODUCTION

Aceclofenac is a non-steroidal anti-inflammatory drug with good analgesic and anti-rheumatic properties. It is used for the various conditions like pain and inflammation in rheumatoid arthritis, osteoarthritis and enclosing spondylitis. [1-3] Aceclofenac is a NSAID that inhibits both isoforms of COX enzyme, a key enzyme involved in the inflammatory cascade. COX-1 enzyme is a constitutive enzyme involved in prostacyclin production and protective functions of gastric mucosa.

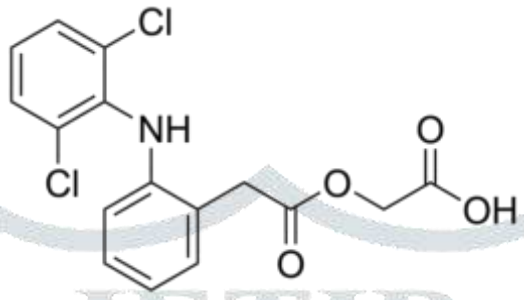

Fig. no. 01: Structure of Aceclofenac

Atovaquone, sold under the brand name Mepron, is a quinone antimicrobial medication for the prevention and treatment of *Pneumocystis jirovecii* pneumonia (PCP).

Atovaquone is a hydroxy-1,4-naphthoquinone, an analog of ubiquinone, with antipneumocystic activity.[4-8]

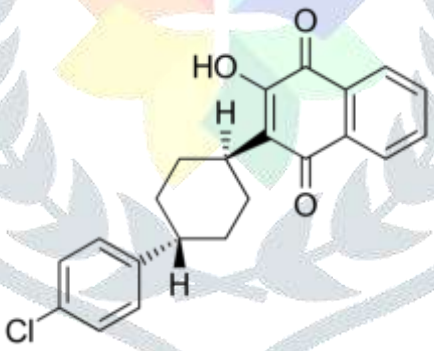

Fig. no. 02: Structure of Atovaquone

Materials and Methods:

Apparatus and Reagents

HPLC strategy will be done for Aceclofenac and Atavoquone mass. The LC20AD Conspicuousness Fluid Chromatography SPD20-A Shimadzu, Japan with UV-Vis indicator and C18 section with measurement on 250×4.6 mm will be utilized for the technique improvement with stream rate 1.0 ml/min. The strategy will be approved by ICH rules were considered. All chemicals used were analytical purpose of AR grade and all solutions prepared in distilled water. [9]

Fixed Stage

C18 section with measurement on 250×4.6 mm will be utilized.

Recognition Frequency by UV Spectroscopy [10]

Precisely gauged and moved about 10mg every one of Aceclofenac and Atavoquone working norm into a 10mL volumetric cup independently, then, at that point added to it around 10 mL of methanol and sonicated for 10 minutes to break down and weakened up to stamp with methanol. This created standard stock arrangement (1mg/mL). Further 1mL of above arrangement was moved into 10mL volumetric flagon and volume was made up with methanol. This created arrangements of fixation 100µg/mL. At last weakened 1mL of above answer for 10mL utilizing methanol and blended well. The centralization of the functioning arrangement along these lines delivered was 10µg/mL. The functioning standard arrangements of Aceclofenac and Atavoquone (10µg/mL) were looked over the scope of 190-400nm. By noticing the overlain spectra of standard arrangements λ274.0 was taken for preliminaries to foster HPLC technique.

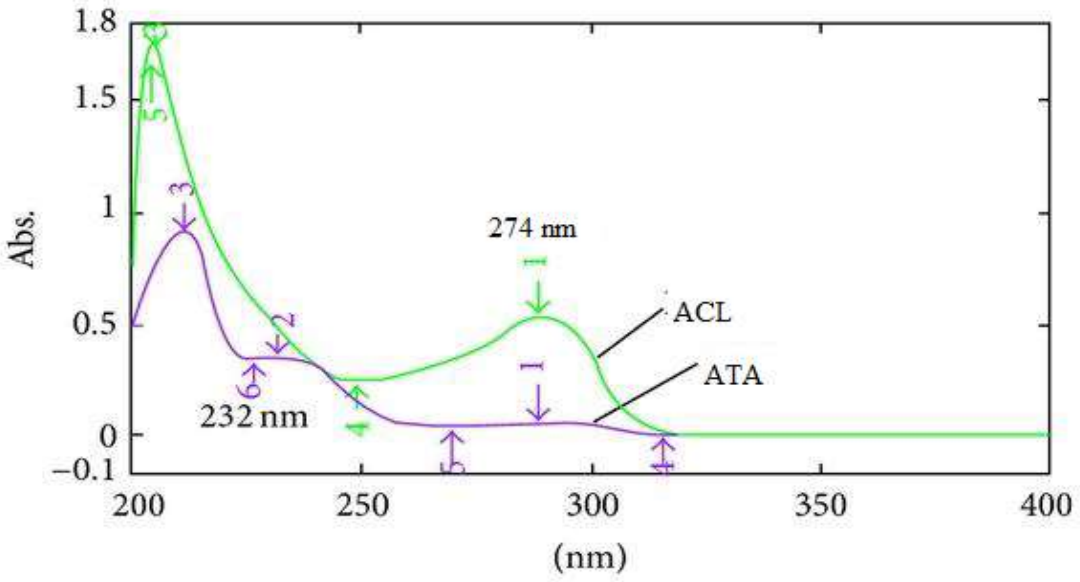

Fig. no. 03: Overlain UV Spectra for Aceclofenac and Atavoquone

Preparation of Stock Solutions [11]

The stock solution of Aceclofenac was prepared by transferring 100 mg of standard Aceclofenac in to a (100 ml) volumetric flask. The flask was sonicated for 10 min. Then volume was made up to the mark with diluent i.e. Methanol to get a solution containing concentration 1000 µg/ml Aceclofenac. The stock solutions of Atavoquone was prepared by transferring 100 mg of standard Atavoquone in to a (100 ml) volumetric flask and dilute up to 100ml with diluent to get 1000 µg/ml. Further transfer 25 ml of above stock solution in another (50 ml) volumetric flask. The flask was sonicated for 10 min. Then volume was made up to the mark with diluent i.e. mobile phase to get a solution containing 500 µg/ml Atavoquone.

Preparation of standard solutions

Withdraw appropriate volume from above Aceclofenac and Atavoquone stock solutions and transferred to 10ml volumetric flasks. The solution was adjusted to the mark with diluent i.e. mobile phase to get a final solution having concentrations 15, 20, 25, 30, 35, and 40 µg/ml Aceclofenac and 5, 10, 15, 20, 25, and 30 µg/ml Atavoquone.

Preparation of sample solution

Accurately weighed powder equivalent to 10 mg of Aceclofenac and 5 mg of Atavoquone and transferred to the 50 ml volumetric flask and add 25 ml mobile phase. The solution was sonicated for 15 min and solution was diluted up to 50 ml with mobile phase. The final resulting solution was filtered through Whatmann paper (pore size 0.45 µm). The solution contains 200 µg/ml of Aceclofenac and 100 µg/ml of Atavoquone. From the final resulting solution adequately transfer 10 ml and diluted up to 100 ml with diluent i.e. mobile phase, resulting solution expected to contain 100 µg/ml Aceclofenac and 50 µg/ml Atavoquone.

Method Validation

System Suitability Parameters:

The area of respective concentrations, theoretical plates, number of theoretical plates per height and the peak symmetry was recorded.

Linearity

Solution preparation for Linearity-

Atavoquone

From the stock solution of 100 ppm concentration a range of solution was prepared with a concentration of 5, 10, 15, 20, 25, and 30 µg/ml Atavoquone.

Aceclofenac

From the stock solution of 100 ppm concentration a range of solution was prepared with a concentration of 15, 20, 25, 30, 35, and 40 µg/ml Aceclofenac.

Precision

Precision of analytical method was studied by multiple injections of homogeneous samples. 6 replicate of 10 ppm solution were prepared and injected for precision at the same flow rate of 1.0 ml/min. The inter-day and intermediate precisions were used to study the variability of the method S.D. and %R.S.D. was calculated for both.

Robustness

Robustness was studied by changing parameters like change in flow rate. The S.D. and %R.S.D. between the change parameter were calculated.

Ruggedness

Ruggedness study was carried out by using different analysts. The S.D. and %R.S.D. were calculated.

Recovery

Recovery of the method was studied using the method of standard addition. Standard Atavoquone and Aceclofenac solutions were added to the unknown bulk and tablet formulation of Atavoquone and Aceclofenac. The percent recovery was determined at three different levels (50%, 100%, and 150%). Impurity content was determined and the percent recovery was calculated.

LOD and LOQ

Limit of detection and limit of Quantitation of the method was calculated by formula given below,

LOD= 3.3×S.D/Slope

LOQ=10×S.D./Slope

RESULT AND DISCUSSION

SYSTEM SUITABILITY STUDIES

The column efficiency, resolution and tailing factor were calculated for the standard solutions (table no. 05). The values obtained demonstrated the suitability of the system for the analysis of the selected drug combinations. System suitability parameters may fall within 2% relative standard deviation range during routine performance of the method. The chromatogram is in the figure no. 05 and system suitability parameter is in the table no. 01.

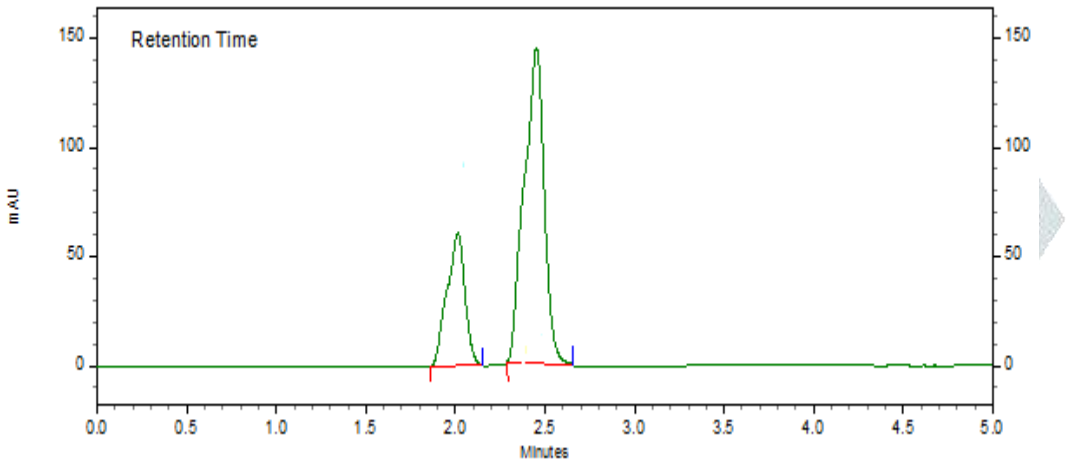

Fig. no. 04 HPLC chromatogram of Aceclofenac and Atavoquone

Table no. 01: System Suitability

Parameters	Aceclofenac	Atavoquone
Retention time (Min)	2.0	2.5
Theoretical plate	7531	10352
Tailing factor	1.2	1.02
Resolution	3.52	

CALIBRATION CURVE (LINEARITY) AND RANGE:

The linearity of the method was determined at six concentration levels. The calibration curve was constructed by plotting response factor against concentration of drugs. Aceclofenac and Atavoquone exhibited linearity of the concentration range of 5-30µg/ml and 15-40 µg/ml. Plot the graph for Area vs. Concentration to get calibration curve.

Table no. 2: Calibration curve of Aceclofenac

Conc. (ppm)	Peak area
5	151005
10	286771
15	456431
20	629274
25	776194
30	955951

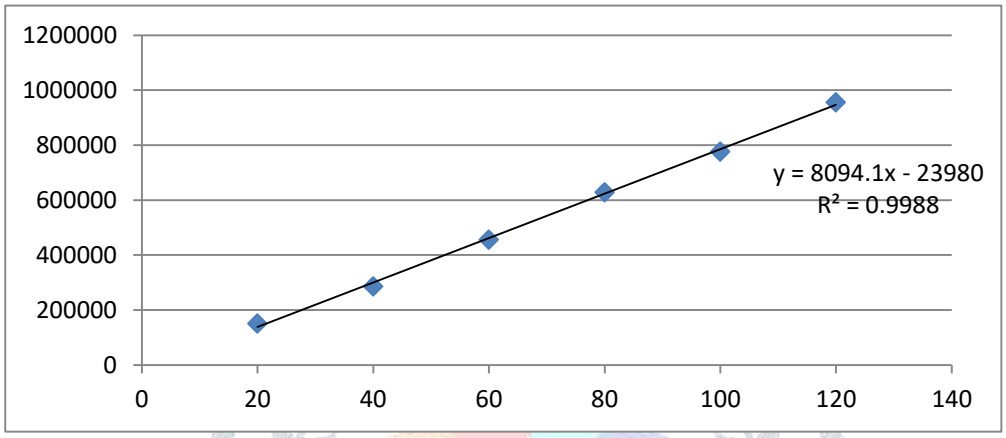

Fig. no. 06: Calibration curve of Aceclofenac

Table no. 3: Calibration curve of Atavoquone

Conc. (ppm)	Peak area
15	385105
20	742803
25	1120592
30	1516931
35	1886593
40	2269990

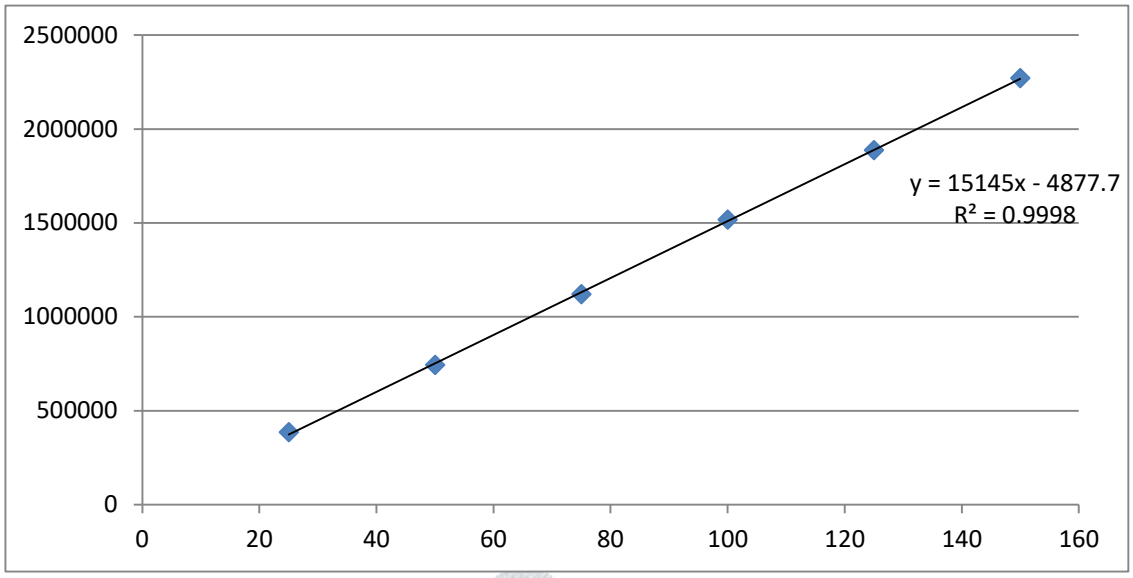

Fig. no. 07: Calibration curve for Atavoquone

RECOVERY STUDIES

Accuracy of the methods was assured by use of the standard addition technique, involving analysis of formulation samples to which certain amounts of authentic drugs were added. The resulting mixtures were assayed, and the results obtained for both drugs were compared to those expected. The good recoveries with the standard addition method prove the good accuracy of the proposed methods. (Table no. 04)

Table no. 04: Recovery data of Aceclofenac and Atavoquone

Drug	Accuracy level (%)	Amount of drug taken (ppm)	Amount of API added (ppm)	Total amount Found (ppm)	% Recovery
Aceclofenac	50	5	2.5	2.48	99.20
	100	5	5	4.87	97.4
	150	5	7.5	7.38	98.40
Atavoquone	50	10	5	4.95	99.00
	100	10	10	9.98	99.80
	150	10	15	14.84	98.93

PRECISION

The precision of the method was demonstrated by inter- day and intra- day variation studies.

Intra-Day Precision: The intra-day was determined by six replicates of the prepared sample solutions. The repeatability of sample application and measurement of peak area for the drugs were calculated by assay six times at three different concentration levels of 5, 10 and 15 ppm for Aceclofenac and 15, 20, and 25 ppm for Atavoquone in the same day at 30 minutes time interval for intra-day precision. Peak areas were determined and % RSD was calculated and found to be less than 2% and it is presented in table no. 05.

Table no. 05: Intraday precision data of Aceclofenac and Atavoquone

Aceclofenac			**Atavoquone**		
Conc. (ppm)	**Avg. Area**	**%RSD**	**Conc. (ppm)**	**Avg. area**	**%RSD**
5	427535	1.587	15	960587	1.851
10	751425	1.857	20	1685457	1.673
15	912536	1.185	25	1857452	1.167

Inter-day Precision: The inter-day precision was determined by six replicates of the prepared sample solutions. The inter-day precision of sample application and measurement of peak area was obtained by the assay of six sample sets on different days at three different concentration levels of 5,10,15 ppm for Aceclofenac and 15,20,25 ppm for Atavoquone represented in table no. 06.

Table no. 06: Inter-day precision data of Aceclofenac and Atavoquone

Aceclofenac			**Atavoquone**		
Conc. (ppm)	**Avg. Area**	**%RSD**	**Conc. (ppm)**	**Avg. area**	**%RSD**
5	435685	0.852	15	958147	0.895
10	742536	0.698	20	1647824	0.678
15	892547	0.579	25	1859763	0.684

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION:

The limit of detection (LOD) and quantitation (LOQ) for both Aceclofenac and Atavoquone were determined according to ICH Guidelines. LOD was defined as 3.3σ/S and LOQ was 10σ/S based on "standard deviation of the response and slope" of the calibration curve specially constructed in a low region of 0.05 to 1% of the target analyte concentration. The standard deviation of the y-intercepts of the regression lines was used as σ (the standard deviation of the response) and S is the slope of the calibration curve. The LOD and LOQ values for the Aceclofenac and Atavoquone are presented table no. 7.

Table no. 07: Result for LOD and LOQ

Sr. no.	Drug	LOD	LOQ
1	Aceclofenac	0.58	1.52
2	Atavoquone	0.62	1.69

ROBUSTNESS

To evaluate the robustness of the developed method, deliberate variations were made in the method parameters such as Column Temperature, Flow Rate and Mobile phase composition. It was observed that there were no marked changes in the analytical performance of the method. The results presented indicate that the low values of % RSD (< 2) of % drug content obtained after introducing small changes in the method parameters were indicative of the robustness.

Table no. 08: Data for Robustness (At Different Flow Rate)

Drug Sample	Flow rate(ml/min)	Area	Mean	SD	%RSD
Aceclofenac	**0.9**	1285756	1284325	1547.6	0.258
	1.0	1282564			
	1.1	1284657			
Atavoquone	**0.9**	395864	397308	1152.34	0.387
	1.0	398675			
	1.1	397387			

CONCLUSION

The current investigation exhibited an approved Opposite Stage Superior Fluid Chromatography technique for the assessment of Aceclofenac and Atovaquone. Writing survey uncovered that solitary few instrumental techniques have been accounted for to decide Aceclofenac and Atovaquone independently in definition. The extent of the current work is to develop the direct and advanced of the chromatographic conditions and RP-HPLC strategy for the assessment of medication in mass. The technique was totally approved and showed good outcomes. Maintenance time and runtime was diminished, so the created technique can be utilized for synchronous examination of Aceclofenac and Atovaquone.

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