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Development and Validation of Analytical Method for Estimation of Antimalarial Drug in Solid Dosage form by HPLC

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Abstract: *A simple, precise, accurate, and robust reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of antimalarial drug in solid dosage form. The chromatographic separation was achieved using a C18 analytical column with an appropriate mobile phase composed of buffer and organic solvent under optimized chromatographic conditions. The mobile phase was pumped at a constant flow rate, and detection was carried out using a UV detector at a suitable wavelength selected based on the maximum absorbance of the drug.*

The developed method showed good separation with a well-resolved peak and acceptable retention time. The method was validated according to the guidelines of the International Council for Harmonisation for parameters including specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantitation (LOQ). The calibration curve exhibited excellent linearity over the selected concentration range with a satisfactory correlation coefficient. Recovery studies confirmed the accuracy of the method, while precision studies demonstrated low percentage relative standard deviation, indicating good repeatability and intermediate precision.

The proposed analytical method was found to be reliable, reproducible, and suitable for routine quantitative analysis of antimalarial drug in pharmaceutical tablet dosage forms. The simplicity and cost-effectiveness of the method make it appropriate for quality control laboratories and pharmaceutical industries..

Keywords: *RP-HPLC*

I. INTRODUCTION

Analytical chemistry deals with methods for determining the chemical composition of samples of matter. Analytical Chemistry plays an important role in the resolution of a chemical compound into its proximate or ultimate parts, determination of its elements or of the foreign substances it may contain. Its application extends to all parts of an industrial society.[1-6]

HISTORY OF ANALYTICAL CHEMISTRY

Analytical chemistry has been important since the early days of chemistry, providing methods for determining which elements and chemicals are present in the world around us. The first instrumental analysis was flame emissive spectrometry developed by Robert Bunsen and Gustav Kirchhoff who discovered rubidium (Rb) and caesium (Cs) in 1860. Most of the major developments in analytical chemistry took place after 1900. During late 20th century analytical chemistry found wide application in forensic, environmental, industrial and medical field.

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Importance of Analytical Chemistry:

It finds numerous applications in various disciplines of chemistry.

It finds wide applications in other fields of related sciences.

Analytical chemistry is concerned with chemical characterization of matter, both qualitative and quantitative.

Qualitative analysis deals with the identification of elements, ions or compounds present in the sample.

Quantitative analysis

Quantitative analytical measurement plays a vital role in many research areas in chemistry, biochemistry, biology, geology and other sciences. It deals with the determination of how much amount of one or more constituents are present in the sample.[1-6]

METHOD DEVELOPMENT

Method development is a challenging and time-consuming process requiring much experience, creativity, logical thinking, and experimentation. With all the software and automated systems available today, method development is still very much a trial-and-error approach, expedited by a logical sequence of generic scouting runs and fine-tuning steps to achieve the requisite resolution and method performance.[7]

EXPERIMENTAL WORK

Material and instruments:

Materials:

The drugs used for the present investigation were obtained from Arrow Chem Mumbai.

Details of Pure drug:

Table No. 8: Details of API

Drug	Supplied by	Quantity	Purity (Assay)
Tafenoquine	Arrow Chem Mumbai.	10 g	99.8 % w/w

Marketed Preparation:

Table No. 9: Details of marketed Preparation

Brand Name	Mfd by	Content	Quantity
Krintafel	GSK	Krintafel 100 mg	10 Tablets

The marketed preparation was obtained from local market and is referred here after in this thesis by the name as such.

Reagents and chemicals:

All reagents and chemicals used were of AR grade and HPLC grade.

Methanol (HPLC grade).

Acetonitrile (HPLC grade)

Potassium dihydrogen phosphate (AR grade).

Distilled Water (HPLC grade).

Ortho Phosphoric Acid (HPLC grade).

Instruments:

Table No. 10: Instruments Used

Sr. No	Instruments	Make	Model
1	**HPLC**	Waters 600	996 PDA Detector
2	**pH Meter**	Hanna	-

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3	**Balance**	Citizen	CY 104 (Micro Analytical Balance)
4	**Ultra Sonicator**	-	1.5 L 50

Study of Functional Group by Using Infra Red Spectroscopy: Determination of wavelength maxima

An accurately weighed quantity of tafenoquine (10 mg) was transferred into a 100 mL volumetric flask and dissolved in methanol. The volume was made up to the mark with methanol to obtain a stock solution of 100 µg/mL.

Further dilution was carried out to obtain a working solution of 10 µg/mL.

The solution was scanned in the UV range of 200–400 nm using methanol as blank. The maximum absorbance (λmax) of tafenoquine was found to be approximately 255 nm, which was selected for further analysis.

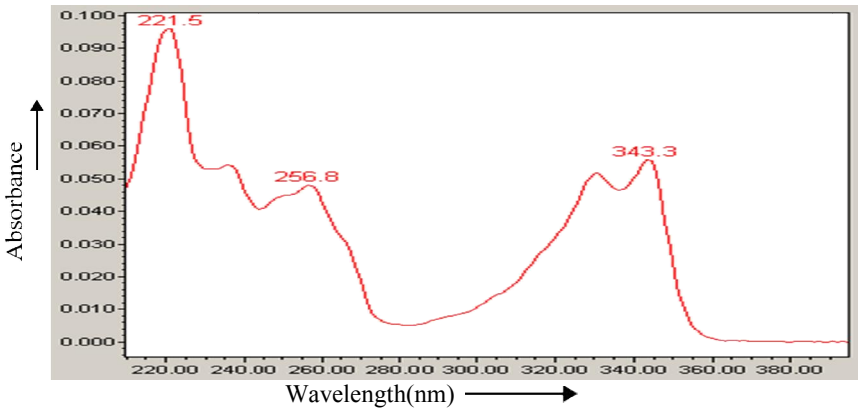

Fig. No. 8: Wavelength Maxima for Tafenoquine.

Development of HPLC method for estimation of Tafenoquine

Method Development Strategy:

Selection of Common Solvent (Diluents)

Methanol was selected as the common solvent for preparation of standard and sample solutions based on its good solubility for tafenoquine. Further dilutions were carried out using the mobile phase.

Preparation of standard stock solution:

Accurately weighted 100 mg Tafenoquine was dissolved in 100ml Methanol. This solution was used as standard stock solution.

Preparation of diluent:

Methanol of HPLC grade were selected as common solvent for preparation of stock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in the Mobile phase.

Procedure:

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The standard solution containing TFQ was injected in different combinations of solvents, to get a stable peak with good peak characters. Each solution was filtered through Membrane filter (size 0.15µ). To achieve peaks with good symmetry various mobile phase compositions were evaluated to achieve acceptable separation using selected chromatographic conditions. The following chromatographic conditions were established by trial and error and were kept constant throughout the method.

Chromatographic Parameters:

Column: C18 (Phenomenex) /4.6 x 250 mm

Flow Rate: 1.0ml/min

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Wavelength: 255 nm
Injection volume: **20µl**
Column oven Temperature: Ambient (25^{0}C)
Run Time: 10 minutes
Mobile Phase: 10mM Phosphate buffer pH 2.5 : ACN (80 : 20 % v/v)
volumetric flask and makeup the volume upto the mark with HPLC water.

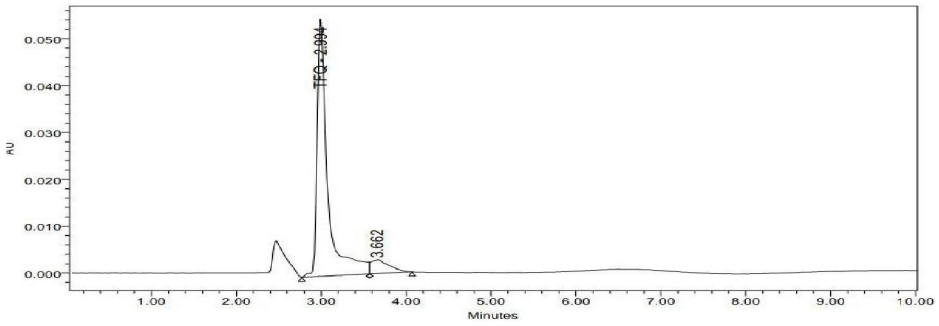

Fig. No. 9: Separation of TFQ in selected mobile phase showing retention time at 2.99 min.

System suitability studies

System suitability is a pharmacopeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The test was performed by collecting data from 5 replicate injections of standard solutions.

The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. Standard working solution of TFQ was injected five times under optimized chromatographic conditions. System suitability parameters were recorded and reported.

B) Procedure:

Filtered mobile phase was allowed to equilibrate with L std. drug solution□stationary phase until steady baseline was obtained. A 20 was injected which was made in five replicates and the system suitability parameters were recorded.

Table No. 11: Result of System suitability test

Sr. No	Peak area	Retention Time	Symmetry	No. of theoretical Plates
	TFQ	TFQ	TFQ	TFQ
1	147820	3.17	1.20	7810
2	159112	3.55	1.10	7875
3	148950	3.88	1.25	7860
4	150780	3.75	1.00	7790
5	149600	3.29	1.20	7818
Mean	149872	3.43	1.15	7825
S.D	551.61	0.057	0.1	46.07
%R.S.D.	0.34	0.47	1.5	0.48

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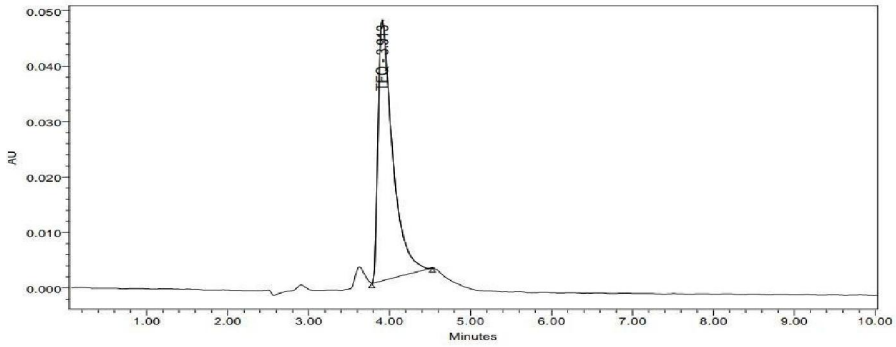

Fig. No 10: Separation of TFQ in selected mobile phase showing retention time at 3.91 min.

Application of proposed method for estimation of TFQ in Solid Dosage: Standard stock solution:

a) Preparation of standard solutions:

Tafenoquine standard stock solution: Accurately weighed quantity of 2.00 mg TFQ was dissolved in Methanol and volume was made up to 100 ml mark by same to obtain 20 µg/ml stock solution.

Tafenoquine standard working solution: Pipette out 1 ml from standard stock solution and dilute it with 10 ml methanol to obtain 2.0µg/ml of TFQ.

Sample solution preparation:

Entire content of Krintafel (100 mg) was transferred to a 10 ml volumetric flask, the volume was made upto the mark with methanol, the resultant concentration was 1000 µg/ml. The whole content was centrifuged at 5000 rpm for 10 min followed by passing through 0.45 µ membrane filter. 1 ml of resultant was transferred to a 10 ml volumetric flask and the volume was made upto the mark with methanol, the concentration of working sample solution was 100 µg/ml.

Procedure:

Equal volume L)□(20 of standard and sample solution were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response

i.e. peak area of major peaks were measured. The content of TFQ was calculated by comparing a sample peak with that of standard.

sample (mg)

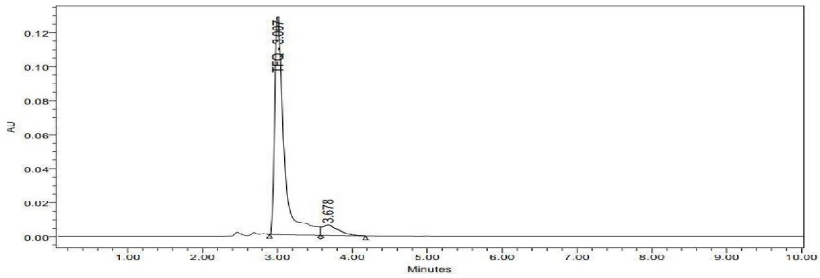

Fig. No.11: Chromatogram of TFQ marketed formulation showing retention time 3.00 min.

Validation parameters:

- Accuracy
- Robustness
- Linearity and range
- Specificity
- Placebo Interference study

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Accuracy:

The accuracy samples were prepared by spiking the standard into the pre-analyzed formulation sample at different concentrations (80%,100% and 120%) and injected each in triplicate. The resultant mix was injected and recovery of standard spiked was calculated.

The % Recovery was then calculated by using formula

% Recovery = A-B x 100/ C

Where- A = Total amount of drug estimated.
B = Amount of drug found on pre analyzed basis.
C = Amount of pure drug added.

Calculate the amount recovered, % recovery, average recovery, % RSD of triplicate sample preparation, overall recovery and overall % RSD. Record the observation into the following table.

	TFQ		
	Levels		
	80%	100%	120%
Amt added (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt taken (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt recovered (µg/ml)	79.90	99.95	119.95
	79.95	99.98	119.98
	79.98	99.97	119.99
% Recovery	98.33	99.33	99.44
	98.33	98.66	98.88
	99.16	99.33	100.00
Mean % recovery	98.60	99.10	99.44
% RSD	0.48	0.39	0.56

Table No 12: Accuracy studies by standard addition method

Acceptance criteria:

The % RSD for the triplicate at each spike level shall be NMT 2.0.

The overall % RSD for % recovery for all spike levels shall be NMT 2.0.

The % recovery at each spike level shall be NLT 98.0 and NMT 102.0 of the added amount.

Robustness:

Effect of Variation in flow rate of mobile phase by ± 10%:

Prepared the system suitability solution (Standard Preparation) and inject into the HPLC system at –10% flow rate (0.9mL/min) and +10% flow rate (1.1mL/min) when compared with the Test method flow rate.

Procedure: Injected standard solution into the HPLC System in normal conditions and followed by the robust conditions. Measured the peak response for the major peaks.

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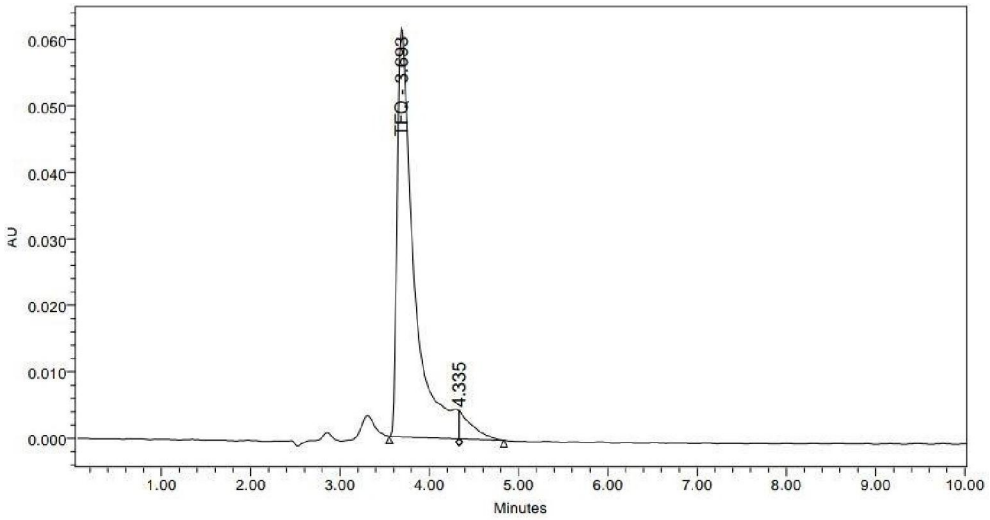
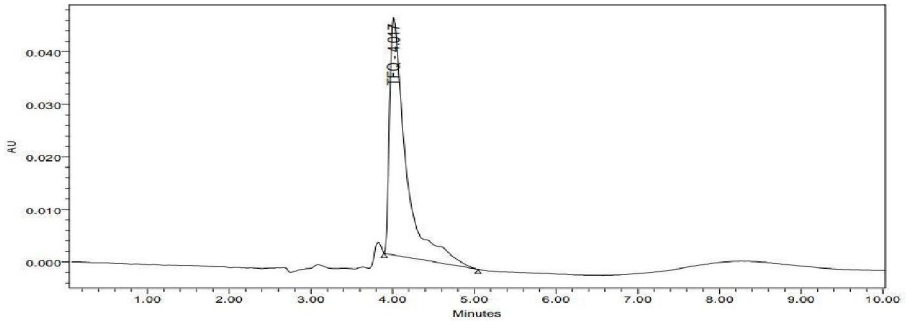


Flow 0.9 ml/min



1.1 ml/min

Fig. No. 12: Chromatograms of Change in Flow Rate

Checked the system suitability and recorded the results in the table.

Sr. No.	System Suitability parameter		Observations for flow rate			Limits
			Unchanged	0.9 ml	1.1 ml	
1	The % RSD of peak area response for five replicate injections	TFQ	1.027	0.92	0.85	NMT 2.0
2	Theoretical plates	TFQ	7805	7810	7805	NLT 2000
3	Tailing factor	TFQ	1.28	1.91	1.10	NMT 2.0
4	Retention Time (Min)	TFQ	3.82	3.69	4.01	

Table No. 13: System suitability of change in Flow Rate

Observation: The allowable variation in flow rate of the method is from 0.9ml/min to 1.1 ml/min

Acceptance criteria: All the system suitability parameters shall pass

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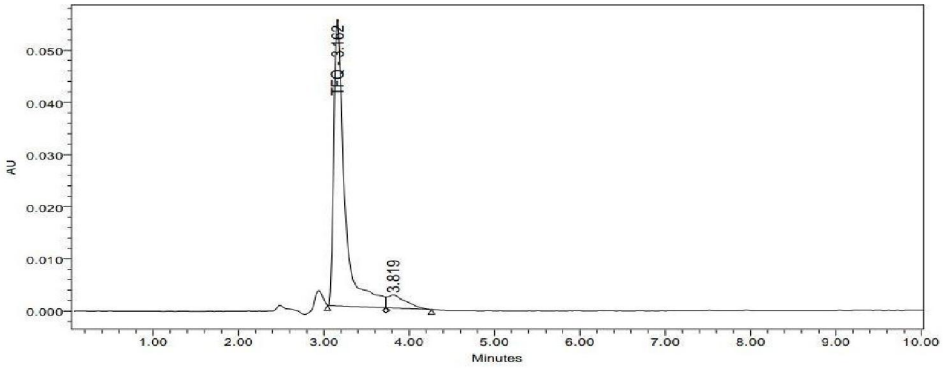
Change in organic composition of mobile phase + 10% (Phosphate Buffer: ACN)

System suitability dilution was prepared and injected into the HPLC system at –10% and + 10 % ACN (Organic phase) compared with the optimized method mobile phase concentration.

Procedure:

Injected standard solution into the HPLC system in normal conditions and followed by the robust conditions. Measure the peak response for the major peaks. Check the system suitability and record the results in the table.

-10% (Phosphate buffer: ACN 77:23)



+10%(Phosphate buffer: ACN 83:17)

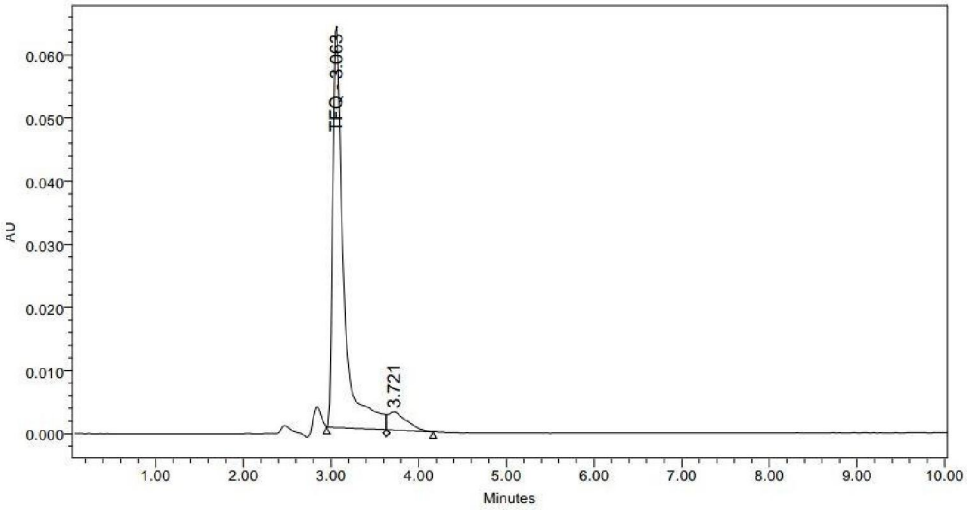


Fig. No. 13: Chromatograms of Change Organic Composition of mobile Phase

Sr. No.	System Suitability parameter		Observations			Limits
			Unchanged	- 10%	+ 10%	
1	The % RSD of peak area response for five replicate injections	TFQ	1.017	0.655	0.046	NMT 2.0
2	Theoretical plates	TFQ	7825	7865	7820	NLT 2000
3	Tailing factor	TFQ	1.28	1.166	1.08	NMT 2.0
4	Retention Time (Min)	TFQ	3.20	3.16	3.06	

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Table No. 14: System suitability of change in Organic Composition Observation: The allowable variation in ACN composition of method is from 90% to
110%. **Acceptance criteria:** 1. All the system suitability parameters shall pass.

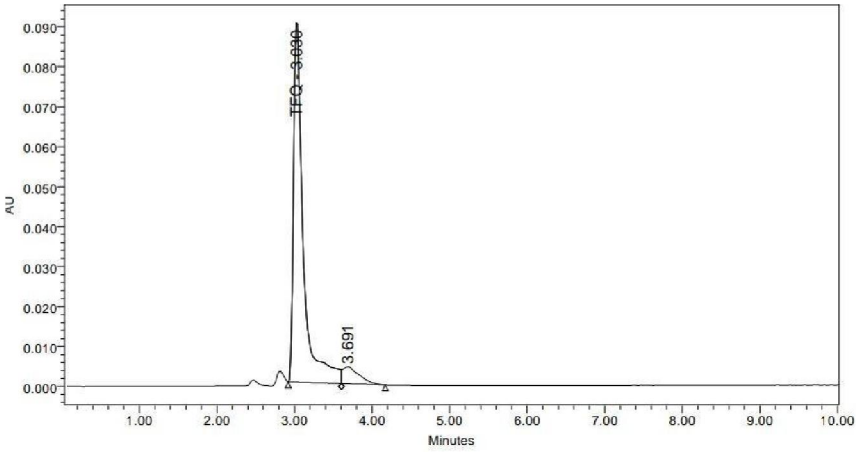
Effect of Variation in Wavelength by ± 2 units:

Prepared the system suitability solution (Standard Preparation) and inject into the HPLC system. Measure the peak area response at different wavelengths at flow rate 1 ml/min.

Procedure:

Injected standard solution into the HPLC System in normal conditions and followed by the robust conditions. Measure the peak response for the major peaks.

At 253 nm wavelength



At 257 nm wavelength

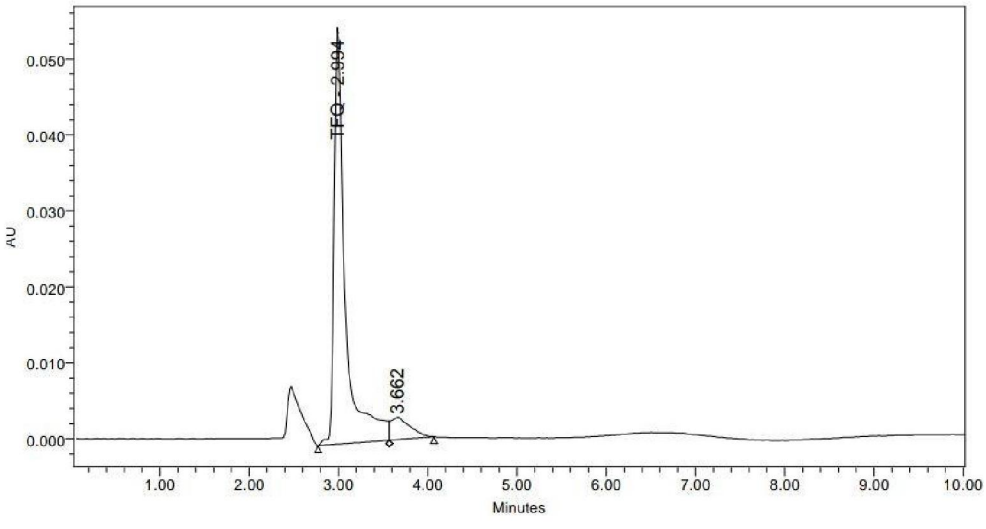


Fig. No. 14: Chromatograms of Change in wavelength.

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Sr. No.	System Suitability parameter		Observations for wavelength			Limits
			Unchanged	253nm	257nm	
1	The % RSD of peak area response for five replicate injections	TFQ	1.017	0.3638	0.141	NMT 2.0
2	Theoretical plates	TFQ	7865	7987.9	7810	NLT 2000
3	Tailing factor	TFQ	1.06	1.00	0.94	NMT 2.0
4	Retention Time (Min)	TFQ	3.00	3.03	2.99	

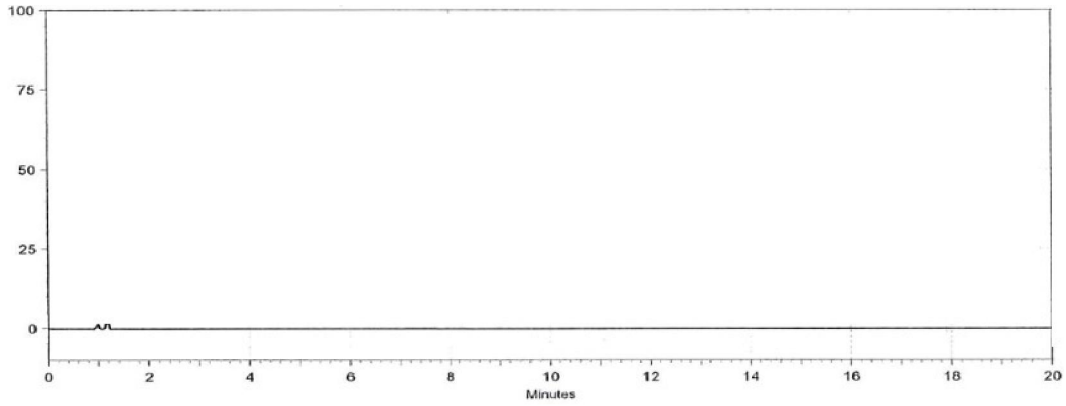
Table No.15: System suitability of change in wavelength

Specificity:

Placebo Interference study:

Prepared the placebo solution by weighing equivalent amount of placebo present in the sample to be taken for assay preparation in triplicate, diluted it as per the test method and injected into the HPLC system. Evaluate the % interference from placebo and recorded the observation.

Sample matrix



Placebo Preparation

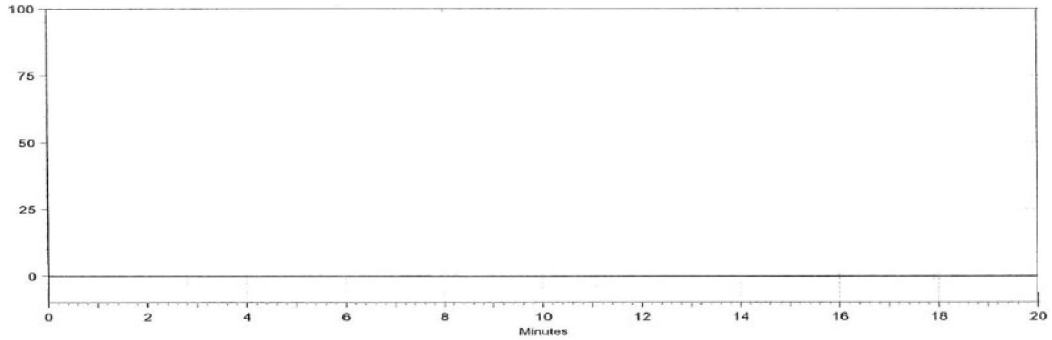


Fig. No. 15: Chromatograms of placebo interference study

Observation	Placebo prep.1	Placebo prep.2	Placebo prep.3
% Interference	No Interference	No Interference	No Interference

Table No. 16: Placebo Interference

Acceptance criteria:

No interference should observe from placebo at the retention time of TFQ

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Linearity and range:

Prepared the series of standard concentrations ranging from 50 % to 150 % of the targeted concentration of TFQ Each of the linearity dilution was injected into the HPLC system with optimized chromatographic parameters.

Procedure:

Separately inject standard preparation and linearity preparations into the HPLC system, record the chromatograms and measure the peak responses for TFQ peaks.

The details of mean peak areas for linearity concentrations are presented in following table and plot the graph of concentration verses average area response for TFQ. the correlation coefficient and equation of regression were recorded.

Sr. No.	% Level	TFQ	
		Conc. (µg/ml)	Mean peak area
1	80	80	119850
2	100	100	14990
3	120	120	178040
4	160	160	236720
5	180	180	266580

Table No.17:- Observations of Linearity and range study for TFQ

Acceptance criteria: The correlation coefficient shall be NLT 0.99

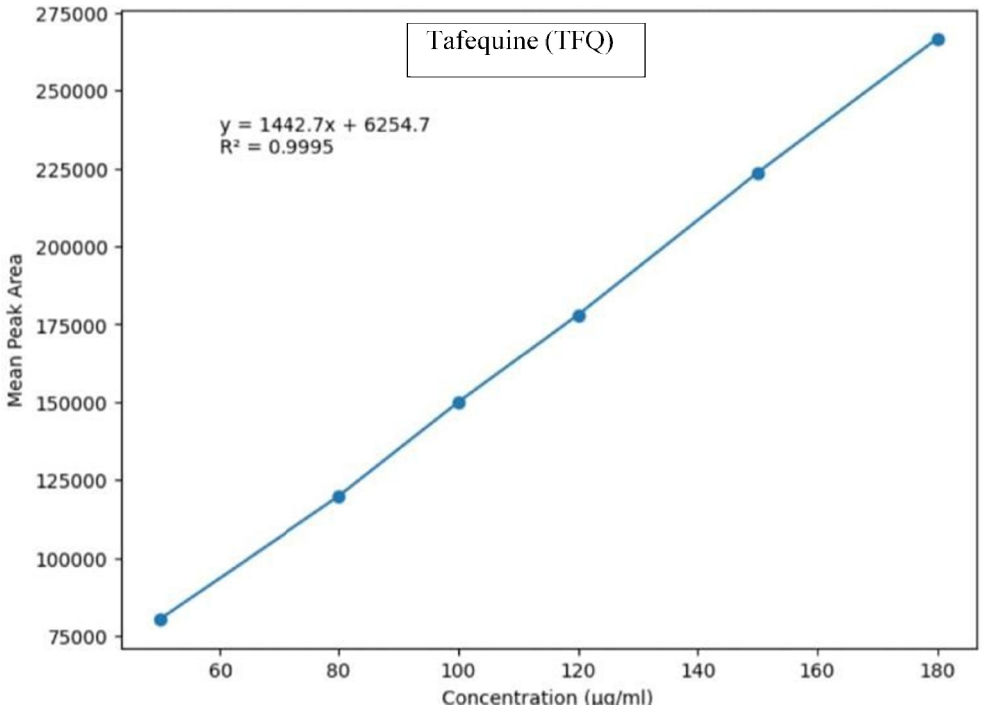

Fig. No. 16: Plot of linearity and range study for TFQ

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II. RESULT AND DISCUSSION

High Performance Liquid Chromatography which is a highly sophisticated technique, it is used for the determination of active pharmaceutical ingredients from their formulations. In the present study, an HPLC method was developed for the analysis of Tafenoquine from its solid dosage form.

Tafenoquine is a long-acting antimalarial drug belonging to the 8-aminoquinoline class and is mainly used for the radical cure and prevention of malaria caused by Plasmodium vivax. Tafenoquine possesses lipophilic characteristics and shows limited aqueous solubility; therefore, careful optimization of chromatographic conditions is required to obtain a sharp, symmetrical, and well-resolved peak. In the present investigation, an attempt has been made to develop a simple, rapid, accurate, precise, and reproducible RP-HPLC method for the estimation of Tafenoquine in pharmaceutical formulation.

Pure Tafenoquine standard was procured from a certified supplier, and the percentage purity of the drug was found to be within acceptable limits as specified by the supplier. The drug sample was prepared using a suitable solvent system and further diluted with the mobile phase for chromatographic analysis. The chromatographic separation was carried out using a reversed-phase C18 column, as this stationary phase provides efficient retention and separation for lipophilic compounds such as Tafenoquine.

A suitable mobile phase consisting of an organic solvent and buffer system was optimized to achieve proper peak symmetry, minimum tailing, and satisfactory resolution within a short retention time. Detection was carried out using a UV detector at an appropriate wavelength where Tafenoquine exhibited good absorbance. The optimized chromatographic conditions produced a well-defined peak without interference from excipients present in the formulation.

The developed RP-HPLC method was validated according to ICH guidelines for parameters such as accuracy, linearity, robustness, specificity, and system suitability. The method showed good linearity over the selected concentration range with a correlation coefficient close to 1. The percentage recovery was found to be within acceptable limits, indicating the accuracy of the method. Precision studies demonstrated low percentage relative standard deviation values, confirming the reproducibility of the method.

The developed analytical method was found to be simple, sensitive, economical, and reliable for routine quantitative estimation of Tafenoquine in solid dosage forms and can be successfully applied in quality control laboratories for regular analysis of the drug formulation.

Pure standards of TFQ were procured from the Arrow chem Mumbai. Percent purity of above mentioned drugs were reported by Supplier Company as follows-

Table No. 18: Details of API

Drug	Supplied by	Quantity	Purity (Assay)
Tafenoquine	Arrow Chem Mumbai.	10 g	99.8 % w/w

These were not analyzed in our study and the % purity stated by the suppliers was taken as standard for comparison studies.

RP-High Performance Liquid Chromatography (HPLC) Method:

HPLC has gained the valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature. This technique is commonly used for the quantitative estimation of the drugs from their formulation as well as for studying their metabolites of drugs and their estimation in their biological fluids. This method offers advantages of estimating the constituents for the multi component system. This technique was employed in the present investigation for estimation of TFQ in solid formulation. Careful evaluation of various parameters influencing analysis is an important aspect for the development of analytical method. In order to establish RP-HPLC method the following parameters were studied.

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HPLC Column Selected:

HPLC Waters 600 system with C18 (Phenomenex) /4.6 x 250 mm column and PDA detector were used for the study. The standard and sample solution of TFQ were prepared in diluent. Different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram.

Mobile Phase selected:

Mobile phase composed of 10mM Phosphate buffer pH2.5 and acetonitrile(80:20 % v/v). An isocratic program was developed contributing a total run time of 10 min. The wavelength 255 nm was selected for the evaluation of the chromatogram of drugs. The selection of the wavelength was based on the λ max obtained by scanning of standard solution. This system gave good resolution and optimum retention time with appropriate tailing factor(<2). The mean values of system suitability test result are depicted in Table below. The following chromatographic conditions were established by trial and error and were kept constant throughout the method.

Table No. 19: Chromatographic Parameters:

Column	C18 (Phenomenex) /4.6 x 250 mm
Flow Rate	1ml/min
Wavelength	255 nm
Injection volume	20µl
Column oven Temperature	Ambient
Run Time	10 minutes
Mobile Phase	Mixture of 10mM Phosphate buffer pH2.5 & Acetonitrile(ACN) in ratio 80:20 % v/v

Mobile phase-preparation

For 1000ml of Phosphate buffer preparation Firstly weigh 0.272 mg of KH2PO4 dissolve in 1000ml of HPLC grade water.Using a 1MOPA adjust the pH2.5. After preparation of Buffer solution sonicate 10-15 minutes. And form Mobile phase 10mM Phosphate buffer pH2.5:ACN(80:20% v/v).Purpose for the Removal of air bubbles and Avoid pressure fluctuations Ensure smooth flow in HPLC.

Preparation of diluent:

Methanol of HPLC grade were selected as common solvent for preparation of stock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in the Mobile phase

Table No. 20: Summary of system suitability of Test results

Sr. No	**Peak area**	**Retention Time**	**Symmetry**	**No. of theoretical Plates**
	TFQ	**TFQ**	**TFQ**	**TFQ**
1	147820	3.17	1.20	7810
2	159112	3.55	1.10	7875
3	148950	3.88	1.25	7860
4	150780	3.75	1.00	7790
5	149600	3.29	1.20	7818
Mean	149872	3.43	1.15	7825
S.D	551.61	0.057	0.1	46.07
%R.S.D.	0.34	0.47	1.5	0.48

Thus the results obtained for such method are given as follow:

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After establishing the chromatographic conditions, Mix standard and marketed preparation solutions were prepared and analyzed by procedure described under experimental work. It gave accurate, reliable results and was extended for estimation of drugs in marketed tablet formulation.

Amount of drug in tablet was calculated using following formula:

$$\text{Assay (mg/ml)} = \frac{A_t}{A_s} \times \frac{D_s}{D_t} \times \frac{W_s}{W_t} \times \frac{p}{100} \times \text{Wt mg/ml of test sample}$$

$$\% \text{ Label claim} = \frac{\text{Assay (mg/ml)}}{\text{Label claim in mg/ml}} \times 100$$

Where,

At = Area count for sample solution. As =Area count for standard solution. Ds = Dilution factor for standard.

Dt = Dilution factor for sample. P = Potency of drug

VALIDATION

Validation of these methods was performed as per the USP guidelines for these following parameters:

Linearity & Range:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. Linearity was carried out for five levels in the range of 80% to 150%. A graph was plotted with concentration on X axis and mean peak areas on Y-axis. The R^2value was found to be 0.999 for tafenoquine. The result show that an excellent correlation exists between concentration and mean peak areas within the selected range. Thus, the developed method can be considered linear, precise, accurate, specific, and suitable for routine quantitative analysis of Tafenoquine in solid dosage form.

Accuracy:

Accuracy of the proposed method was ascertained from the recovery studies by standard addition method. Recovery results werewell within the range **99-101%.** Thus the method was found to be accurate.

Table No. 21: Result of Accuracy Studies

	TFQ		
	Levels		
	80%	100%	120%
Amt added (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt taken (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt recovered (µg/ml)	79.90	99.95	119.95
	79.95	99.98	119.98
	79.98	99.97	119.99
% Recovery	98.33	99.33	99.44
	98.33	98.66	98.88
	99.16	99.33	100.00
Mean % recovery	98.60	99.10	99.44
% RSD	0.48	0.39	0.56

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Robustness:

Robustness of the proposed analytical method was evaluated by making deliberate changes in the chromatographic system method parameters, the standard solution and test solutions were injected for each of the changes made to access the Robustness of proposed analytical method.

Following Parameters were covered under robustness parameter.

Effect of variation in flow rate of mobile phase by ± 10%

Organic phase composition (± 10%)

Change in Wavelength by ± 2 units

The results suggested all the system suitability parameters were within limits.

Specificity:

Is the ability of an analytical method to assess the analyte unequivocally in the presence of excipients, impurities, degradants, and other matrix components. It was evaluated for tafenoquine by injecting the blank, placebo, and the control sample solution prepared as per the proposed method to check for any interference at the retention time of Tafenoquine. Thus, no interference was observed at the retention time of tafenoquine, confirming that the developed method is specific for the drug in its formulation.

III. SUMMARY AND CONCLUSION

SUMMARY

Tafenoquine, an antimalarial drug, has been recently introduced in the pharmaceutical market for the radical cure and prophylaxis of malaria. Literature survey revealed that only limited analytical methods are available for the estimation of tafenoquine in solid dosage forms.

The present study was undertaken with the objective of developing a simple, sensitive, accurate, and reliable RP-HPLC method for the estimation of tafenoquine in tablet dosage form.

In the developed RP-HPLC method, the analyte was effectively separated using a mobile phase consisting of Phosphate buffer and ACN(1MOPA) in the ratio of 80:20%v/v under isocratic conditions. The chromatographic separation was carried out using a C18 column (Phenomenex, 4.6 × 250 mm) with a flow rate of 1.0 mL/min. The total run time was 10 minutes, and detection was performed using a PDA detector at a wavelength of 255nm. The method provided good peak shape, satisfactory retention time, and excellent resolution of Tafenoquine.

The developed method was validated as per ICH guidelines for various parameters including accuracy, linearity, range, robustness, and specificity. The results obtained were within acceptable limits, indicating that the method is reliable and reproducible.

Hence, the proposed RP-HPLC method was found to be simple, rapid, sensitive, precise, and cost-effective, making it suitable for routine quality control analysis of Tafenoquine in solid dosage forms.

The results of analysis in all the method were validated in terms of accuracy, ruggedness, linearity & range. The method were found to be sensitive, reliable, reproducible, rapid & economic also.

CONCLUSION:

From the results of the study it can be concluded that the present RP-HPLC technique was successfully used for the estimation of the Antimalarial drug Tafenoquine in solid dosage form by HPLC.

The method showed good reproducibility and was found to be accurate, specific, and sensitive. The analysis of tafenoquine dosage form was successfully performed by the developed and validated RP-HPLC method.

The RP-HPLC method was also simple, accurate, reproducible, and economical, making it suitable for routine quality control analysis of tafenoquine alone in pharmaceutical dosage forms.

No interference of additives or excipients was observed in the method, confirming its specificity for the drug. Further studies on other pharmaceutical formulations and biological samples may provide additional support for wider application of this analytical method.

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