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Research Article

Development and Validation of Tafenoquine by High Performance Liquid Chromatography Technique along with Stress Degradation Study of Tafenoquine

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ABSTRACT

The most prevalent parasite disease in humans is malaria. According to estimates, 3 billion individuals worldwide are at danger of developing this illness. For the quantitative detection of tafenoquine, a brand-new, quick, and accurate stability-indicating high performance liquid chromatographic method was created and validated. On an Inertsil ODS-3V column (150 mm 4.6 mm, 5.0 m) with mobile phase methanol and water (80:20) at a flow rate of 1 mL/min, effective chromatographic separation was accomplished. The analyte was seen using a photo-diode array detector at a wavelength of 254 nm. tafenoquine was subjected to acidic, basic, oxidative, thermal, and photolytic conditions in order to force its destruction. The peaks of the degradation products produced were distinct from those of tafenoquine and showed the specificity and stability of the technique. The method was also validated using criteria like specificity, precision, linearity, accuracy, and robustness in accordance with the International Conference on harmonization of technical requirements for registration of pharmaceuticals for human use. The major goal of this research was to create a fast, sensitive, and stable HPLC method for analysing and assessing the purity and stability of tafenoquine in formulations.

Introduction

Tafenoquine (SB-252263), racemic 8-[(4-amino- than primaquine.[1,2] Compound I is effective 1-methylbutyl) amino]-2,6-dimethoxy-4-methyl-5-(3 trifluoromethyl-phenoxy) quinoline succinate (I:1) as shown in Fig. 1. GlaxoSmithKline and the US Army are now working on a prophylactic malaria treatment for *Plasmodium falciparum* and *Plasmodium vivax*. Compound I is an antimalarial drug made up of 8 aminoquinolines that has higher anti-malarial activity *in-vitro* than primaquine. Compound I is now undergoing phase III clinical studies and is effective against all stages of Plasmodium. It is broken down into a vast number of tiny components throughout time.

In response to primaquine deficiencies, tafenoquine was developed. It is more effective than primaquine at preventing malaria in both the blood and the liver stages.[3-5] Tafenoquine was 7-10 fold more effective than primaquine in the rhesus monkey malaria model as a radical curative and causal preventive medication.[6-7] *In-vitro* investigations also shown that Tafenoquine is at least 5-fold more effective than primaquine against multidrug-resistant *P. falciparum* asexual blood stages.[8] Tafenoquine has been shown to be well tolerated in human tests, with very minor, temporary gastrointestinal side effects.[3]

Tafenoquine totally protected three of the four volunteers in a challenge trial in which they were administered *P. falciparum* sporozoites and considerably slowed the onset of parasitaemia in the fourth.[9] In a holoendemic location, the prophylactic benefit of Tafenoquine against

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P. falciparum was proven in semi-immune black adult Kenyans.[10] The Kenyans were given either of two tafenoquine regimens after being presumptively treated with halofantrine (250 mg daily for 3 days): 200 mg for 3 days followed by 200 mg weekly or 400 mg for 3 days followed by 400 mg weekly. The preventive efficacy of weekly 200 mg and 400 mg after 13 weeks was 88 percent and 90 percent, respectively. Short-term treatments of Tafenoquine have recently been found to be efficacious and well tolerated in Thai individuals for the radical cure of *P. vivax*.[11]

High-Performance Liquid Chromatography (HPLC) is one of the most successful separation analytical tools for determining and quantifying contaminants. We can separate a mixture of substances into discrete components using HPLC to identify and quantify them.

To our knowledge, no HPLC method for the determination of tafenoquine has been described, and no stability indicating method for tafenoquine has been identified. The major goal of this research was to create a fast, sensitive, and stable HPLC method for analysing and assessing the purity and stability of tafenoquine in formulations.

This study is highly efficient on the basis of purity and stability of the pharmaceutical product because we need more stable formulation as well as their purity. Nowadays, there are many types of impurities are formed in the formulation many of that impurities are carcinogenic that's why we need to develop pure and stable product.

Materials and Methods

Chemicals and Reagents

Manus Aktteva Biopharma, Ahmedabad, India, has gifted a tafenoquine sample. Unless otherwise noted, all additional compounds employed in the HPLC technique development were analytical grade. The mobile phase was prepared with high-quality clean water.

Instrumentation

The HPLC system included a Jasco MD-2010 Plus Multi-wavelength detector and a Plus Intelligent LC pump PU-2080 from Jasco (Tokyo, Japan). ChromNav was used to analyse and monitor the detector output (Version 1.02.04). An Inertsil ODS-3V column (150 mm × 4.6 mm, 5.0 µm) was used to achieve chromatographic separation methanol and water were used for the mobile phase. The mobile phase flow rate was set at 1.0 milliliters per minute. A total of 20 µL were injected. A photo-diode array detector tuned to 254 nm was used to analyse the resulting products.

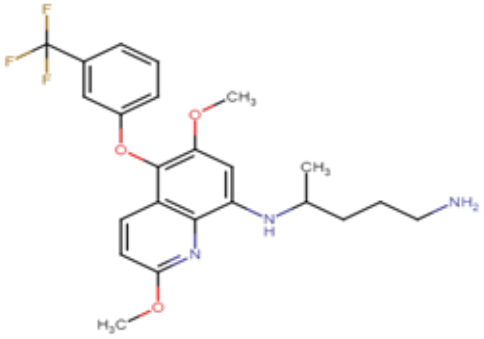

Fig. 1: Structure of Tafenoquine

Preparation of Stock and Standard Solutions

By dissolving accurately weighed tafenoquine in methanol using a 25 mL volumetric flask, a stock solution of Tafenoquine (5 mg/mL) was prepared. Tafenoquine standard solutions (concentration range of 10–500 g/mL) were prepared by diluting the stock solution with methanol to the desired concentration.

Stability of Tafenoquine in Methanol and Mobile Phase

Tafenoquine solutions produced in methanol and mobile phase (500 g/mL) were left in amber-colored stoppered test tubes for 6 hours to check their stability. The quantity of tafenoquine was determined by injecting the solutions into an HPLC system.

Mobile Phase Optimization

After trying several permutations the mobile phase Methanol: Water (80:20) was discovered to be the effective chromatographic separation. More polar solvents may effect on to the separation of drug sample. Water plays a vital role for separation after some trial we came to know water is necessary for separation of drug we try to replace water and methanol with n-Butanol and DMSO it may fluctuate the result.

Acidic, Basic and Degradation of Tafenoquine

Tafenoquine was tested for acid- and base-induced degradation by adding 1-mL of 0.1 N HCl and 0.1 N NaOH to 1-mL of methanolic stock solution of Tafenoquine, respectively. After that, the combinations were heated for 6 hours at 60°C. After cooling to room temperature, the solutions were neutralised to pH 7 with sodium hydroxide (NaOH) for acid-induced drug degradation and hydrochloric acid (HCl) for base-induced drug degradation, and then diluted to 10 mL with diluents and injected into the HPLC system. The forced degradation in acidic and basic conditions was carried out in 25 mL amber-colored volumetric flasks to rule out the possibility of light-induced degradation. The solutions were neutralised before being injected into the HPLC system, and chromatograms were generated as previously reported. The quantity of Tafenoquine in the solutions was then determined using an HPLC system.

Oxidative Degradation of Tafenoquine

The methanolic stock solution of Tafenoquine was treated with hydrogen peroxide (H_2O_2), resulting in a final reaction

mixture with a concentration of H_2O_2 of 10%. For 6 hours, the mixture was allowed to left at room temperature. To avoid any potential degradation owing to exposure to light, the drug was forced to degrade in a 25 mL amber-colored volumetric flask. The finished solution was injected into the HPLC, and the chromatogram was conducted.

Photo Degradation of Tafenoquine

One ml of methanolic stock solution of Tafenoquine was diluted to 10 mL with methanol (500 µg/mL) and the drug solution was exposed to direct sunlight for 6 hours. The solution was then suitably diluted with the mobile phase and injected into the HPLC and the chromatogram was run.

Thermal Degradation of Tafenoquine

In a 25 mL amber-colored volumetric flask, a methanolic stock solution of Tafenoquine was heated for 24 hours at 80°C. The solution was then allowed to cool before being injected into the HPLC and the chromatogram was conducted.

Method Validation

According to the standards mentioned in the international conference on harmonization of technical requirements for Registration for human use guidelines (EMEA,1995), the validation parameters were carried out.

Effect of blank

To check any interference from unrelated substances present during the retention time (RT) of the main peak, mobile phase was injected into the column.

Accuracy and precision

Tafenoquine at a concentration of 10 µg/mL was used to test precision and accuracy. Variations detected during intra- and inter-day determinations were reported as percent RSD for the concentrations used. This was also done to check if the drug could be recovered in the formulations at various concentrations. The accuracy and precision acceptance standards are 99.5–101%.

Linearity

Solutions were prepared by diluting the stock solution to the needed concentrations in order to ensure that the HPLC method developed was linear for various concentrations of tafenoquine. The calibration curves for the peak regions of tafenoquine against the relevant concentrations were plotted for solutions ranging from 2 to 12 g/mL. Acceptance criteria must show that the approach is linear across a certain range. A higher R2 number suggests that the system is more linear.

Robustness

Small but deliberate changes were introduced in the current method to study its effect on tafenoquine's retention time and symmetry factor.

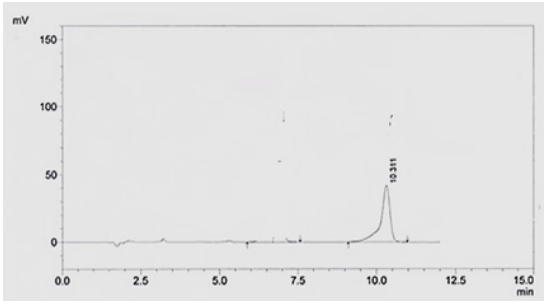

Fig. 2: HPLC chromatogram of Tafenoquine

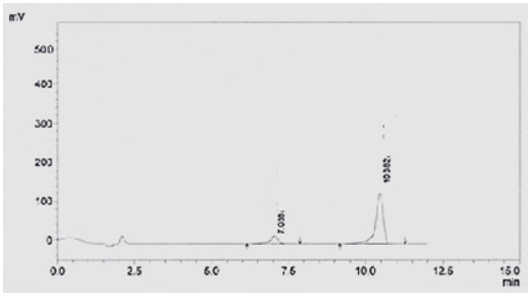

Fig. 3: HPLC chromatogram of acid hydrolysis

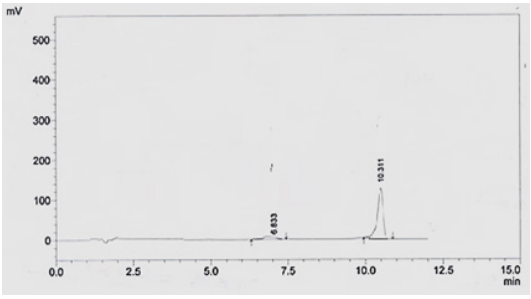

Fig. 4 : HPLC chromatogram of alkaline hydrolysis

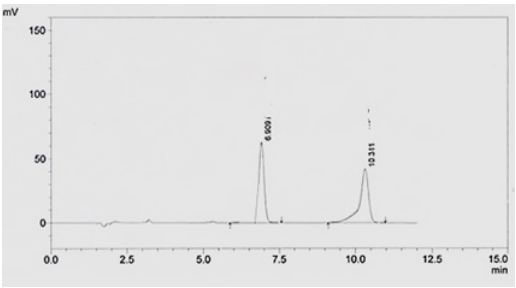

Fig. 5: HPLC chromatogram of Thermal degradation at 6 hour.

Result

Development of the Chromatographic Conditions

The pure drug was injected in different solvent systems under ambient and subjected to forced degradation conditions. An isocratic method was employed for methanol and water prepared at various ratios (80:20, 75:25, 70:30), the peak nature of the pure drug as well as the impurities was good and symmetrical. Also, the method

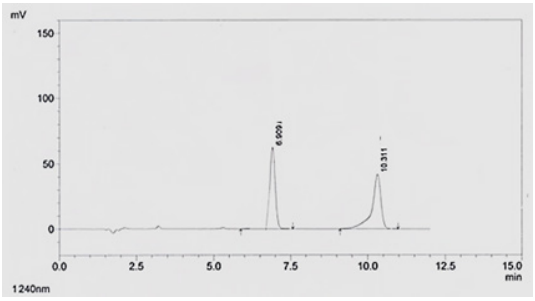

Fig. 6: HPLC chromatogram of Photo degradation at 240nm for 6 hour.

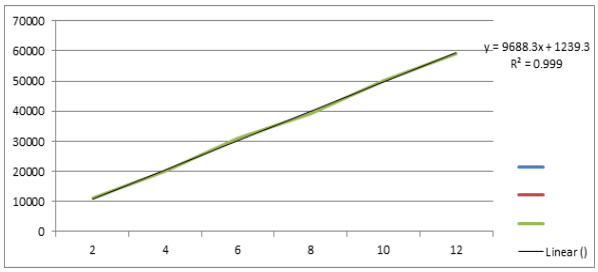

Fig. 7: Linearity of Tafenoquine

Table 1: Degradation TAF at different stress conditions at 6 hour by HPLC

Stress condition	*Standard concentration For HPLC (µg/mL)*		*% Area For HPLC (µg/mL)*	
	Tafenoquine	*Tafenoquine*	*Tafenoquine*	*Tafenoquine*
0.1N HCL	100	1000	65.85	59.89
0.1N NaOH	100	1000	80.15	81.58
6% H_2O_2	100	1000	31.49	35.08
Thermal	100	1000	3.69	2.40
UV (240nm)	100	1000	5.81	3.64

Table 2: Data of recovery study of TAF by HPLC

Recovery level (%)	*Conc. of test solution (µg/mL)*		*Conc. of Standard solution (µg/mL)*		*% Recovery*		*%RSD*	
80	10	10	8	8	98.26	98.49	0.7607	0.7216
100	10	10	10	10	100.54	100.69	0.8520	0.7326
120	10	10	12	12	99.26	98.55	0.7490	0.7357

Table 3: Data of recovery study of TAF by HPLC

Recovery level (%)	*Conc. of test solution (µg/mL)*		*Conc. of Standard solution (µg/mL)*		*% Recovery*		*%RSD*	
80	1000	1000	800	800	98.74	99.36	0.5062	0.5088
100	1000	1000	1000	1000	100.20	99.47	0.5501	0.6227
120	1000	1000	1200	1200	99.66	98.42	0.5258	0.5259

Table 4: Data of precision of TAF by HPLC

Precision	*Concentration*	*S.D*	*%RSD*
	For HPLC (µg/mL)	*For HPLC (µg/mL)*	*For HPLC (µg/mL)*
Repeatability	10	0.04226	0.4282
Intermediate	10	0.05244	0.5299
Reproducibility	10	0.04326	0.4382

Table 5: Data of precision of TAF by HPLC

Precision	*Concentration*	*S.D*	*%RSD*
	For HPLC (µg/mL)	*For HPLC (µg/mL)*	*For HPLC (µg/mL)*
Repeatability	10	0.04226	0.4282
Intermediate	10	0.05244	0.5299
Reproducibility	10	0.04326	0.4382

also showed good baseline separation of tafenoquine from its degradation products.

Stability of Tafenoquine in Methanol and Mobile Phase

No significant changes were observed in the chromatograms of the standard and the experimental solutions and the %RSD for RT and area was within 1%. It was also observed that the mobile phase did not interfere with the RT of the main peak of tafenoquine or any other impurity.

Forced-degradation of Tafenoquine

The chromatograms of the samples exposed to acidic, basic, H_2O_2, photo and thermal degradation showed symmetrical peaks of tafenoquine and some degradation products at different RT (Fig. 2-5), which were well separated as shown in Table 1.

Validation of the Method

Accuracy

The method's accuracy was determined to confirm its reliability by dissolving a known amount of tafenoquine in the solvent and analysing the contents using the same approach as stated above. By injecting three concentrations, six times each, of a known concentration of tafenoquine at various levels (low, medium, and high) of the required limit, and then measuring the % recovery of

Table 6: Linearity data of TAF by HPLC

S. no	*Concentration for HPLC (µg/mL)*	*Area for HPLC*		
1	2	2	10224	11062
2	4	4	21131	20132
3	6	6	30123	31082
4	8	8	41243	39216
5	10	10	50143	50164
6	12	12	61042	59234

Table 7: Robustness parameter for HPLC method.

S. no	*Parameter*	*Normal condition*		*Variable 1*		*Variable 2*	
1	Wavelength	240 nm		238 nm		242 nm	
		TAF	TAF	TAF	TAF	TAF	TAF
	Area	926821	834564	937402	833561	934248	834648
		938786	841153	928201	824240	934851	824860
		917742	824551	926402	822130	921213	842130
	Average	938786	833422	929768	826643	930104	833879
	SD	40956.0	8359.6	4366.3	6082.7	7705.7	8660.6
	%RSD	1.36	1.00	0.46	0.73	0.83	1.04
2	Flow rate	1.0 mL/min.		0.8 mL/min.		1.2 mL/min.	
		TAF	TAF	TAF	TAF	TAF	TAF
	Area	921545	838551	924140	826450	938082	824551
		938554	843655	933148	838552	921810	834564
		919852	826451	933842	843655	921416	841153
	Average	926650	833422	930376	833422	927102.7	833422
	SD	10343.5	8359.6	5412.2	8359.6	9510.4	8359.4
	%RSD	1.11	1.00	0.58	1.00	1.02	1.00
3	Ph	5.0		4.0		6.0	
		TAF	TAF	TAF	TAF	TAF	TAF
	Area	927072	826742	921545	841254	932350	836471
		924640	838421	919852	831820	913242	847363
		923542	833440	938554	834224	901106	848252
	Average	925084	832867	926650	835766	915566	844028
	SD	1806.5	5860.4	10343.5	4902.38	15751.1	6560.2
	%RSD	0.19	0.70	1.11	0.58	1.72	0.77

drug for each concentration, accuracy in terms of sample recovery was examined. At a concentration of 10 µg/mL, the accuracy of the drug assay was determined. The accuracy, as measured by mean percent recovery, ranged from 99.29 to 100.61%, as shown in Tables 2 and 3.

Precision

Six repeated injections of the material were used to assess the precision of the developed method. Six replicates of tafenoquine samples were analysed to determine the intra-day precision. The accuracy and precision between days were determined by analysing three batches on three different days. The intra-day and inter-day % RSD values for RT and area were 0.465 and 0.584, respectively, indicating that the method was repeatable. Tables 4 and 5 summarise the results for intra-day and inter-day precision.

Linearity

The method was found to be linear over a range from 2-12 µg/mL (Table 6).

Robustness

As shown in Table 7

Discussion

From the above experimental results it was concluded that, the pure drug, both under ambient and subjected to forced degradation conditions, was injected in different solvent systems. An isocratic method was employed for methanol and water prepared at various ratios (80:20, 75:25, 70:30), the peak nature of the pure drug as well as the impurities was good and symmetrical. Also, the method also showed good baseline separation of tafenoquine from its degradation products. The newly developed HPLC method for determining tafenoquine was found to be linear, precise, specific, accurate and stable. Statistical analysis also proves that the method is reproducible and specific to the analysis of tafenoquine. The method was found to be versatile in the analysis of tafenoquine as a pure drug.

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Conflict of interest

None

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