STABILITY INDICATING REVERSE PHASE ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND VALIDATION FOR TAFENOQUINE SUCCINATE IN THE PRESENCE OF DEGRADATION PRODUCTS AND APPLICATION TO GREEN ANALYTICAL CHEMISTRY.

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Abstract:
Tafenoquine Succinate is the succinate salt form of tafenoquine, an orally bioavailable 8-aminoquinoline derivative, with antimalarial activity. In this study, we developed and validated a method to determine Tafenoquine Succinate and its related impurities in pharmaceutical dosage forms using a reverse phase-UPLC technique. All the impurities are separated in the analytical column Waters X bridge C18 (50mm X 4.6 mm, 3.5 µm) using a suitable mobile phase was 0.1% Formic acid and Ethanol in the 55:45 v/v isocratic mode. The flow rate is 0.3 mL/min with the gradient programme the injection volume is 3 µL, detection at 215 nm in UV and the total run time is 10 minutes. The samples were made for forced degradation under hydrolysis, oxidation, thermal and photolytic conditions. The method was validated according to the international conference on Harmonisation (ICH) guidelines and found to be specific, linear, rugged, robust and accurate. The method was linear from LOQ to 150% concentration level for all impurities. The recovery was performed from LOQ to 150% concentration, and the mean recovery was found acceptable. The degradation and validated study results indicate its stable nature. Therefore, this method can be used in pharmaceutical research and development and quality control departments. Green analytical chemistry tools are used to assess the greenness of the method and calculated using GAPI, AGREE and Eco-scale and found excellent green of >75%.

Key words: Tafenoquine Succinate, SIAM, Green Analytical Chemistry, ICH

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1. Introduction:

Tafenoquine is used for the treatment and prevention of relapse of Vivax malaria in patients 16 years and older. Tafenoquine is not indicated to treat acute vivax malaria. Malaria is a disease that remains to occur in many tropical countries. Vivax malaria, caused by _Plasmodium vivax_, is known to be less virulent and seldom causes death. However, it causes a substantive illness-related burden in endemic areas and it is known to present dormant forms in the hepatocytes named hypnozoites which can remain dormant for weeks or even months. This dormant form produces ongoing relapses. Its empirical formula $C_{24}H2_8F_3N_3O3$ and a 463.5 g/mol molecular weight[1]. Tafenoquine Succinate is described chemically as 4-N-[2,6-dimethoxy-4-methyl-5-[3-(trifluoromethyl) phenoxy] quinolin-8-yl] pentane-1,4-diamine and has the following chemical structure in **Figure 1**[2].

A literature survey indicated that there is no chromatographic methods had been utilised to estimate Tafenoquine Succinate in pharmaceutical preparations separately [3–5].

This study aimed to develop a simple, specific, linear, robust, rugged, and stability-indicating method for determining Tafenoquine Succinate and its related impurities in pharmaceutical dosage forms. The stability-indicating method is a validated quantitative analytical procedure generally involving forced degradation and validation studies. Based on these previous results, we herein performed degradation studies, and during stress studies, we found one potential impurities in the drug product in the presence of peroxides[6, 7]. The compound is stable under both physical and hydrolysis degradation conditions, which is also confirmed by validation parameters. Hence, this method can be used to determine Tafenoquine succinate and its related impurities and this method is cost-effective. The Green Analytical principles (GAP) were developed by Galuszka et al. These principles aim to minimize or eliminate the utilization of harmful or hazardous substances. The objective of this study is to minimize the utilization of organic solvents while assessing the environmental sustainability of the existing approach. Several metric tools exist for evaluating the level of environmental sustainability, such the Green Analytical Procedure Index (GAPI), National Environmental Method Index (NEMI), Analytical GREEness (AGREE), and analytical eco-scale[8–13].

The GAPI is a useful tool for assessing the level of environmental sustainability in relation to several factors such as sample preparation, procedures employed, and sample determination. The pictograph is depicted using a color scheme consisting of red, yellow, and green colors. The pictogram is comprised of six separate sections, namely: 1. Sample source, 2. Method type, 3. Sample preparation, 4. Reagents and chemicals employed, 5. Instrumentation, and 6. Symbol O indicates the qualitative or quantitative nature of the method.

The Analytical Eco-scale is a metric tool utilized for assessing the environmental sustainability of a given technique. It achieves this by calculating penalty points associated with various aspects of the method's ecological impact. The Eco-scale quantifies the cumulative penalty points derived from a comprehensive scale of 100. A method is deemed excellent if the cumulative penalty point exceeds 75%. The AGREE metric is a technique utilized to quantify the level of environmental sustainability of a particular method, drawing upon the 12 principles of green analytical chemistry. The present study employed the AGREE, GAPI, and Eco-scale tools to evaluate the environmental sustainability of the analytical approach as theses tool are advanced and can calculate both qualitative and quantitative.

2. Materials and Methods:

2.1. Instrumentation and software:

The experiment was performed on a Water Alliance UPLC 2695 UPLC (Water Corporation, Milford, MA, USA) consisting of a quaternary pump, a column thermostat, and an autosampler using a Waters 2489 PDA detector, instrument control and data acquisition were performed using Waters Empower 3 software. An ultrasonication, analytical balances, and vacuum microfiltration unit with 0.22µm PVDF filters from Millipore were used.

2.2. Chemicals, reagents and standards:

AR grade Formic acid was purchased from SD Fine chemical, Mumbai, India, Ethanol was purchased from Honeywell, Mumbai, India. Milli-Q water was obtained from Millipore. Samples of the finished products tablets were procured from local pharmacy.

2.3. Chromatographic conditions:

The buffer was prepared using 0.1% Formic acid and filtered through a 0.22µm membrane filter. Mobile phase was composed of buffer and Ethanol

in a isocratic mode and total run time is 10 minutes. the specificity and selectivity achieved using Waters Xbridge C18 (50mm X 4.6mm, 3.5µm). Isocratic elution at a ration of 55:45 v/v (Buffer: Ethanol) at a flow rate of 0.5 mL/min was employed. The column and sample temperature were maintained at 40°C and 25°C respectively. The analytes and impurities were detected at 215 nm in UV. The injection volume is 3 µL and the total run time is 10 minutes with a isocratic mode. The diluent consists of 500:500 v/v of Ethanol and Milli-Q water mixture[14, 15].

2.4. Preparation of the Tafenoquine Succinate Standard Stock Solution:

An Tafenoquine Succinate standard solution was prepared (Concentration of Tafenoquine Succinate is about 250 µg/mL) by accurately weighing and transferring 100 mg of Tafenoquine Succinate working standard into a 100 mL volumetric flask and adding about 70mL of diluent and sonicate to dissolve and dilute volume with diluent (Stock solution). Further, Transfer 5 mL of Stock Solution into a 25 mL volumetric flask, makeup to the volume with diluent and mix.

2.5. Preparation of the API Sample preparation:

Weigh and transfer the 100 mg of Tafenoquine Succinate into a 100 mL volumetric flask. Add about 60 mL of diluent and sonicate for 20 minutes with intermittent shaking, dilute to volume with diluent and mix well. Centrifuge the solution at 5000 RPM for 10 min and use the supernatant solution. Further, Transfer 5 mL of Stock Solution into a 25 mL volumetric flask, makeup to the volume with diluent and mix.

2.6. Preparation of the Marketed Sample preparation:

An Tafenoquine Succinate sample solution was prepared (the concentration is about 250 µg/mL) by weighed not less than 10 tablets (each 300 mg) and determining the average weight. Crush the tablets into fine homogeneous powder. Weigh and transfer the tablet powder equivalent to about 100 mg of Tafenoquine Succinate into a 100 mL volumetric flask. Add about 60 mL of diluent and sonicate for 20 minutes with intermittent shaking, dilute to volume with diluent and mix well. Centrifuge the solution at 5000 RPM for 10 min and use the supernatant solution. Further, Transfer 5 mL of Stock Solution into a 25 mL volumetric flask, makeup to the volume with diluent and mix.

3. Results and Discussions:

3.1. Method Development:

This study aimed to develop a stability-indicating chromatographic method for the quantification of analyte in both API and marketed formulation under extreme stability conditions. An LC-DAD (Liquid Chromatography equipped with a Diode array detector) method was developed to provide the suitability of routine stability studies and quality control analysis. The method was optimised to separate degradation products obtained during the forced degradation studies (stress studies). The important criteria in developing this method are to achieve sufficient resolution of the impurities, peak asymmetry and total run time. To achieve the criteria several experiments were performed to optimise the stationary and mobile phases. The preliminary method development was initiated by an isocratic method using mobile phase A as 20 mM ammonium acetate buffer adjusted pH to 4.0 with acetic acid and mobile phase B as Ethanol in the ratio of 60:40 v/v using the Symmetry C8 column (50mm X 4.6mm, 3.5µm) with a flow rate of 0.5 mL/min. The analyte and impurities were not resolved and the analyte peak shape was distorted. Even after multiple trials under the isocratic mode, we could not successfully resolve all the impurities and still the peak shape is not improved. In the isocratic mode of elution, several mobile phase compositions were evaluated using various proportions of different buffers and Ethanol. The best chromatographic separation was achieved by using a gradient mode with a Mobile phase with 0.1% Formic acid and Ethanol in the isocratic mode (55:45 v/v) using Waters Xbridge C18 (50mm X 4.6mm, 3.5µm) at a flow rate of 0.5 mL/min. The final optimised chromatograms are shown in **Figure 2-5.**

3.2. Method Validation

The method was validated (In terms of System suitability, Specificity, Sensitivity, Accuracy, Precision, Linearity, Range, LOD, LOQ, Robustness, and Solution stability) following ICH guidelines[16–18].

3.3. System Suitability:

The system suitability was evaluated by six replicate injections of standard solution and a single injection of LOQ solution according to the recommendations given in United States Pharmacopeia (USP). The peak asymmetry, theoretical plates, and %RSD for main peak areas were calculated. The system suitability results are

shown in **Table 1**. The representative chromatogram of Standard is shown in **Figure 3.**

3.4. Specificity and Forced degradation studies:
The specificity of the method was evaluated by injecting the blank, standard (API) as such sample, at the specified concentration (250 µg/mL). The method was found specific as there is no interference observed in blank chromatograms at the main peak retention time, The representative chromatogram of blank and sample were shown in **Figure 2 and 4 respectively**. The individual retention time (RTs), Relative retention time (RRTs), Purity angle and Purity threshold are given in the **Table 2**. The specificity of the method is also evaluated using forced degradation studies following ICH Q1A guidelines. The sample degradation was performed as per the below experimental conditions[19–23].

3.4.1 Acid Degradation:
Weighed and transferred equivalent to about 100 mg of sample into a 100 mL volumetric flask, added 5 mL of 1N HCl solution and kept the sample in a water bath for 6 hrs at 80°C. The sample was allowed to cool down to room temperature and then neutralise with 5mL of 1N NaOH solution. Added 30 mL of diluent and sonicated for 10 minutes to dissolve. Finally, the volume was made up to the mark with diluent and mixed well. The sample was centrifuged at 5000 rpm for 10 minutes and supernatant solution was used. Further, transfer 5 mL of Stock Solution into a 25 mL volumetric flask, makeup the volume with diluent and mix and injected to the UPLC analysis. The obtained chromatogram shows no significant degradation under the acidic condition. The results of percentage assay, percentage degradation, mass balance and peak purity of Tafenoquine Succinate in **Table 3**.

3.4.2 Base Degradation:
Weighed and transferred equivalent to about 50 mg of sample into a 50 mL volumetric flask, added 5 mL of 1N NaOH solution and kept the sample in a water bath for 3 hrs at 80°C. The sample was allowed to cool down to room temperature and then neutralise with 5mL of 1N HCl solution. Added 30 mL of diluent and sonicated for 10 minutes to dissolve. Finally, the volume was made up to the mark with diluent and mixed well. The sample was centrifuged at 5000 rpm for 10 minutes and supernatant solution was used further, transfer 5 mL of Stock Solution into a 25 mL volumetric flask, makeup the volume with diluent and mix and injected to the UPLC analysis. The obtained chromatogram shows no significant degradation under the basic condition. The results of percentage assay, percentage degradation, mass balance and peak purity of Tafenoquine Succinate in **Table 3**.

3.4.3 Thermal Degradation:
The samples were kept in a hot air oven maintained at 120 °C for 24 hours. The exposed samples were weighed and transferred equivalent to about 50 mg of sample into a 50 mL volumetric flask, added 30 mL of diluent and sonicated for 10 minutes to dissolve. The sample was allowed to cool down to room temperature and finally, the volume was made up to the mark with diluent and mixed well. The sample was centrifuged at 5000 rpm for 10 minutes and supernatant solution was used Further, transfer 5 mL of Stock Solution into a 25 mL volumetric flask, makeup the volume with diluent and mix and injected to the UPLC analysis. The obtained chromatogram shows no significant degradation under the thermal condition. The results of percentage assay, percentage degradation, mass balance and peak purity of Tafenoquine Succinate in **Table 3**.

3.4.4 Photo Degradation:
The sample was exposed in a photostability chamber set for 1.2 million LUX hours and 200 watts hrs./sq. meters of UV-Visible light. The exposed samples were weighed and transferred equivalent to about 50 mg of sample into a 50 mL volumetric flask, added 30 mL of diluent and sonicated for 10 minutes to dissolve. finally, the volume was made up to the mark with diluent and mixed well. The sample was centrifuged at 5000 rpm for 10 minutes and supernatant solution was used Further, transfer 5 mL of Stock Solution into a 25 mL volumetric flask, makeup the volume with diluent and mix and injected to the UPLC analysis. The obtained chromatogram shows no significant degradation under the Photo condition. The results of percentage assay, percentage degradation, mass balance and peak purity of Tafenoquine Succinate in **Table 3**.

3.4.5 Humidity Degradation:
The samples were kept in a humidity chamber maintained at 25°C/80%RH for 7 days. The exposed samples were weighed and transferred equivalent to about 50 mg of sample into a 50 mL volumetric flask, added 30 mL of diluent and sonicated for 10 minutes to dissolve. The sample was allowed to cool down to room temperature and finally, the volume was made up to the mark with diluent and mixed well. The sample was centrifuged at 5000 rpm for 10 minutes and

supernatant solution was used further, transfer 5 mL of Stock Solution into a 25 mL volumetric flask, makeup the volume with diluent and mix and injected to the UPLC analysis. The obtained chromatogram shows no significant degradation under the humidity condition. The results of percentage assay, percentage degradation, mass balance and peak purity of Tafenoquine Succinate in **Table 3**.

3.4.6 Peroxide Degradation:

Weighed and transferred equivalent to about 50 mg of sample into a 50 mL volumetric flask, added 5 mL of 10%H_2O_2 and kept the sample in a water bath for 5 hrs at 80°C. The sample was allowed to cool down to room temperature. Added 30 mL of diluent and sonicated for 10 minutes to dissolve. Finally, the volume was made up to the mark with diluent and mixed well. The sample was centrifuged at 5000 rpm for 10 minutes and supernatant solution was used Further, transfer 5 mL of Stock Solution into a 25 mL volumetric flask, makeup the volume with diluent and mix and injected to the UPLC analysis. The obtained chromatogram shows significant degradation under the oxidative condition. From the above results, the drug is more susceptible to oxidative stress conditions The results of percentage assay, percentage degradation, mass balance and peak purity of Tafenoquine Succinate in **Table 3**.

3.5. Linearity:

Linearity of the method was determined by injecting the standard solutions of Tafenoquine Succinate at concentrations ranging from five (n=5) levels from 10 to 150%. The calibration curve was obtained by plotting a graph between the peak areas and the concentrations of Tafenoquine Succinate. The obtained calibration curve showed a correlation coefficient greater than 0.999 for Tafenoquine Succinate and the method is found to be linear. The tabulated results are shown in **Table 4**.

3.6. Precision and intermediate precision:

3.4.7 System Precision:

The system precision was evaluated by six replicate injections of Tafenoquine Succinate standard into the instrument and the % RSD was found to be 0.53. The results obtained from the system precision study concluded that the system is precise for determining Tafenoquine Succinate in tablets by UPLC and results shown in **Table 4**.

3.4.8 Method Precision:

The method precision was determined by analysing sample solution at 100% specification limit with respect to the sample concentration and the %RSD of the area of each analyte was calculated. The results are shown in **Table 4** and confirm that the method is rugged for the determination of Tafenoquine Succinate.

3.4.9 Intermediate Precision:

The intermediate precision of the method was evaluated on different days on different equipment. The %RSD of Tafenoquine Succinate were calculated. The results are shown in **Table 4** and confirm the rugged for determination of Tafenoquine Succinate.

3.7. Accuracy:

The accuracy of the method was evaluated by using the standard addition method. The study was carried out in triplicate at 10%, 50%, 100%, and 150% levels and the percentage recoveries were calculated. The percentage recovery values for the analyte are in the range of 100.0 to 106.0 for Tafenoquine Succinate which were in the acceptance criteria. The relative standard deviation values of recoveries obtained for analyte were less than 5.0. The results are shown in the **Table 4.**

3.8. Solution Stability:

The solution stability of Tafenoquine Succinate were determined by storing the samples in tightly capped volumetric flasks at 25°C and 2-8°C for 24 hrs and 48 hrs. The % difference in the area of samples was calculated against freshly prepared sample solution. The results were shown in the **Table 4** and found that Tafenoquine Succinate were stable at 2-8°C after 24 hrs.

3.9. Robustness:

The robustness of the method was evaluated by deliberate change in the experimental conditions and the impurities RRTs were recorded. The variables evaluated in the study were column temperature from 25°C to 35°C, Wavelength from 250 nm to 260 nm, the Flow rate from 0.1 to 0.3 mL/min. The results were meeting the acceptance criteria and the results are shown in the **Table 4.**

3.10 Green Analytical metric assessment:

The Analytical Eco-scale, a metric tool, is employed to measure penalty points. The total penalty points amount to 17, thus the current analytical method of analysis obtains a score of 83, indicating excellent performance. For detailed results; please refer to **Table 5**. The AGREE

software was developed by The Gdansk University of Technology in Poland, using a structure that consists of 12 green analytical principles. Each principle is assigned a score ranging from 0.1 to 1.0, denoting its level of adherence to sustainability criteria. The method's overall AGREE score of 0.74 is depicted in **Table 6** and **Figure 7**. The GAPI tool displays a collection of 15 pictograms and 5 pentagrams. Among the several pictograms utilized, it is significant that the pictogram denoted by the number 8 is depicted in the color red, symbolizing the specific sample treatment used. Conversely, the pictograms about sample preparation and sample handling are illustrated in yellow. The reagents and instruments utilized in the study are indicated by a green pictogram. The results obtained from the Green Analytical Process Index (GAPI) are presented in **Table 8** and **Figure 7**.

4. Conclusion:

The Experimental and validated results confirm that the developed method can determine Tafenoquine Succinate, and also well separate all impurities by suitable stationary phase and mobile phase. The proposed method has been validated according to ICH Q2 guidelines. We found one potential impurities during the degradation of the peroxide sample. The Tafenoquine Succinate is found susceptible to the peroxide degradation conditions but remained stable under acid, base, thermal, humidity, and Photo (UV-Visible) environment conditions. The degradation and validation suggest that the method is a specific, linear, accurate, precise, robust and stability-indicating method. It could be used to estimate Tafenoquine Succinate-related impurities in the drug product dosage form. The developed analytical method was assessed with green analytical metric tools GAPI, AGREE and Eco-scale and found that the method is green.

5. Acknowledgement

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6. Conflict of Interest

The author has no conflict of interest to declare.

7. References

1. Peters W (1999) The Evolution of Tafenoquine—Antimalarial for a New Millennium? J R Soc Med 92:345–352. https://doi.org/10.1177/014107689909200705
2. Markus MB (2021) Safety and Efficacy of Tafenoquine for Plasmodium vivax Malaria Prophylaxis and Radical Cure: Overview and Perspectives. Ther Clin Risk Manag 17:989–999. https://doi.org/10.2147/TCRM.S269336
3. Lobo AMG, Magalhães IRS (2020) Determination of tafenoquine in human plasma by dispersive liquid-liquid micro-extraction. Separation Science Plus 3:44–50. https://doi.org/10.1002/sscp.201900088
4. Birrell GW, Van Breda K, Barber B, Webster R, McCarthy JS, Shanks GD, Edstein MD (2022) Quantification of Tafenoquine and 5,6-Orthoquinone Tafenoquine by UHPLC-MS/MS in Blood, Plasma, and Urine, and Application to a Pharmacokinetic Study. Molecules 27:8186. https://doi.org/10.3390/molecules27238186
5. Doyle E, Fowles SE, Summerfield S, White TJ (2002) Rapid determination of tafenoquine in small volume human plasma samples by high-performance liquid chromatography–tandem mass spectrometry. Journal of Chromatography B 769:127–132. https://doi.org/10.1016/S1570-0232(02)00003-X
6. Haid M, Muschet C, Wahl S, Römisch-Margl W, Prehn C, Möller G, Adamski J (2018) Long-Term Stability of Human Plasma Metabolites during Storage at −80 °C. J Proteome Res 17:203–211. https://doi.org/10.1021/acs.jproteome.7b00518
7. Iqbal M, Ezzeldin E, Al-Rashood KA, Asiri YA, Rezk NL (2015) Rapid determination of canagliflozin in rat plasma by UHPLC–MS/MS using negative ionization mode to avoid adduct-ions formation. Talanta 132:29–36. https://doi.org/10.1016/j.talanta.2014.08.041
8. Mohamed HM, Lamie NT (2016) Analytical Eco-Scale for Assessing the Greenness of a Developed RP-HPLC Method Used for Simultaneous Analysis of Combined Antihypertensive Medications. Journal of AOAC INTERNATIONAL 99:1260–1265. https://doi.org/10.5740/jaoacint.16-0124
9. Elsheikh SG, Hassan AME, Fayez YM, El-Mosallamy SS (2023) Green analytical chemistry and experimental design: a combined approach for the analysis of zonisamide. BMC Chemistry 17:38. https://doi.org/10.1186/s13065-023-00942-1

10. Kowtharapu LP, Katari NK, Sandoval CA, Muchakayala SK, Rekulapally VK (2022) Green Liquid Chromatography Method for the Determination of Related Substances Present in Olopatadine HCl Nasal Spray Formulation, Robustness by Design Expert. Journal of AOAC INTERNATIONAL 105:1247–1257. https://doi.org/10.1093/jaoacint/qsac072
11. Yabré M, Ferey L, Somé I, Gaudin K (2018) Greening Reversed-Phase Liquid Chromatography Methods Using Alternative Solvents for Pharmaceutical Analysis. Molecules 23:1065. https://doi.org/10.3390/molecules23051065
12. Muchakayala SK, Katari NK, Saripella KK, Schaaf H, Marisetti VM, Ettaboina SK, Rekulapally VK (2022) Implementation of analytical quality by design and green chemistry principles to develop an ultra-high performance liquid chromatography method for the determination of Fluocinolone Acetonide impurities from its drug substance and topical oil formulations. Journal of Chromatography A 1679:463380. https://doi.org/10.1016/j.chroma.2022.463380
13. Fares MY, Hegazy MA, El-Sayed GM, Abdelrahman MM, Abdelwahab NS (2022) Quality by design approach for green HPLC method development for simultaneous analysis of two thalassemia drugs in biological fluid with pharmacokinetic study. RSC Adv 12:13896–13916. https://doi.org/10.1039/D2RA00966H
14. Žigart N, Časar Z (2020) Development of a Stability-Indicating Analytical Method for Determination of Venetoclax Using AQbD Principles. ACS Omega 5:17726–17742. https://doi.org/10.1021/acsomega.0c02338
15. Bakshi M, Singh S (2002) Development of validated stability-indicating assay methods —critical review. Journal of Pharmaceutical and Biomedical Analysis 28:1011–1040. https://doi.org/10.1016/S0731-7085(02)00047-X
16. (2005) ICH Guideline, Validation of analytical procedures: text and methodology Q2 (R1)
17. (2003) ICH Guideline: Stability testing of new drug substances and products, Q1A (R2)
18. (1996) ICH Guidline: Stability testing: photostability tesing of new drug substances and products, Q1B
19. Felton LA (2006) A Review of: "Pharmaceutical Stress Testing: Predicting Drug Degradation": S. W. Baertschi, ed. Taylor & Francis, Boca Raton, Fl, 2005, ISBN: 0824740211; Hardback. 482 pages. Drug Development and Industrial Pharmacy 32:505–505. https://doi.org/10.1080/03639040500529234
20. Harmon PA, Kosuda K, Nelson E, Mowery M, Reed RA (2006) A Novel peroxy Radical Based Oxidative Stressing System for Ranking the Oxidizability of Drug Substances. Journal of Pharmaceutical Sciences 95:2014–2028. https://doi.org/10.1002/jps.20668
21. Tamizi E, Jouyban A (2016) Forced degradation studies of biopharmaceuticals: Selection of stress conditions. European Journal of Pharmaceutics and Biopharmaceutics 98:26–46. https://doi.org/10.1016/j.ejpb.2015.10.016
22. Baertschi SW, Alsante KM, Reed RA (2016) Pharmaceutical Stress Testing: Predicting Drug Degradation, Second Edition, 0 ed. CRC Press
23. (2002) Stability of Drugs and Dosage Forms. Kluwer Academic Publishers, Boston

Table 1: System suitability parameters and results

Injection No.	Area of Tafenoquine Succinate Peak
1	63535
2	65217
3	65339
4	65535
5	65634
6	65598
Mean	65143
SD	803.90
% RSD	1.2

Theoretical Plate Count	14107
Tailing Factor	1.0

Table 2: Specificity and peak purity summary

Peak Name	Retention Time (minutes)		Relative Retention Time	Purity Angle	Purity Threshold
	Standard Solution	Sample Solution			
Tafenoquine Succinate	2.98	2.96	1.00	0.150	0.427

Table 3: Forced degradation conditions and data

Stress Condition	% Assay of Tafenoquine Succinate	% Degradation	Total (%w/w)	% Mass Balance	Purity Angle	Purity Threshold
As Such	98.52	NA	0.0	NA	0.028	0.222
Acid degradation	97.22	0.0	0.0	98.51	0.028	0.225
Base degradation	98.01	0.0	0.0	98.54	0.027	0.226
Thermal degradation	98.54	0.0	0.0	97.72	0.028	0.239
Photolytic degradation	98.52	0.0.	0.0	98.43	0.026	0.224
Humidity degradation	98.45	0.0	0.0	99.27	0.026	0.223
Peroxide degradation	89.95	8.57	8.57	95.21	0.027	0.537

Table 4: Method Validation data Summary

Parameters	Tafenoquine Succinate
System Suitability n=6	
%RSD, Theoretical plates, Tailing Factor	1.2, 14107, 1.0
Linearity	
Correlation coefficient	0.9999
Accuracy (% of recovery) n=3	
10% mean, %RSD	106.033, 4.08
50% mean, %RSD	104.233, 2.41
100% mean, %RSD	104.298, 0.12
150% mean, %RSD	104.006, 0.11
Repeatability %RSD (n=6)	
System Precision, %RSD	0.53
Method Precision, %RSD	0.70
Intermediate Precision, Overall %RSD	0.82
Solution Stability	
In Sample at 25°C after 24 hrs, % Difference	0.97
In Sample at 25°C after 48 hrs, % Difference	1.40
In Sample at 2-8°C after 24 hrs, % Difference	0.37
In Sample at 2-8°C after 48 hrs, % Difference	0.20
Robustness	
Temperature as Such (40° C), RRT	1.00
Temperature Minus (35° C), RRT	1.00
Temperature Plus (45° C), RRT	1.00
Wavelength as Such (254 nm), RRT	1.00
Wavelength Minus (250 nm), RRT	1.00
Wavelength Plus (260 nm), RRT	1.00
Flow Rate as Such (1.0 mL/Min)	1.00

Flow Minus (0.9 mL/Min)	1.00
Flow Plus (1.1 mL/Min)	1.00

Abbreviations: NA: Not Applicable, RSD: Relative Standard Deviation, LOD: Limit of detection, LOQ: Limit of Quantification.

Table 5 Represents the GAC tool Eco scale to assess the Greenness analytical method

Analytical Eco Scale for assessing the analytical method		
S.No	**Name**	**Penalty Points**
	Chemicals or reagents	
1	Formic acid	3
2	Ethanol	4
	Instruments	
1	Energy- 1.5kWh of energy per sample for UPLC and LC-MS	1
2	Occupational Waste- Procedure release vapours into the environment	3
	Waste	
1	Total Amount of waste generated (Waste Generated >10mL)	5
2	Management (The generated waste has a degradation process)	1
	Total Penalty Points	17
	Analytical Eco Scale Total Score (100-Total Penalty Points)	83
	Green ness Evaluation	Excellent

Table 6 Represents the GAC tool AGREE to assess the Greenness analytical method

AGREE tool for assessing the Analytical Method on UPLC and LC-MS/MS		
S.No	**Green analytical Chemistry Principles**	**Sample Procedure**
1	Direct analytical techniques should be applied to avoid sample treatment	Off-Line Analysis
2	Minimal sample size and minimal number of samples are goals	1 g
3	If possible, measurement should be performed in-situ	At-line
4	Integration of analytical processes and operations saves energy and reduces the use of reagents	3 distinct steps involved in the sample preparation procedure
5	Automated and miniaturized methods should be selected	Semi-Automatic and miniaturized
6	Derivatization should be avoided	No Derivatization
7	Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided	1 g
8	Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time	4 analytes determined with single run, 3 samples analysed per hour
9	**The use of energy should be minimized:** Select the most energy-intensive technique used in the method, or the closest equivalent	UPLC
	Alternatively, estimate the total power consumption of a single analysis in kWh	0.15
10	Reagents obtained from renewable sources should be preferred.	All reagents are bio-based
11	Toxic reagents should be eliminated or replaced	No toxic reagents or solvents used
12	Operator's safety should be increased.	The threats that are not avoided are a. Bioaccumulative b. Highly flammable c. Explosive

Table 8 Represents the GAC tool GAPI to assess the Greenness analytical method

GAPI tool for assessing the analytical method on UPLC and LC-MS/MS		
Sample Sourcing		
1	Collection	Off-line
2	Preservation	Physical or Chemical
3	Transport	None
4	Storage	None
Method Type and Sample Preparation		
5	Type of Method	Simple Procedure
6	Scale of extraction	Micro-Extraction
7	Solvent or reagent used	Green Solvents/ Reagents used
8	Additional Treatment	Simple Treatment
Reagents and Solvents		
9	Amount	<10 g used
10	Health hazard	Slightly toxic, slight irritant; NFPA health hazard score of 0 to 1.
11	Safety hazard	No special hazards. Highest NFPA flammability or instability score of 0 or 1. No Special hazards.
Instrumentation		
12	Energy	<=0.1 kWh per sample
13	Occupational hazard	Hermetic sealing of the analytical process
14	Waste	< 1g
15	Waste treatment	Degradation, Passivation
Quantification/Qualification		
1	Symbol O	Procedure for qualification and quantification

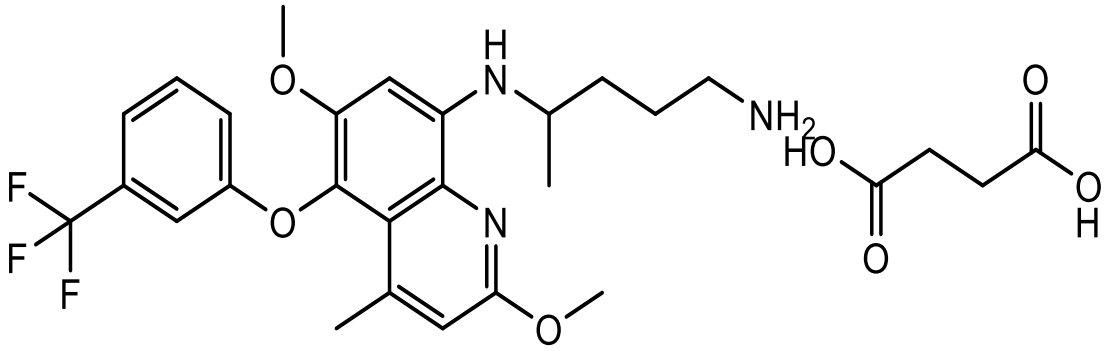

Figure 1: Representative chemical structure of Tafenoquine Succinate

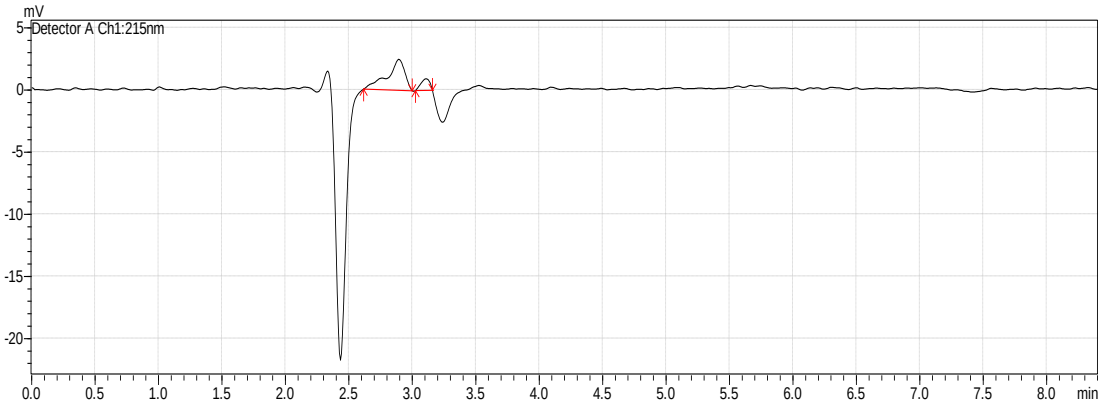

Figure 2: Representative chromatogram of Blank

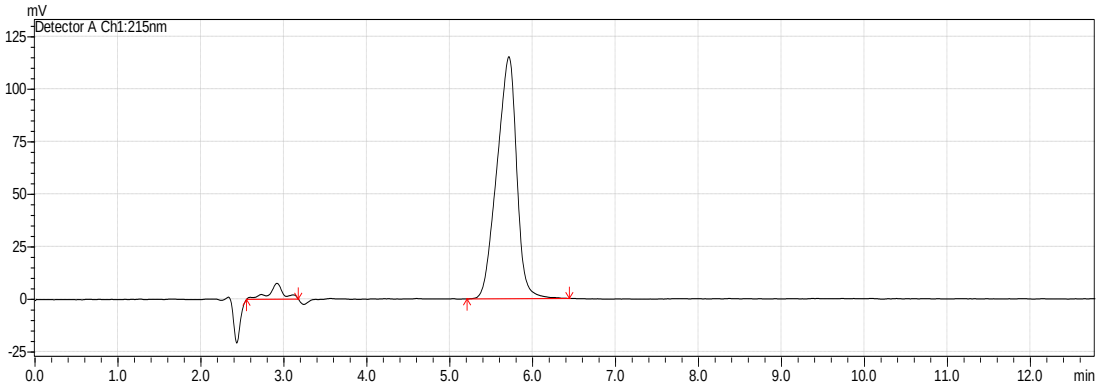

Figure 3: Representative chromatogram of Standard

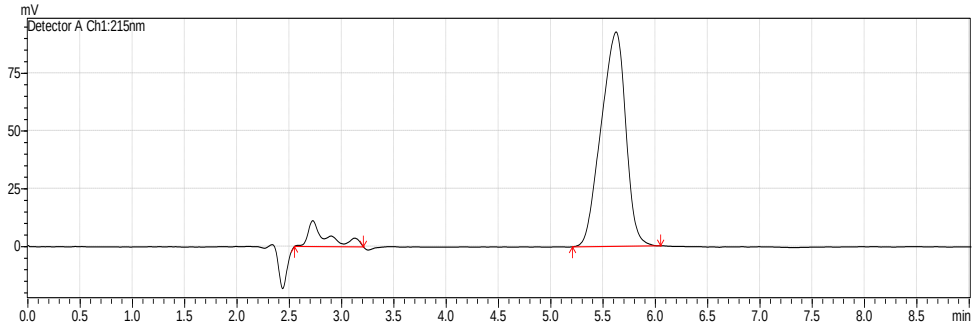

Figure 4: Representative chromatogram of API

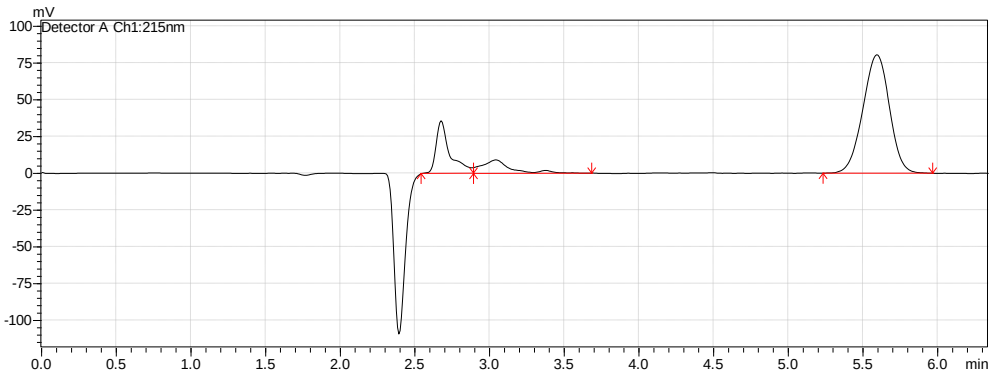

Figure 5: Representative chromatogram of Marketed formulation

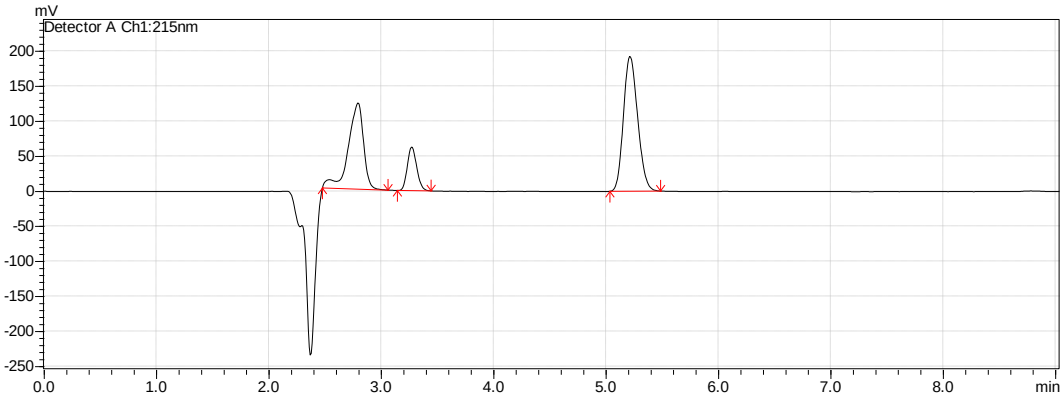

Figure 6: Representative chromatogram at Oxidative degradation condition

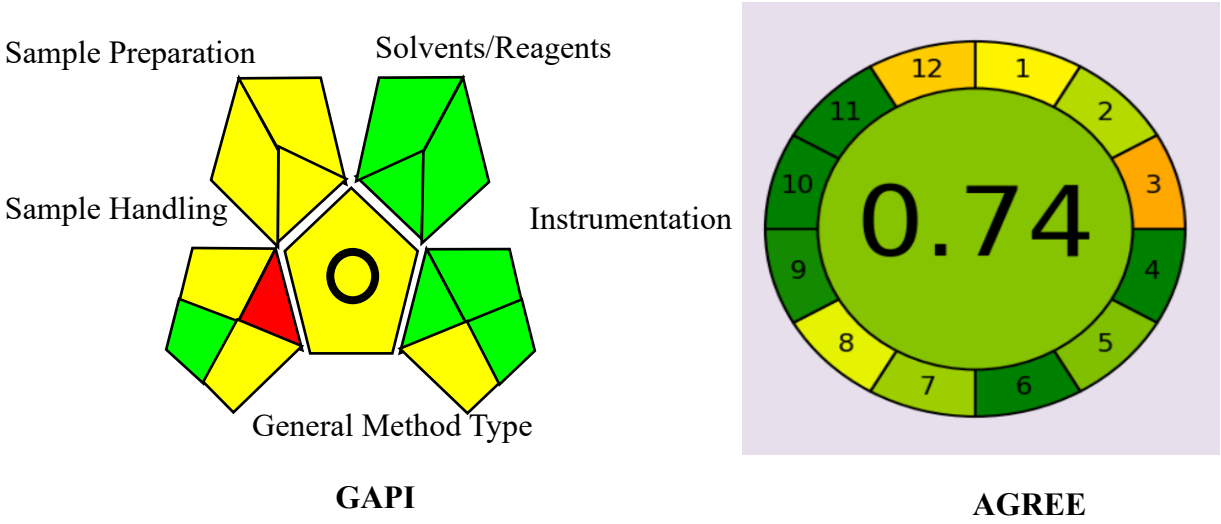

Figure 7: Representative Pictograms of GAPI and AGREE