Asian Journal of Chemistry; Vol. 35, No. 10 (2023), 2517-2526

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2023.28204

A Novel Approach to AQbD-Assisted Green and Robust UPLC Method for the Determination of Selected Aminoquinoline Antimalarial Drugs: Assessment of Green Method

JAYA RAJU CHEERLA[1,2] and T. BHASKARA RAO[1,*]

[1]Department of Engineering Chemistry, College of Engineering, Koneru Lakshmaiah Education Foundation, Vaddeswaram-522302, India
[2]Analytical Research & Development, United States Pharmacopeia-India Private Limited, Plot No. D6 and D8, IKP Knowledge Park, Genome Valley, Shameerpet, Hyderabad-500101, India

*Corresponding author: E-mail: tbhaskararao@kluniversity.in

Received: 15 July 2023; *Accepted*: 23 August 2023; *Published online*: 28 September 2023; AJC-21405

Greening analytical procedures have gained popularity in the area of pharmaceutical industries in order to safeguard the environment. In this study, an innovative methodology by integrating analytical quality-by-design (AQbD) along with Green Chemistry concepts with a systematic approach has been used to develop an environmentally-friendly and more robust ultra performance liquid chromatography (UPLC) method for the determination of three selected aminoquinoline antimalarial drugs namely amodiaquine hydrochloride (AMD), chloroquine phosphate (CHQ) and piperaquine phosphate (PPQ). An environmentally benign solvent ethanol has been chosen as an alternative to the commonly used acetonitrile and methanol, which are widely used. Based on the comprehensive risk analysis, the critical method parameters (CMP's) that affect the critical method attributes (CMA's) have been identified and the final method parameters were further optimized with the help of a full factorial design and the design of experiments (DOE), established the "design space"(DS) and identified the "method-operable-design-region (MODR) for the developed method. Achieved the separation with the use of an Acquity-UPLC BEH Shield RP-18 (100 mm × 2.1 mm), 1.7 µm column by using an isocratic elution of mobile phase containing 0.1% trifluoroacetic acid in water, ethanol in a mixture of 90:10% v/v and maintained a flow rate of 0.2 mL min^{-1} and the column oven temperature was 40 ºC and the UV detection wavelength of 225 nm was employed. A statistical tool Analysis of Variance (ANNOVA) has been used to assess the model's statistical significance and found that the model's p-values is < 0.00005 and its lack of fit is > 0.05 respectively, which demonstrates that the selected model is the most appropriate predictive tool for the analyzed responses R1 & R2 (*i.e.* peak resolutions R1 & R2). The proposed method's linearity, accuracy & precision studies has been successfully validated in compliance with USP <1225> and ICH Q2 (R1) guidelines. Further assessed the proposed method's greenness using various green evaluation tools such as: "complex green analytical procedure index"-cGAPI, "analytical greenness"-AGREE, "analytical method green score"-AMGS and found acceptable results. This novel AQbD-assisted eco-friendly analytical method for the quantification of AMD, CHQ & PPQ in bulk drugs (APIs) or finished dosage forms is simple, rapid, precise and accurate, robust and environmentally benign.

Keywords: Green analytical chemistry, Analytical Quality-by-design, UPLC, Design of experiments, Method-Operable-Design-Region.

INTRODUCTION

The concepts of Green Chemistry have become more significant in light of the issue of environmental contamination. Green chemistry refers to the practice of developing chemical processes and products with the goal of reducing or eliminating the production and use of substances that may be harmful [1]. The concept of green chemistry supports to the decrease in air, water, air and soil contamination, as well as the improvement of living conditions for humans and animals. Therefore, research laboratories are continuously focusing on developing an eco-friendly methods that uses sustainable solvents. Relatively new terminology, green analytical chemistry has been used to refer the efforts in the area of chemistry to safeguard the environment [2-4]. This concept of "green analytical chemistry" (GAC) is a subset of the concept of sustainability. Therefore, developing green liquid chromatographic analytical methods provides an alternative and is more environmentally benign for analyzing pharmaceutical samples [5-8]. The aim of the present research is to develop an eco-friendly analytical procedure that minimizes

the use of hazardous organic solvents by employing environmentally benign solvents. Various evaluation tools, including complex green analytical procedure index (cGAPI) [9,10], Analytical GREEnness (AGREE) [11,12] and analytical method green score (AMGS) [13] were utilized to evaluate the ecological friendliness nature and greenness of the developed method.

Ultra-performance liquid chromatography (UPLC) has been identified as one of the most effective techniques for the separation of diverse analytical substances in liquid chromatography. When comparing HPLC and UPLC, it is an evident that UPLC improves the separation of analytes efficiency and sensitivity, which demonstrates that it is a time and cost-effective approach [14]. The conventional method development process varying with the one-factor-at-a-time (OFAT) methodology, is time-taking and trial-and-error method. The application of quality-by-design (QbD) in the method development of green analytical procedures provides better knowledge on the critical method parameters (CMP's) and their effects on critical method attributes (CMA's). According to ICH Q14 guidelines, the QbD concept is a systematic approach that entails a predefined analytical target profile (ATP), choosing of critical method attributes (CMA's) and detailed risk evaluation to find out the possible risks that lead to failures. Identification of critical method parameters (CMP's) is the next step, followed by optimization with the use of design of experiments (DOE) to create the design space (DS). Method operable design region (MODR) is a set of analytical method parameters that can vary within which the analytic method performance requirements are met and the quality of the measured outcome is ensured [15]. Furthermore, the QbD approach helps to understand the extent of specific effects as well as the interaction of CMP's on method performance [16-20].

Malaria is a huge public health concern across the globe, particularly in underdeveloped countries, where many instances and fatalities occur. As per the latest world malaria report released by World Health Organization (WHO), malaria affected 247 million people in 2021, an increase from the 245 million affected in 2020. The estimated number of malaria fatalities in 2021 was 619,000, a decrease from 625,000 in 2020 [21]. Malaria is a life-threatening illness that arises from infection with the Plasmodium parasite, which is transmitted to humans through mosquito bites, posing a significant risk to vulnerable populations such as children under the age of five and pregnant women. Several methods of treatment have been documented and advised to control this disease [22,23].

Chloroquine (Fig. 1), a 4-aminoquinoline category drug has been widely used for many years to treat and prevent malaria caused by *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malaria*. It is typically not used to treat malaria caused by *Plasmodium falciparum* due to its extensive resistance [24]. Amodiaquine (Fig. 1) is another orally active 4-aminoquinoline derivative with antimalarial and anti-inflammatory properties [25]. It works similarly to chloroquine against some chloroquine resistant strains namely *Plasmodium falciparum*, the most lethal malaria parasite and is used to treat simple malaria especially *Plasmodium falciparum* malaria [25].

Piperaquine (Fig. 1) is a chemically synthesized 4-aminoquinoline derivative with significant blood schizonticidal action, similar as chloroquine and shown to be effective against the two kinds of malaria parasites, *P. falciparum* and *P. vivax*, which are causing the majority of malaria cases worldwide. This drug is often combined with another antimalarial drugs such as artemisinin or dihydro artemisinin to make a fixed-dose combination therapy [26,27]. This combination therapy is highly effective in treating uncomplicated malaria and has been advised by WHO as the first-line therapy for malaria in several countries.

Various techniques were documented for the determination of the investigated pharmaceutical compounds and their formulations, encompassing UV spectrophotometric methods, HPTLC and HPLC methods [28-38]. There are limited research articles reported for the quantification of various antimalarial drugs by using the quality-by-design methodology [39]. A wide range of analytical techniques are currently available for the quantitative analysis of antimalarial drugs in different pharmaceutical formulations. However, it is essential to develop a single chromatographic method for the quantification of aforementioned drugs. The current research study was aimed to develop a straightforward and robust analytical method for selected aminoquinoline antimalarial drugs quantification by employing analytical quality-by-design (AQbD)-assisted green chemistry concepts. According to our knowledge, this is the first-ever UPLC method for the determination of selected aminoquinoline antimalarial drugs with the combination approach of analytical QbD and green analytical chemistry principles based on the available literature. The proposed method will be validated

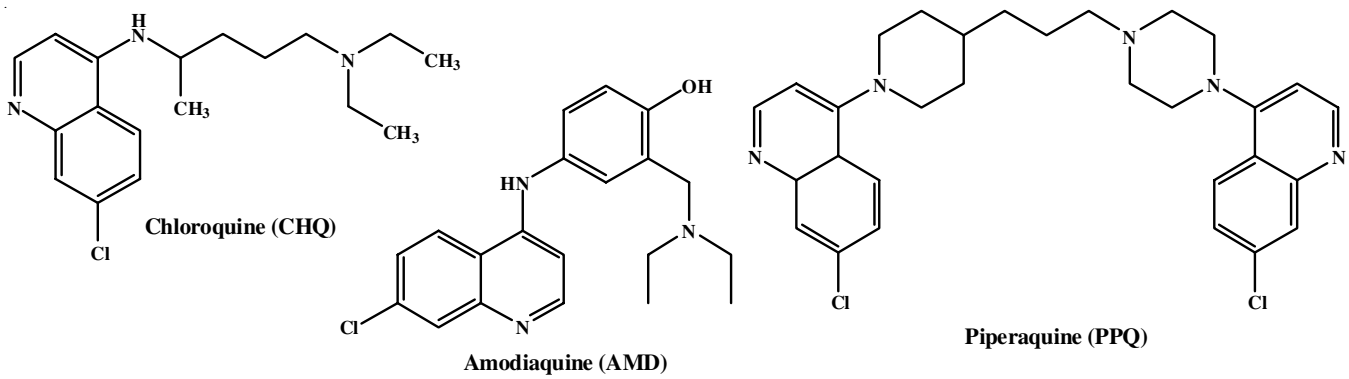

Fig. 1. Chemical structures of selected aminoquinoline antimalarial drugs

according to USP <1225> and ICH Q2 (R1) recomm-endations [40,41].

EXPERIMENTAL

Drug substances of amodiaquine hydrochloride (AMD), chloroquine phosphate (CHQ) and piperaquine phosphate (PPQ) samples has been provided by United States Pharmacopeia-India Pvt. Ltd. Hyderabad, India. Honeywell Research Chemicals supplied the AR-grade solvent ethanol, whereas Sisco Research Laboratories Pvt. Ltd (India) supplied the analytical reagent grade trifluoroacetic acid (TFA). All solutions were prepared using Milli-Q water collected from the Sartorius water system.

Instrumentation: The analysis was carried out using the Waters Acquity H-Class UPLC instrument from the manufacturer of Waters, USA. The equipment comprises a quaternary pump, a solvent mixture to degas the mobile phases and a PDA detector (photodiode array detector), a sample cooler compartment and a column compartment with a thermostat. The system is operated by Empower 3 version software. Sartorius Balances were used for sample preparations.

Chromatographic conditions: An optimum separation was achieved using the UPLC BEH shield RP-18 column with a dimensions of 100 mm × 2.1 mm, 1.7 µm manufactured by the Waters Acquity. The mobile phase consists of 0.1% trifluoroacetic acid in water, ethanol in a mixture of 90:10% v/v with an isocratic mode of elution using 0.2 mL min^{-1} flow rate and column oven temperature is 40 ºC with a 2 µL of injection volume and the wavelength opted as 225 nm for UV detection.

Software: The Design Expert software 13.0.5.0 version (Stat-Ease, Inc., Minneapolis, United States) has been used to analyze the impact of chromatographic method parameters such as mobile phase ratio, flow rate and column temperature, upon the critical method attributes of the developed method.

Preparations of solutions

Mobile phase: Prepared 0.1% TFA in water and ethanol in a mixture of 90:10 (v/v) and used as mobile phase.

Diluent: Mobile phase used as diluent.

Standard solution: Prepared an individual standard stock solutions of amodiaquine hydrochloride (AMD), chloroquine phosphate (CHQ) and piperaquine phosphate (PPQ) at a 1000 µg mL^{-1} concentration by dissolving the appropriate quantity in the diluent. This solution was further diluted to obtain the standard solution having 100 µg mL^{-1} of AMD, 200 µg mL^{-1} of CHQ and PPQ. The system suitability criteria were established using the same solution.

Assay sample solution: Prepared in a similar manner for samples to get the sample solution having 100 µg mL^{-1} of AMD, 200 µg mL^{-1} of CHQ and PPQ.

Robustness solution: The standard solution was used to assess the method's robustness.

Method validation: Validated the developed UPLC procedure in compliance with the USP <1225> & ICH Q2 (R1) guidelines for the system suitability, specificity study, linearity, precision and accuracy.

System suitability solution: Standard solution was injected with five replicate injections to ensure the system suitability in terms of area %RSD criteria for system performance. Also considered the minimum resolution criteria is NLT 2.0 for all analytes.

Specificity: Injected diluent, system suitability solution and all drug substances individually to confirm that there is no blank interference at the main analyte and the peak purity of each analyte.

Linearity: Prepared the linearity solutions from the range of 50% to 150% of the nominal sample concentration of 100 µg mL^{-1} of AMD, 200 µg mL^{-1} of CHQ and PPQ at 5 levels and reported the correlation coefficient value.

Accuracy: The accuracy of an analytical procedure refers to the extent to which the observed value is consistent with the true value. Determined the accuracy study by preparing the sample solutions at three distinct levels of concentrations (50%, 100% and 150%) and calculated the mean recovery and % RSD.

Precision and intermediate precision: Precision was determined by analyzing six preparations of AMD, CHQ and PPQ samples at various intervals, *i.e*. intra-day and inter-day intervals and calculated the recovery %RSD.

Robustness studies: To assess the robustness of developed method, a full-factorial design was used. Using the mobile phase flow rate, mobile phase ratio and column oven temperature as the independent variables, a mathematical model was constructed using a 2 level 3 factorial layout. The interaction and effect on system suitability parameters like retention time, resolution (not less than 2.0) and tailing factor (not more than 2.0) were evaluated by making deliberate changes in the chromatographic parameters.

RESULTS AND DISCUSSION

Method development and optimization: Initiated the method development by following the AQbD workflow, which begins with a pre-defined analytical target profile (ATP) followed by the selection of critical method attributes (CMA). A quality risk assessment was used to identify the critical method parameters (CMP) and used design of experiments (DOE) for robustness studies to generate the design space (DS), is a multi-dimensional combination and interactions of CMPs that fulfils the CMAs requirements with a defined probability.

Analytical target profile (ATP), identification of critical method attributes (CMAs) and selection of critical method parameters (CMPs): An analytical target profile (ATP) is a predefined set of objectives for an analytical method that defines the desired performance characteristics and its acceptance criteria. The quantification of the selected drug substances should be within the range of 50% to 150%, with an acceptable recovery rate of 98% to 102%. The relative standard deviation (%RSD) for repeatability should be ≤ 2% with the nominal sample concentrations of amodiaquine HCl at 100 µg mL^{-1} and 200 µg mL^{-1} of chloroquine phosphate and piperaquine phosphate. Furthermore, a potential strategy for the green UPLC method is the replacement of hazardous solvents with more environmentally benign ones.

The selection of critical method attributes (CMAs) and the critical material parameters (CMPs) identification are the most important steps in the analytical quality by design (AQbD)

process. In current study, the resolutions (Rs) among all the peaks were considered as CMAs and targeted a resolution of not less than 2.0 for all peaks for better separation and quantification. Mobile phase organic solvent ratio, column oven temperature and mobile phase flow rate were considered as CMPs.

Risk assessment: In the QbD framework, the initial risk assessment plays an important role to identify the potential impact of critical method parameters (CMPs), which may result in failure of the critical method attributes (CMAs) and the established analytical target profile (ATP). Therefore, during the development of QbD-based methods, it is an important to meticulously consider the risks that might have an impact on CMAs. Gathering all the information related to the sample's physicochemical properties, molecular structures and their nature in polarity, mode of detection, types of stationary phases and their dimensions and the effects of aqueous and organic phases on CMAs will help to identify the probable risk factors. Based on this risk assessment, it was found that pH of the mobile phase, ratio of organic solvent, type of stationary phase and column temperature may have an impact on CMAs and can influence the ATP. Initial screening and optimization of analytical method parameters associated with significant risk factors were carried out according to the initial risk analysis.

Critical method parameters (CMPs) screening and optimization: In liquid chromatographic techniques, many variables may have an impact on peak elution and separation. A comprehensive screening process must be carried out to minimize the factors that have no or nominal impact. As a number of factors can influence the analytical method performance, a quality risk assessment based approach has been used to understand the parameters that may have a high amount of impact on CMAs. A systematic screening studies have been carried out to identify the critical method parameters and narrow down them to achieve the final optimized method conditions. Therefore, the initial screening experiments were carried out with wide pH ranges of mobile phases: 0.1% TFA in water opted for acidic pH, 10 mM ammonium acetate buffer at pH 5.0 adjusted with acetic acid opted for mid-level pH (at pH 5.0) and for basic pH selected 10 mM ammonium bicarbonate pH 8.0 with acetic acid chosen with the use of a variety of column chemistries like C18, C8, phenyl and cyano columns by using 0.2 mL/min as an optimal flow rate. Ethanol was chosen as an organic solvent due to its eco-friendly nature and as an ideal alternative for hazardous solvents. Based on the initial screening data, it was found that the required separation was not obtained on cyano, phenyl and C8 columns with mid-level pH (at pH 5.0) and basic-level pH (at pH 8.0). The acidic pH resulted in greater separation using C18 column chemistry and the details were stated in the chromatographic conditions. Further optimization studies were conducted to assess the impact of the optimized method conditions (CMPs) on selected method attributes (CMAs). The mobile phase flow rate, organic solvent ratio in the mobile phase and column oven temperature are the most significant CMPs for the current chromatographic procedure. The optimum flow rate was 0.2 mL/min, finalized the column oven temperature as 40 ºC, selected 225 nm as a detection wave length and the mobile phase is a premixed solution contains water and ethanol in a ratio of 90:10 % v/v having 0.1% TFA with an isocratic elution time up to 15 min. A chromatogram (Fig. 2) demonstrates the findings achieved with the optimized method conditions and the system suitability results were summarized in Table-1.

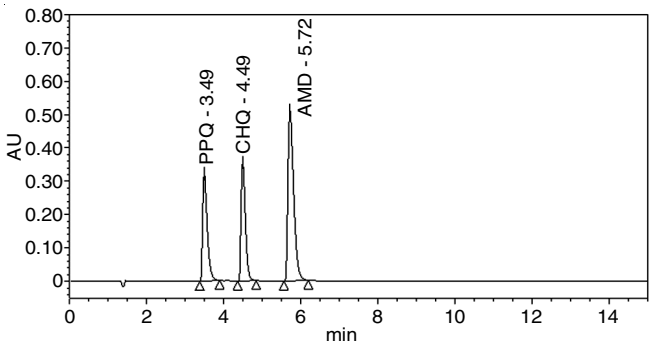

Fig. 2. Obtained chromatogram using the optimized analytical conditions for determination of PPQ, CHQ and AMD

TABLE-1
SYSTEM SUITABILITY RESULTS
FROM STANDARD SOLUTION

Name	Retention time	USP resolution	USP tailing	USP plate count	RSD (%)
Piperaquine	3.49	–	1.27	6165	0.14
Chloroquine	4.49	4.82	1.12	8651	0.09
Amodiaquine	5.72	5.21	1.04	9931	0.11

Full factorial design: To assess the effect of CMPs on CMAs, a mathematical model was established by utilizing design expert software tools. A full factorial experimental design considered as one of the most effective statistical approach for method optimization was employed to develop the model, which can help to evaluate both individual as well as cumulative effects of the selected CMPs on CMAs. The CMPs considered for the current study are mobile phase flow rate in mL min^{-1} (0.8-1.2 mL min^{-1}) as Factor-A, column oven temperature (30 to 50 ºC) as Factor-B and organic solvent ratio composition in mobile phase (07% to 13%) as Factor-C. The lower, actual and higher values for Factor-A are 0.18, 0.20 & 0.22 mL/min and 30, 40 and 50 ºC, respectively are considered for Factor-B and the percent of organic solvent ratio in the mobile phase were 07%, 10% and 13% for Factor-C. The total design of experiments with their variables is listed in Table-2. In this study, the resolution between all the analytes was considered as CMAs, *i.e.*

TABLE-2
FULL FACTORIAL DESIGN OF EXPERIMENTS AND
THEIR VARIABLES FOR ROBUSTNESS STUDIES

Factors	Code levels	Actual levels
A: Flow rate (mL/min)	-1.0	0.18
	0.0	0.20
	+1.0	0.22
B: Column oven temperature (ºC)	-1.0	25
	0.0	30
	+1.0	35
C: Organic ratio	-1.0	07
	0.0	10
	+1.0	13

the resolution between PPQ and CHQ considered as response R1 and the resolution between CHQ and AMD is considered as response R2. The minimum resolution should not be less than 2.0 between all the peaks, which was considered as a criteria to assess the effect of CMPs on CMAs. The impact of these variables was assessed and observed that the resolution value is not less than 2.5 and the tailing factor is not more than 1.5 across all experiments. Thus, the developed method will be treated as a robust method.

ANOVA (Analysis of variance) has been employed to investigate the model's statistical significance and the ANOVA findings are summarized in Table-3. Observed that the *p*-value is < 0.0005 and its lack of fit value is > 0.05 for both responses R1 (resolution b/w PPQ and CHQ), R2 (resolution b/w CHQ and AMD) indicating the model's significance. The model fits to the current study appropriately, as shown by its significance and the lack of fit's non-significance.

Generated perturbation plots & cube plots, 3D-response surface and 2D counter plots and Pareto charts by analyzing the obtained data from the design of experiments to understand the influence of these three variables (CMPs) such as mobile phase flow rate, organic solvent ratio and the column temperature on responses (CMAs). Based on the perturbation plots (Fig. 3), it was deduced that the organic ratio and column temperature have a substantial impact on PPQ and CHQ (R1), CHQ & AMD (R2) resolutions. According to the data presented in Fig. 3, the cube plots indicate that the lowest resolution for response R1 is 2.19, while the highest resolution is 5.33. Similarly, for response R2, the lowest resolution is 2.72 and the highest resolution is 8.20, as observed throughout the entire study.

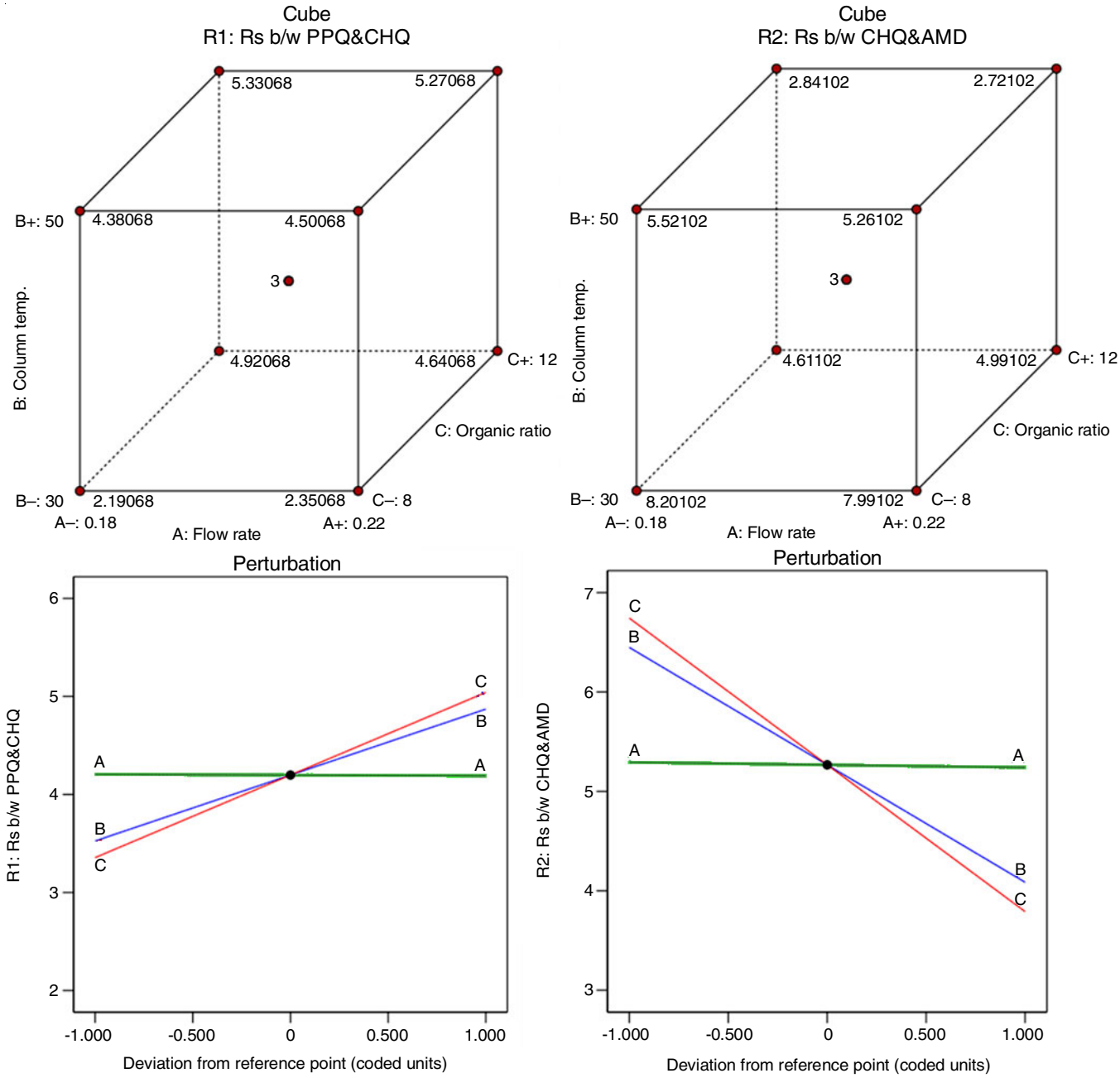

Fig. 3. Perturbation plots and Cube plots for responses R1 & R2 (Factors: flow rate, column temperature and organic ratio)

TABLE-3
ANNOVA RESULTS FOR RESPONSES R1, R2 FROM THE FULL FACTORIAL DESIGN STUDY

	Source	Sum of squares	Df	Mean square	Model F-value	Model *p*-value	Prob > F
Response 1 (R1) Resolution b/w PPQ&CHQ	Model	10.72	7	1.53	21502.66	< 0.0001	Significant
	Flow rate	0.0005	1	0.0005	6.32	0.0866	
	Temperature	3.62	1	3.62	50806.66	< 0.0001	
	Organic ratio	5.68	1	5.68	79739.94	< 0.0001	
Response 2 (R2) Resolution b/w CHQ&AMD	Model	28.97	7	4.14	590.30	0.0001	Significant
	Flow rate	0.0055	1	0.0055	0.7864	0.4405	
	Temperature	11.16	1	11.16	1592.36	< 0.0001	
	Organic ratio	17.43	1	17.43	2487.01	< 0.0001	

The three-dimensional surface plots (Fig. 4) depict all three variables influence on the responses R1 and R2. It was observed that the influence of flow rate was largely independent for both responses, whereas the column oven temperature and the organic solvent ratio had a significant impact on response R1 (resolution between PPQ & CHQ). The 2D counter plots were used to understand the three selected variables effect on the responses R1 and R2 as shown in Fig. 5. Response R1 (resolution between PPQ & CHQ) increased with increasing the column oven temperature & organic solvent ratio. On the other hand, decreased the response R2 (resolution between CHQ & AMD) with increasing organic solvent ratio and column oven temperature. Furthermore, column temperature and organic solvent ratio present in mobile phase have a greater effect on responses whereas the mobile phase flowrate has a minimal effect on the same. The 2D contour plots produced a comparable interpretation which further strengthened the findings from the 3D response surface plots studies.

Pareto charts: Pareto charts can additionally be used to add a further illustration to the statistical results [42]. Pareto charts with interaction plots provide visual information on the magnitude of effects produced by each variable to consider them as significant and not significant [43]. The most statistically significant parameters that had effects on various responses were determined using Pareto charts. All the effects were studied thoroughly and depicted in Fig. 6 in the form of Pareto charts. The interaction between column oven temperature (Factor-B) and organic solvent ratio (Factor-C) has a significant impact on both the responses R1 and R2. However, the mobile phase flow rate (Factor-A) was not found to be statistically significant.

Method operable design region (MODR): The concept of MODR as stated in ICH Q(14) refers to a combination of

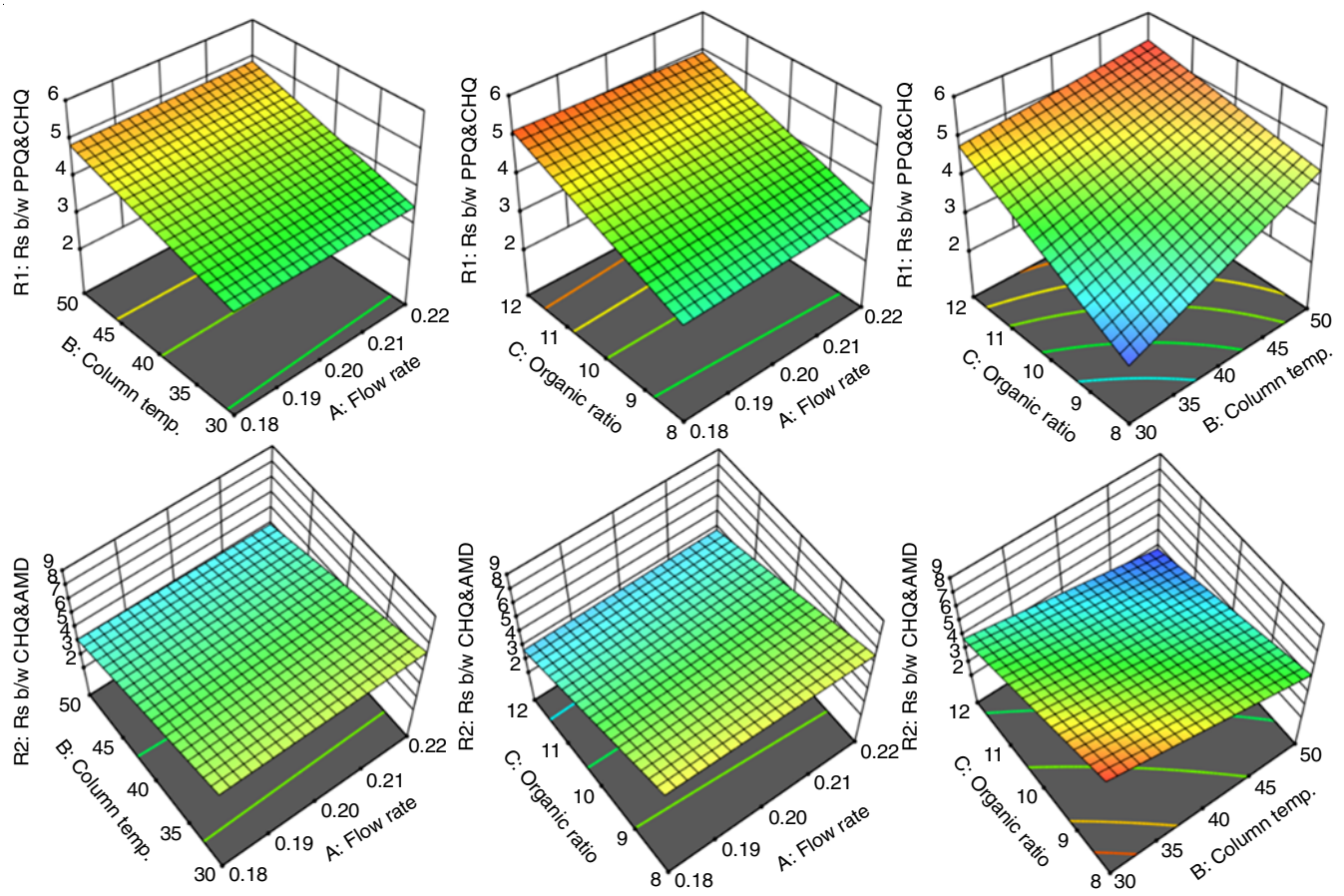

Fig. 4. 3D-Response surface plots for responses R1 & R2 (Factors: flow rate, column temperature and organic ratio)

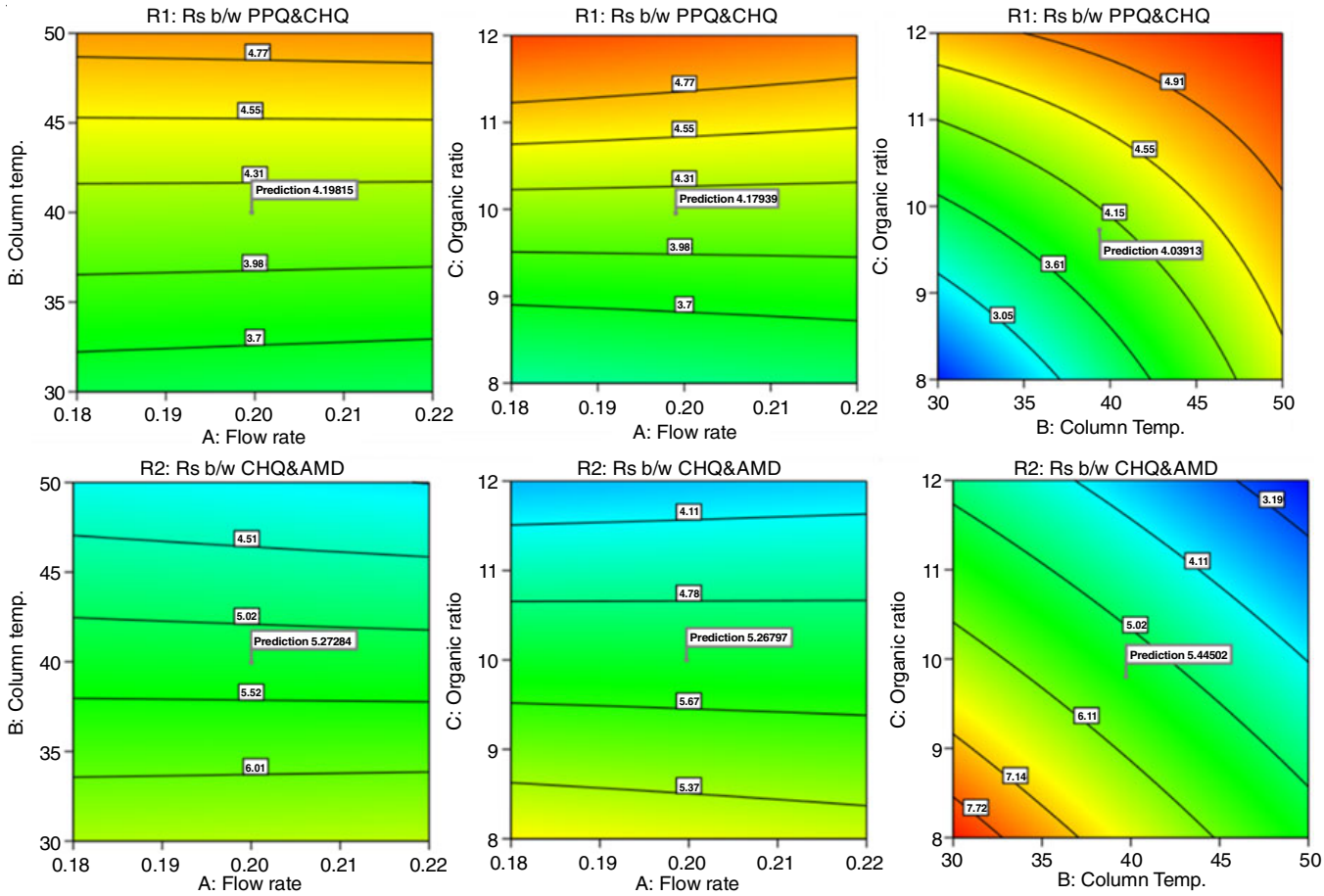

Fig. 5. 2D Counter plots for depicting the interactions among chosen CMPs, *i.e.*, of flow rate, column oven temperature and ethanol ratio on CMAs, *i.e.*, responses R1 & R2

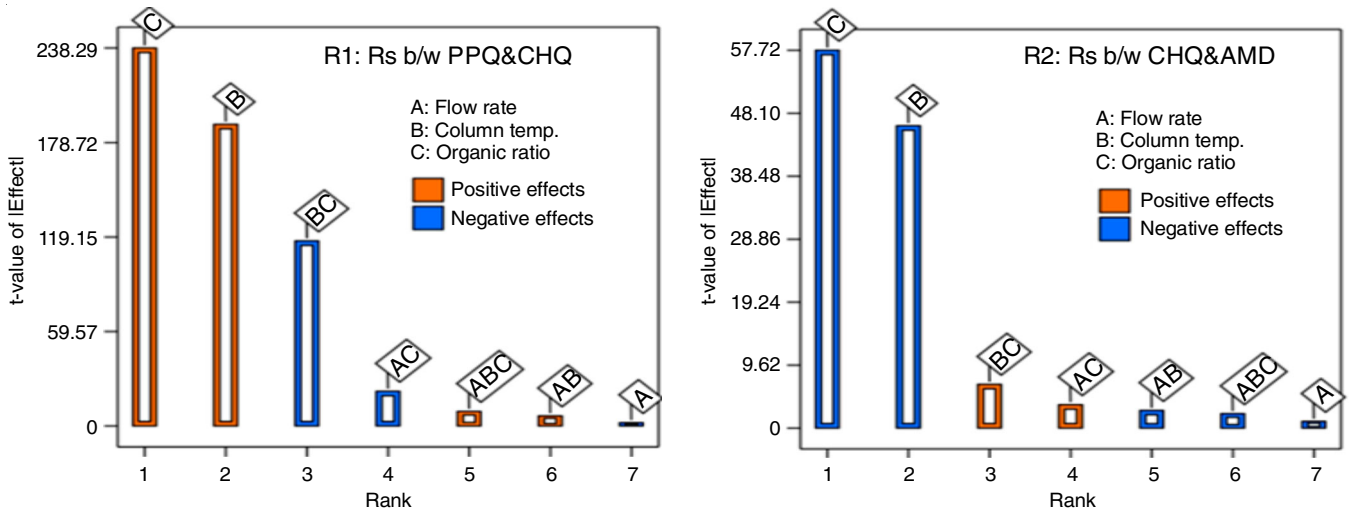

Fig. 6. Pareto charts of the effect of flow rate (A), column temperature (B) and organic ratio (C) on responses R1 and R2 (resolutions)

analytical method parameters ranges wherein the performance criteria of the analytical method are fulfilled, thereby ensuring the quality of the obtained result [15]. In this study, the MODR represents all possible combinations of interactions between critical method parameters (CMPs) and its influences on critical method attributes (CMAs), which were evaluated at the time of optimization process thereby providing assurance on the quality of the developed method. However, achieved the minimum resolution criteria for all the responses was more than 2.0 for all the variables with a range of 0.18 to 0.22 mL/min flow rate, column oven temperature range between 30 to 50 ºC, the organic solvent ratio composition in the mobile phase is from 08% to 12%. Identified the MODR by keeping the minimum resolution criteria at 2.0 as desirability for both the responses R1 and R2. An overlay plots (Fig. 7) from all the experimental runs demonstrates that the optimal operating point for responses R1 and

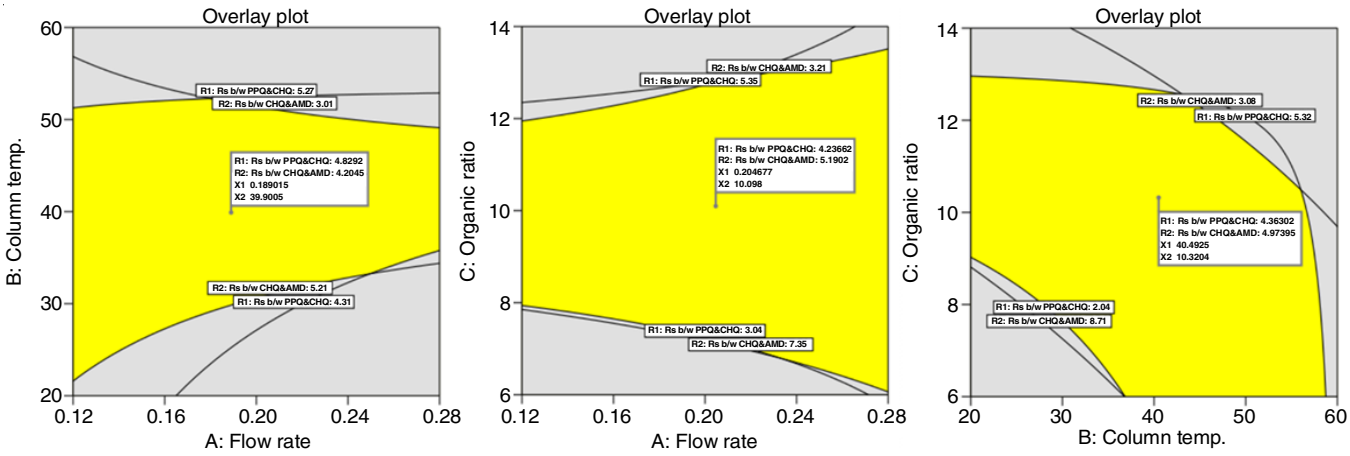

Fig. 7. Overlay plots for the responses R1, R2 and the identified method operable design region (MODR) from the graphical optimization (yellow region: design space, gray region: undesirable region)

R2 has greater design space (DS). The yellow colour region, which met the desirability criteria was considered as favourable, whereas the grey colour does not fit into the model. Thus, the developed method in this region (*i.e.* yellow region) is more robust and the changes made within this region would not have any major impact on the quality (CMAs) of the proposed method. Therefore, this area was chosen as the working point region.

Chromatographic method validation: The proposed method has been validated in compliance with USP <1225> and ICH Q2 (R1) guidelines to evaluate the method's linearity, specificity, precision and accuracy.

System suitability: Standard solution was injected with five replicate injections and found that the peak area response in terms of % RSD is < 0.73% for all peaks, resolution between all the analytes were NLT 2.0 and the peak tailing is NMT 2.0. Summarized the system suitability results and tabulated in Table-1.

Specificity: Specificity refers to a method's efficiency to assess the analyte's response in the presence of additional interferences. Injected diluent, system suitability solution and all drug substances individually and confirmed that all the analytes are free from diluent interference and the spectral homogeneity has been proved by confirming that the purity angel is lesser than the purity threshold for each analyte and summarized the results in Table-4.

Linearity: Linearity studies were carried out with the linearity solutions from 50 to 150% the range of the analytes optimized concentration and reported the correlation coefficient value for this range. The resulted values from the linearity and range were found to be more than 0.999 and summarized all results in Table-4.

Precision and intermediate precision: The precision results of the six replicates of AMD, CHQ and PPQ analytes are in the acceptable range of 100 ± 2% for both intraday and inter day determinations, the %RSD for peak area responses from the standard is below 0.73 % and the summarized the validation study results in Table-4. Based on these results, the method is found to be precise.

Accuracy: Determined the accuracy data from the sample preparations at three distinct concentrations levels namely 50%, 100% and 150% and calculated the mean recovery and % RSD. It can be seen that the % recoveries of aforementioned drugs were found to be 98% to 102%, which confirms the method's accuracy and tabulated the results in Table-4.

TABLE-4
SUMMARY OF VALIDATION RESULTS

Parameter	PPQ	CHQ	AMD
Linearity and range (%)	50 to 150%	50 to 150%	50 to 150%
Correlation-coefficient	0.9996	0.9999	0.9993
Accuracy at 50 (%) level			
% Recovery	99.46	99.05	99.32
% RSD	0.41	0.33	0.27
Accuracy at 100 (%) level			
% Recovery	99.87	99.33	99.27
% RSD	0.14	0.51	0.47
Accuracy at 150 (%) level			
% Recovery	99.18	99.55	99.11
% RSD	0.35	0.52	0.28
Intermediate precision			
% Recovery	99.37	99.48	99.44
% RSD	0.47	0.34	0.39
Specificity			
Purity1 angle	0.044	0.069	0.048
Purity1 threshold	0.225	0.236	0.237

Greenness assessment of proposed method: By keeping this green analytical chemistry concept in consideration, an UPLC method is developed with a shorter runtime at lower flow rates using environmentally benign solvents such as ethanol. The total run time for the proposed method is 15 min at 0.2 mL/min and the organic solvent (ethanol) consumption is less than 1 mL per each injection run. Each sample preparation requires less than 2 mL of ethanol and a cumulative volume of less than 10 mL for each sample analysis. In present study, a few green assessment tools were utilized to assess the optimized method's greenness namely, complex green analytical procedure Index-cGAPI, analytical method greenness score-AMGS and analytical greenness metrics-AGREE.

Analytical greenness metrics (AGREE): A green assessment tool "analytical greenness metrics" (AGREE) that takes into consideration of all 12 green analytical principles with a clock shaped pictogram. Each principle or component is scored

with values ranging from 0 to 1, with 1 being the most friendly to the environment. The total average value is depicted at the middle of the clock-shaped pictogram, the value close to 1 shows the procedure is eco-friendly. The current approach utilized AGREE tool and achieved a score of 0.92 as shown in Fig. 8, indicates that the overall impact of the proposed method on the ecosystems is highly benign and processes a long-term sustainability aspect.

Complex green analytical procedure index (cGAPI): cGAPI is another method to estimate the degree of greenness and assess the sustainability level of the proposed method. It combines all phases of the study into a unified visual representation using a colour-codded pictogram. The tool comprises multiple steps for analyzing the greenness of processes and their final portrayal using a different colour-coding system based on the method's impact on the environment. Based on the pictogram in Fig. 8, the developed UPLC method's overall effect on the environment is quite benign and environmentally friendly.

Analytical method's greenness score-AMGS: AMGS is another evaluation tool which combines HPLC-EAT (environmental assessment) together with SHE (safety, health and environmental assessment). The AMGS results are separated into 3 distinct groups: related to the solvent energy, EHS score of the solvents and the instrument score. The total score of these three evaluations determines the approach's overall result, which is recommended to be as low as possible in order to ensure the method is as environmentally friendly as possible. After providing the appropriate data in the ACS Green Chemistry Institute's green evaluation spreadsheet, the overall score achieved for suggested technique is 720.12 as shown in Fig. 8 indicated that the proposed method has a beneficial effect on the environment.

Conclusion

A novel methodology has been adopted by integrating the AQbD approach with green analytical chemistry concept and developed an environmentally friendly and robust UPLC procedure for the quantification of selected aminoquinoline antimalarial drugs namely PPQ, CHQ and AMD within a 15 min run time and with very less solvent consumption. Stated the analytical target profile (ATP) distinctly, CMAs and CMPs have been identified and thoroughly evaluated the effect of CMPs and their influence on CMAs. The inclusion of AQbD into the developed method has shown greater robustness and increased CMAs performance. The critical method parameters (CMPs) were thoroughly evaluated by using design of experiments (DOE) as well as overlay plots, which is helped to identify the method operable design region (MODR). The design of experiments (DOE) as well as the overlay plots were used for a thorough evaluation of critical method parameters (CMPs) and has identified the method operable design region (MODR). The success of this study shows the applicability and potential advantages of AQbD focused analytical method development using green chemistry principles. In compliance with USP <1225> and ICH Q2 guidelines, the proposed method was validated, observed that the proposed method is linear, specific, sensitive, precise, accurate, robust and eco-friendly (environmentally benign) and the same has been used to determine the selected drugs in commercially available dosage forms. Finally, the method has been verified employing green assessment tools: cGAPI, AMGS (720.12), AGREE score (0.92) shown to provide the best environmentally-friendly results and found that the method is green. The results of all the approaches employed indicate that the developed method is environmentally friendly and suitable for future study without encountering any problems. The progress made in this work has the potential to generate possibilities for the development of more environmentally friendly and resilient Analytical Quality by Design (AQbD) methodologies for the analysis of various pharmaceutical compounds using sustainable solvents.

ACKNOWLEDGEMENTS

The authors thank to USP-India Pvt. Ltd. Hyderabad, India for providing all required samples, chemicals and research facilities for this study to be conducted. The authors acknowledge the support from Koneru Lakshmaiah Education Foundation (KLEF) Department of Engineering Chemistry for the publication of this research work.

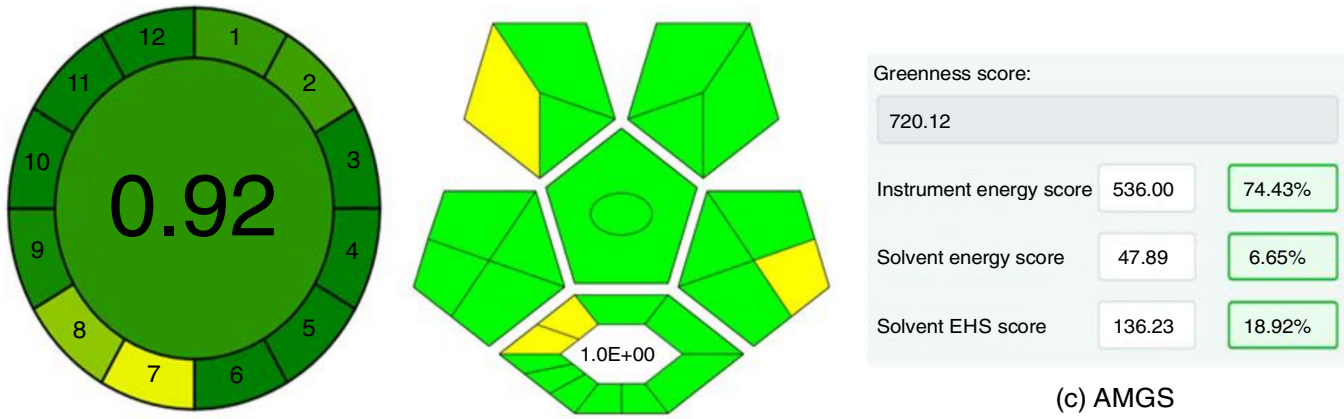

Fig. 8. Greenness assessment tools (a) AGREE, (b) cGAPI, (c) AMGS

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. P. Anastas and N. Eghbali, *Chem. Soc. Rev.*, **39**, 301 (2010); https://doi.org/10.1039/B918763B
2. P.T. Anastas and J.C. Warner, Green Chemistry: Theory and Practice, Oxford University Press, Oxford [England]: New York (1998).
3. A. Galuszka, Z. Migaszewski and J. Namie'snik, *Trends Analyt. Chem.*, **50**, 78 (2013); https://doi.org/10.1016/j.trac.2013.04.010
4. C.J. Welch, N. Wu, M. Biba, R. Hartman, T. Brkovic, X. Gong, R. Helmy, W. Schafer, J. Cuff, Z. Pirzada and L. Zhou, *Trends Analyt. Chem.*, **29**, 667 (2010); https://doi.org/10.1016/j.trac.2010.03.008
5. N. Haq, S. Alshehri, P. Alam, M.M. Ghoneim, Z. Hasan and F. Shakeel, *Sustain. Chem. Pharm.*, **26**, 100648 (2022); https://doi.org/10.1016/j.scp.2022.100648
6. A. Galuszka, Z.M. Migaszewski, P. Konieczka and J. Namiesnik, *Trends Analyt. Chem.*, **37**, 61 (2012); https://doi.org/10.1016/j.trac.2012.03.013
7. H. Shaaban and T. Górecki, *Talanta*, **132**, 739 (2015); https://doi.org/10.1016/j.talanta.2014.09.050
8. H.M. Mohamed and N.T. Lamie, *J. AOAC Int.*, **99**, 1260 (2016); https://doi.org/10.5740/jaoacint.16-0124
9. J. Plotka-Wasylka and W. Wojnowski, *Green Chem.*, **23**, 8657 (2021); https://doi.org/10.1039/D1GC02318G
10. J. Plotka-Wasylka, *Talanta*, **181**, 204 (2018); https://doi.org/10.1016/j.talanta.2018.01.013
11. F. Pena-Pereira, W. Wojnowski and M. Tobiszewski, *Anal. Chem.*, **92**, 10076 (2020); https://doi.org/10.1021/acs.analchem.0c01887
12. F. Pena-Pereira, M. Tobiszewski, W. Wojnowski and E. Psillakis, *Adv. Sample Prep.*, **3**, 100025 (2022); https://doi.org/10.1016/j.sampre.2022.100025
13. M.B. Hicks, W. Farrell, C. Aurigemma, L. Lehmann, L. Weisel, K. Nadeau, H. Lee, C. Moraff, M. Wong, Y. Huang and P. Ferguson, *Green Chem.*, **21**, 1816 (2019); https://doi.org/10.1039/C8GC03875A
14. S.A.C. Wren and P. Tchelitcheff, *J. Chromatogr. A*, **1119**, 140 (2006); https://doi.org/10.1016/j.chroma.2006.02.052
15. ICH Q14 Guideline, Analytical Procedure Development Draft Version, Geneva, Switzerland (2022); http://www.capa.org.tw/upfiles/1649667779.pdf
16. S.K. Muchakayala, N.K. Katari, K.K. Saripella, H. Schaaf, V.M. Marisetti, L.P. Kowtharapu and S.B. Jonnalagadda, *Sci. Rep.*, **12**, 19138 (2022); https://doi.org/10.1038/s41598-022-22998-0
17. M.K. Parr and A.H. Schmidt, *J. Pharm. Biomed. Anal.*, **147**, 506 (2018); https://doi.org/10.1016/j.jpba.2017.06.020
18. Y.V.S. Veerendra, P.K. Brahman, S.D. Mankumare, J.R. Ch and J. Satish, *Asian J. Chem.*, **32**, 219 (2020); https://doi.org/10.14233/ajchem.2020.22224
19. J. Teng, C. Zhu, J. Lyu, L. Pan, M. Zhang, F. Zhang and H. Wu, *J. Pharm. Biomed. Anal.*, **207**, 114417 (2022); https://doi.org/10.1016/j.jpba.2021.114417
20. S.K. Muchakayala, N.K. Katari, K.K. Saripella, H. Schaaf, V.M. Marisetti, S.K. Ettaboina and V.K. Rekulapally, *J. Chromatogr. A*, **1679**, 463380 (2022); https://doi.org/10.1016/j.chroma.2022.463380
21. WHO, World Malaria Report 2022 (2023) (Accessed on 19th June 2023); https://www.who.int/news-room/fact-sheets/detail/malaria
22. A. Manirakiza, E. Serdouma, R.N. Ngbalé, S. Moussa, S. Gondjé, R. Mbetid Degana, G.G. Banthas Bata, J.M. Moyen, G. Grésenguet, J. Delmont and A. Sepou, *J. Public Health Africa*, **8**, 668 (2017); https://doi.org/10.4081/jphia.2017.668
23. A. Hafiz, M.A. Alam, O.A. Alghamdi and A. Mohammed, Combination Therapy and Multidrug Resistance in Malaria Parasite, In: Combination Therapy against Multidrug Resistance, Academic Press, pp. 141-156 (2020).
24. A.R. Parhizgar and A. Tahghighi, *Iran. J. Med. Sci.*, 42, 115 (2017).
25. L. Ravindar, S.A. Hasbullah, K.P. Rakesh and N.I. Hassan, *Eur. J. Med. Chem.*, **256**, 115458 (2023); https://doi.org/10.1016/j.ejmech.2023.115458
26. L.K. Basco and P. Ringwald, *Antimicrob. Agents Chemother.*, **47**, 1391 (2003); https://doi.org/10.1128/AAC.47.4.1391-1394.2003
27. J.K. Aronson, Meyler's Side Effects of Drugs, Elsevier, edn. 16, p. 781 (2016).
28. The International Pharmacopoeia, WHO Department of Essential Medicines and Health Product, edn. 10 (2020).
29. P. Nagaraja, A.K. Shrestha, A. Shivakumar and A.K. Gowda, *J. Food Drug Anal.*, **18**, 7 (2010); https://doi.org/10.38212/2224-6614.2244
30. M. Yabré, L. Ferey, T.I. Somé, G. Sivadier and K. Gaudin, *J. Pharm. Biomed. Anal.*, **19**, 113507 (2020); https://doi.org/10.1016/j.jpba.2020.113507
31. Y. Le Vaillant, C. Brenier, Y. Grange, A. Nicolas, P.A. Bonnet, L.R. Massing-Bias, P. Rakotomanga, B. Koumaré, A. Mahly, M. Absi, M. Ciss, M.H. Loueslati and D. Chauvey, *Chromatographia*, **75**, 617 (2012); https://doi.org/10.1007/s10337-012-2241-5
32. A.T. Miranda, P.H.R. Silva, G.A. Pianetti and I.C. César, *Malar. J.*, **14**, 29 (2015); https://doi.org/10.1186/s12936-015-0570-1
33. V.F. Samanidou, E.N. Evaggelopoulou and I.N. Papadoyannis, *J. Pharm. Biomed. Anal.*, **38**, 21 (2005); https://doi.org/10.1016/j.jpba.2004.12.005
34. D.M. Pawde, N.R.R. Syed, S. Ponneganti, A. Goswami, R.M. Borkar and T. Shunmugaperumal, *J. Chromatogr. Sci.*, **61**, 665 (2023); https://doi.org/10.1093/chromsci/bmac088
35. G.S. Reddy, S.L.N. Prasad Reddy and S. Reddy, *Orient. J. Chem.*, **29**, 1371 (2013).
36. A. Choemang and K. Na-Bangchang, *J. Chromatogr. Sci.*, **57**, 27 (2019); https://doi.org/10.1093/chromsci/bmy077
37. L. Huang, V. Sok, U. Aslam-Mir, F. Marzan, M. Whalen, P.J. Rosenthal and F. Aweeka, *J. Chromatogr. Open*, **2**, 100042 (2022); https://doi.org/10.1016/j.jcoa.2022.100042
38. S. Gaikwad, A. Bansode, N. Patade and S. Tathe, *J. Planar Chromatogr. Mod. TLC*, **33**, 131 (2020); https://doi.org/10.1007/s00764-020-00021-4
39. Jaya Raju Ch Bhaskara Rao T, Sanath Kumar Goud P, Satish J &Rajashekhar K, *J. Liq. Chromatogr. Relat. Technol.*, **41**, 17 (2018); https://doi.org/10.1080/10826076.2018.1492936
40. USP General Chapter 1225, Validation of Compendial Procedures, USPNF 2023 Issue 1 (2023).
41. ICH Guideline, Validation of Analytical Procedures: Text and Methodology, Q2 (R1), International Conference on Harmonization, Geneva, Switzerland (2005); https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf
42. H. Fabre, *J. Pharm. Biomed. Anal.*, **14**, 1125 (1996); https://doi.org/10.1016/S0731-7085(96)01770-0
43. K.D. Altria and S.D. Filbey, *Chromatographia*, **39**, 306 (1994); https://doi.org/10.1007/BF02274518