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Method operable design region for robust RP-HPLC analysis of pioglitazone hydrochloride and teneligliptin hydrobromide hydrate: incorporating hybrid principles of white analytical chemistry and design of experiments

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Abstract

Background A combination of pioglitazone hydrochloride with teneligliptin hydrobromide hydrate is used to treat type-2 diabetes. Several chromatographic techniques have been described in the literature for determination of each of these medications separately. But these procedures used organic solvents that could be dangerous for humans and animals, not to mention harmful to the environment. It is vital to substitute or reduce the use of these neurotoxic and teratogenic solvents in the chromatographic analysis of these anti-diabetic medications to ensure the safety of life and safeguard the environment. The chromatographic technique used for sample analysis should have characteristics like robustness, eco-friendliness, cost-effectiveness, and user-friendliness following the recently developed idea of white analytical chemistry. Teneligliptin and pioglitazone have not yet been simultaneously estimated using a chromatographic method that has been documented in the literature.

Methods A white analytical chemistry-assisted RP-HPLC method has been developed to fill this gap, using economical and eco-friendly solvents. The design of the experiment approach was used during the development of the RP-HPLC method to reduce organic waste and guarantee that the procedure complied with all applicable regulations. Response surface models were built using the full factorial design approach, and the analytical design space was investigated. This method allowed for the identification of an optimal chromatographic condition within the method's operational design region, allowing reliable RP-HPLC analysis of pioglitazone and teneligliptin.

Results The developed RP-HPLC technique underwent validation and was used effectively to assess these drugs in their fixed-dose combinations. Assessments were made of the suggested and published RP-HPLC techniques' validation status, process greenness, cost, and analysis time. For a thorough examination, this review included white analytical chemistry-based RGB models and different green analytical chemistry-based tools.

Conclusion In allowing the simultaneous estimate of teneligliptin and pioglitazone, the devised approach demonstrated robustness, eco-friendliness, and cost-effectiveness.

Keywords Teneligliptin hydrobromide hydrate, Pioglitazone hydrochloride, White analytical chemistry, Design of experiments, Analytical quality by design

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Background

The RP-HPLC technique is often used in the pharmaceutical sector for regular analysis and quality control of medicinal ingredients and products. The International Council for Harmonization (ICH) Q3 (R8) guideline and Indian Pharmacopoeia (IP) 2018 both recognize that the two most frequently used organic solvents for RP-HPLC analysis, acetonitrile and methanol are potentially neurotoxic and teratogenic to people and aquatic animals, as well as harmful to the environment. These organic solvents are also categorized as ones that should be used cautiously owing to their possible dangers in several solvent selection guides provided by the pharmaceutical sectors. According to the Globally Harmonized System of Classification and Labeling of Chemicals, acetonitrile and methanol, two widely used organic solvents in RP-HPLC analysis, are categorized as extremely flammable and volatile chemicals and are marked with the signal word "Danger." The values of 26.8 and 15.7 are their respective dangerous levels. According to the ICH Q3 (R8) and Indian Pharmacopoeia 2018 recommendations, the permissible daily exposure (PDE) levels for acetonitrile and methanol are 410 and 3000 ppm, respectively. When analyzing drug samples, washing columns, and purging systems, RP-HPLC is known to use a lot of solvents and produce a lot of organic waste. The environment, human health, and aquatic life are all seriously in danger from the hazardous solvents like acetonitrile and methanol found in the organic waste produced by RP-HPLC analysis. During the chromatographic analysis of drugs substances and products, it is crucial to replace or decrease the use of these hazardous solvents in line with the principles of white analytical chemistry (WAC). The WAC idea not only encourages environmental safety but also makes it possible to assess the success of analytical procedures in terms of cost, time, and validation power. Therefore, it is essential to create WAC-driven RP-HPLC techniques for reliable, eco-friendly, time-efficient, and cost-effective analysis of drug substances and goods to protect the environment, ensure public safety, and protect aquatic life [1–5].

Using the principles of Analytical Quality Risk Management (AQRM) and Design of Experiments (DoE), Analytical Quality by Design (AQbD) is a systematic approach used in the development of analytical methods that focuses on achieving robust, accurate, and precise analysis of drug substances and products. The development of WAC-driven RP-HPLC analysis uses the AQbD methodology to reduce the production of organic waste during drug sample analysis. Critical Method Performance Attributes (CMPAs) and Critical Procedure Parameters (CPPs), which are essential for the development of analytical methods, may be easily identified using AQRM. The DoE is an essential tool for doing response surface analysis of key process variables and responses and optimizing analytical techniques to ensure their robustness, accuracy, and precision while analyzing drug samples. The use of the Analytical Quality by Design (AQbD) methodology in the establishment of analytical techniques will be required for receiving new medication approval, according to the upcoming ICH Q14 guideline. Therefore, the RP-HPLC technique should be developed utilizing the AQbD methodology to assure future regulatory compliance with the analytical method. Several RP-HPLC techniques have recently been published in the literature that uses the AQbD, Green Analytical Chemistry (GAC), Quality Risk Management (QRM), and Design of Experiments (DoE) tenets. These approaches show how these cutting-edge methodologies may be integrated, showing their value in achieving analytical excellence while taking environmental sustainability and risk management into account [6–12].

Today's demanding lifestyles and unhealthful eating patterns have made diabetes mellitus an illness that is frequently seen. Dipeptidyl Peptidase-4 (DPP-4) inhibitor teneligliptin hydrobromide hydrate (THH) is recommended for the treatment of type 2 diabetes. On the other hand, pioglitazone hydrochloride (PIO) is a thiazolidinedione used in individuals with type 2 diabetes mellitus to control glycemic levels together with diet and exercise. The Central Drugs Standards Control Organization (CDSCO) authorized the fixed-dose combination of these anti-diabetic medications in 2022, providing a therapy option for people with type 2 diabetes mellitus [13]. The chemical structures of pioglitazone hydrochloride (PIO) and teneligliptin hydrobromide hydrate (THH) are shown in Fig. 1. Numerous RP-HPLC techniques have been described for the measurement of THH and PIO

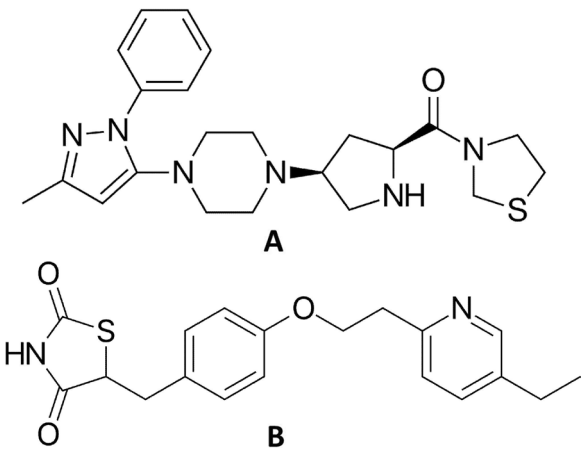


Fig. 1 Chemical structures of anti-diabetic drugs **A** teneligliptin hydrobromide hydrate (THH) and **B** pioglitazone hydrochloride (PIO)

separately [14–23], and several HPTLC techniques have also been reported [24–27]. However, hazardous organic solvents including acetonitrile, toluene, and methanol are often used in these RP-HPLC procedures. These solvents are harmful to the environment and provide dangers to aquatic and terrestrial life. In comparison, ethanol is a less flammable and hazardous substitute that is also biodegradable because it is made from natural resources. For RP-HPLC analysis of drug samples, ethanol is frequently suggested as a more cost-effective, environmentally friendly alternative to acetonitrile and methanol (1–5). Ethanol is categorized as a class 3 solvent, signifying minimal toxicity, with a permitted daily exposure (PDE) limit surpassing 5000 ppm, following the ICH Q3C (R8) guideline and IP 2018 criteria. With a total analytical risk value of just 2.6, ethanol is considered to be a safe organic solvent by the worldwide standardized system of categorization and labeling of chemicals, outperforming acetonitrile and methanol. There are no published studies that use RP-HPLC to simultaneously estimate THH and PIO in their fixed-dose combination (FDC). As a result, a WAC-driven, cost-efficient, quick, and environmentally friendly RP-HPLC approach has been developed that uses ethanol as the solvent under a single chromatographic condition. Following the ICH Q14 guideline, the RP-HPLC method was developed by implementing the AQbD methodology. This strategy aims to lessen the production of organic waste and guarantee the analytical method's regulatory compliance. Using the risk priority number (RPN) ranking and filtering method, an analytical quality risk assessment was carried out to identify crucial procedure variables and responses. Using a central composite design and the trial version of Minitab 18.0 software, a DoE-based response surface modeling (RSM) was carried out to optimize the RP-HPLC technique. AGREE, NEMI, ESA, and complex GAPI software were used to assess the greenness profile of the proposed RP-HPLC method to simultaneously quantify THH and PIO. The proposed RP-HPLC analysis of THH and PIO's validation power, greenness profiles, cost, and time efficiency were evaluated utilizing the WAC and RGB models' principles (Fig. 2).

Methods

Instruments and softwares

The chromatographic separation of THH and PIO was performed using a Shimadzu HPLC 2030 prominence i-series model equipped with UV (Ultra Violet) and PDA (Photo-Diode Array) detectors, which was purchased from Shimadzu Scientific Instruments (India) Private Limited, Bangalore, India. The mobile phase's components were filtered using the Rocker 300 vacuum

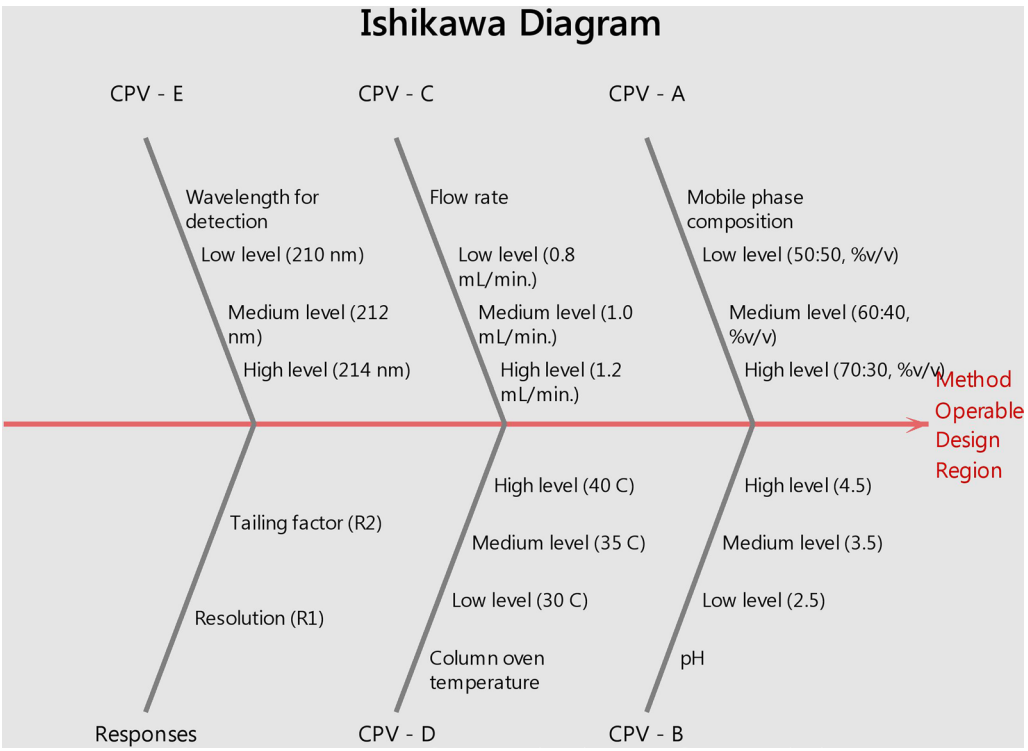


Fig. 2 Minitab 18 software generated Ishikawa diagram showing the list of critical procedure variables and responses for the development of RP-HPLC method for estimation of PIO and THH

filtration assembly, which was bought from PCI Analytics Private Limited in Mumbai, Maharashtra. A sonicator water bath from PCI analytics private limited, Mumbai, Maharashtra, was used for drug extraction from FDCs and the deaeration of mobile phase components. Sample and standard powder were weighed using an electronic weighing scale that was purchased from Shimadzu Scientific Instruments (India) Private Limited, Bangalore, India. Using Stat-Ease's Design-Expert 10.0 software (trial version), the analytical quality risk assessment and design of experiments-based response surface modeling were completed. The AGREE calculator, which can be found at https://mostwiedzy.pl/AGREE, was used to determine the method's greenness rating. Last but not least, Complex GAPI software from mostwiedzy. pl/complexgapi was used to examine the method's greenness profile.

Reagents and materials

The THH and PIO active pharmaceutical ingredients (APIs) gift samples were acquired from reputed drug manufacturers in Gujarat, India. It was decided to buy ethanol of the analytical reagent (AR) quality from a Shree Khedut Sahakari Khand Udhyod Mandali Limited, Bardoli, Gujarat, India. In our institute laboratory, double-distilled water was produced using distillation assembly. From Avantor Performance Materials India Limited in Thane, Maharashtra, India, we purchased glacial acetic acid, orthophosphoric acid, and triethylamine of AR grade. Shimadzu Scientific Instruments (India) Private Limited in Bangalore, India, supplied the Shim-Pack C18 RP (250 mm, 4.6 mm, 5.0 m) column. Both the sample syringe filter and the membrane filter were purchased from Pall India Private Limited in Andheri, Mumbai, India. The FDCs for THH and PIO were bought at a nearby drug store in Surat, Gujarat, India.

Combined working standard solution

A 1.0 mL aliquot was collected from each standard stock solution of THH (200 g/mL) and PIO (150 g/mL) to generate the combined working standard solution. The mobile phase was used as the diluent to dilute these aliquots in a 10 mL volumetric flask. The combined working standard solution had final concentrations of 20 g/mL for THH and 15 g/mL for PIO.

Risk priority number ranking and filtering

Following preliminary trials, the risk priority number (RPN) ranking and filtering approach was used to analyze the prospective procedure variables to ascertain their impact on method responses. An analytical quality risk evaluation of prospective critical process parameters (CPPs) was then carried out using Minitab 18 software using the specified experimental trials and their accompanying responses. The critical procedural variables were then examined for main effects, two-way interactions, and quadratic effects using a central composite design and Minitab 18 software using a DoE-based response surface methodology (RSM). To optimize the desired RP-HPLC procedure, the experimental trials and their results were subjected to analysis of variances (ANOVA), response surface contour plots and multiple regression analysis using Minitab 18 software.

Central composite design and optimization

The Analytical Target Profile (ATP) of the targeted RP-HPLC technique was achieved by navigating the Method's Operable Design Region (MODR) using the DoE-based response surface methodology (RSM) and looking at response surface contour plots. To enable simultaneous determination of THH and PIO using the RP-HPLC method, a single analytical control point based on the MODR was used. THH and PIO were separated by isocratic chromatography using a Shim-pack C18 column (250 mm 4.6 mm, 5.0 m) and a mobile phase made of ethanol/water (65 + 35, v/v) that had been pH-adjusted with orthophosphoric acid to 3.0. The column oven temperature was set at 30˚C, and the chromatographic conditions included a flow rate of 0.8 mL/minute. At 210 nm, the chromatographic peaks for THH and PIO were detected.

Calibration curve for pioglitazone and teneligliptin

From combined working standard solution of THH and PIO, aliquots of 2, 4, 6, 8, and 10 µL were injected into RP-HPLC analytical columns to generate the calibration curve. The corresponding peak areas were determined, and their concentrations of THH and PIO were linked. These data points were plotted to generate calibration curves, which then allowed for the calculation of the correlation coefficient and the regression line equation. To accurately determine the analytes in subsequent samples, these calibration curves offer a quantitative link between the peak areas and concentrations of THH and PIO.

Validation study of method

The targeted RP-HPLC method's validation involves several steps, including system suitability testing, specificity, linearity, precision, accuracy, and robustness assessments, as well as the selection of the LOD (Limit of Detection) and LOQ (Limit of Quantitation). To validate the accuracy and applicability of the method for the simultaneous estimate of THH and PIO, these validation parameters were assessed. The chromatographic system was tested for system appropriateness to make sure it was performing within acceptable performance standards.

The method's precision in measuring THH and PIO in the presence of any interfering chemicals was evaluated by its specificity. The analyte concentrations and accompanying detector response were compared using linearity. The method's repeatability and intermediate precision were determined using precision. The degree of accuracy indicated how well the results matched the actual data. Robustness evaluated a method's resilience to minute changes in experimental circumstances. The lowest detectable and measurable quantities of THH and PIO, respectively, were established by the LOD and LOQ.

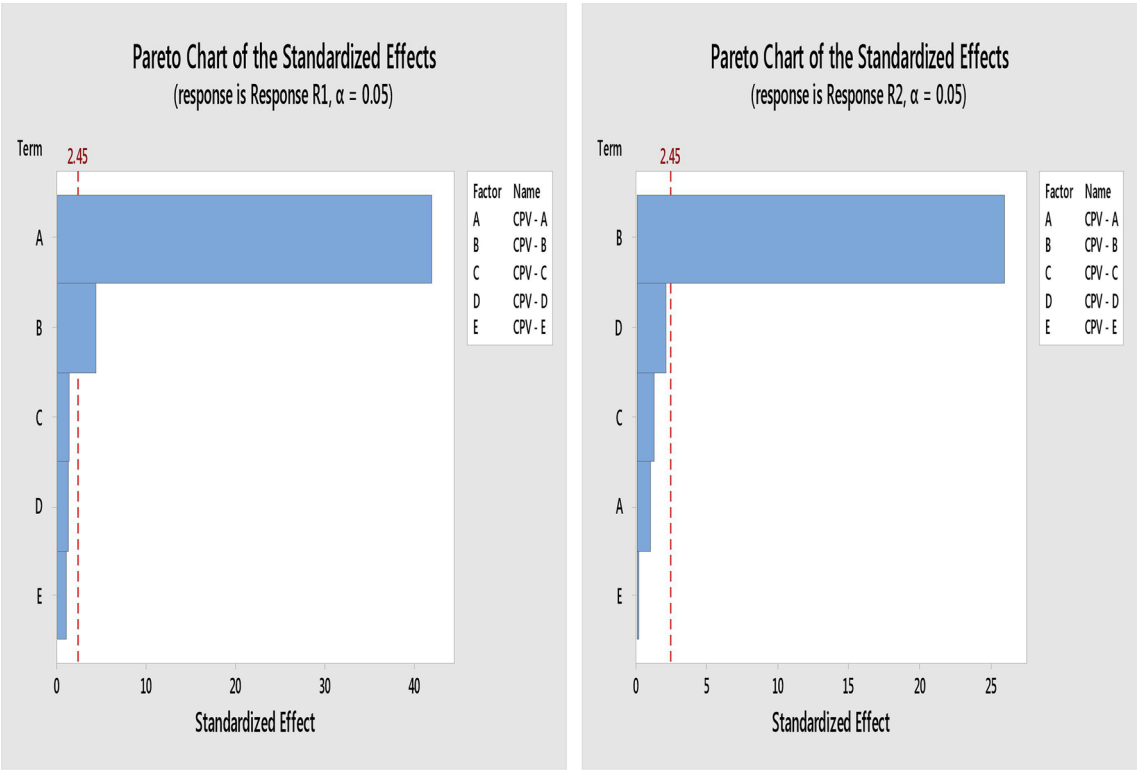
Analysis of fixed-dose combinations

The concurrent evaluation of THH and PIO in their fixed-dose combinations (FDCs) was effectively accomplished using the devised RP-HPLC method, which is distinguished by its robustness and greenness. Tablet powder corresponding to the unit dose of the medications was dissolved and diluted in a 100 mL volumetric flask using ethanol as the solvent to carry out the analysis for each FDC. After 20 min of sonication, Whatman filter paper was used to filter the resultant solution. A sample from the filtrate after it had been properly diluted with mobile phase was injected and subjected to optimal chromatographic conditions. The regression line equation unique to each drug was used to calculate how much of each was present in the FDC.

Results

Analytical quality risk assessment

An analytical quality risk assessment was performed on the potential procedure variables to determine how they would affect the chosen responses (R1 and R2). The risk priority number (RPN) ranking and filtering method through the Plackett–Burman design was used to conduct this evaluation. To generate design metrics for two levels, all selected essential procedure variables were depicted in an Ishikawa diagram and entered into the Minitab 18 software. The related responses were then measured during experimental runs in the lab. Using Minitab 18, a Pareto chart analysis was carried out on the measured responses. Critical procedural variables were classified as high-risk CPVs by the Pareto chart analysis if their *p*-values were less than 0.05 and low-risk CPVs if their *p*-values were more than 0.05.



A **B**

Fig. 3 Analytical quality risk assessment using Plackett–Burman design and Minitab 18 software **A** Pareto analysis showing high-risk CPVs for response R1 **B** Pareto analysis showing high-risk CPVs for response R2

Table 1 Analytical quality risk assessment for identification of high-risk CPVs (Critical Procedure Variables) for variation in responses (resolution and tailing factor) using RPN (Risk Priority Numbering) ranking and filtering method

Source	DF	Adjusted SS	Adjusted MS	F-Value	P-Value	Risk priority number ranking	Risk priority number filtering
Risk priority ranking and filtering for Response 1 using Plackett–Burman design and Minitab software							
Model	5	92.1244	18.4249	357.40	0.000	–	–
Linear	5	92.1244	18.4249	357.40	0.000	–	–
CPV—A	1	90.9151	90.9151	1763.53	0.000	Rank 1	High-risk
CPV—B	1	0.9804	0.9804	19.02	0.005	Rank 2	High-risk
CPV—C	1	0.0919	0.0919	1.78	0.230	Rank 3	Low-risk
CPV—D	1	0.0850	0.0850	1.65	0.246	Rank 4	Low-risk
CPV—E	1	0.0520	0.0520	1.01	0.354	Rank 5	Low-risk
Error	6	0.3093	0.0516	–	–	–	–
Total	11	92.4337	–	–	–	–	–
Risk priority ranking and filtering for Response 2 using Plackett–Burman design and Minitab software							
CPV—A	1	0.000408	0.000408	0.92	0.373	Rank 4	Low-risk
CPV—B	1	0.297675	0.297675	673.98	0.000	Rank 1	High-risk
CPV—C	1	0.000675	0.000675	1.53	0.263	Rank 3	Low-risk
CPV—D	1	0.001875	0.001875	4.25	0.085	Rank 2	Low-risk
CPV—E	1	0.000008	0.000008	0.02	0.895	Rank 5	Low-risk
Error	6	0.002650	0.000442	–	–	–	–
Total	11	0.303292	–	–	–	–	–

Figure 3 and Table 1 show the outcomes of the Pareto chart analysis for the two chosen responses.

Response surface methodology and optimization

Using DoE-based response surface methodology, the high-risk critical procedural variables A and B were further examined for their substantial main effects, two-way interactions, and quadratic effects on responses R1 and R2. A central composite design was implemented using the Minitab 18 software to carry out this process. The program suggested 13 experimental trials, and these experiments were conducted in the laboratory following the recommendations listed in Table 2. The measured responses from each experimental trial were entered into the program to perform an analysis of variance (ANOVA), contour plots of the response surface, and

Table 2 Design metrics for DoE-based response surface methodology for high-risk CPVs (Critical Procedure Variables) and critical responses using central composite design and MINITAB 18 software

Std Order	Run Order	Point Type	Blocks	CPV—A	CPV—B	Response–R1	Response–R2
7	1	−1	1	60.0000	2.08579	5.55	1.32
11	2	0	1	60.0000	3.50000	5.56	1.11
6	3	−1	1	74.1421	3.50000	7.85	1.11
9	4	0	1	60.0000	3.50000	5.45	1.12
13	5	0	1	60.0000	3.50000	5.62	1.13
3	6	1	1	50.0000	4.50000	1.35	2.20
5	7	−1	1	45.8579	3.50000	1.34	1.14
4	8	1	1	70.0000	4.50000	6.54	2.22
8	9	−1	1	60.0000	4.91421	5.45	2.55
1	10	1	1	50.0000	2.50000	1.32	1.23
2	11	1	1	70.0000	2.50000	6.85	1.25
10	12	0	1	60.0000	3.50000	5.61	1.10
12	13	0	1	60.0000	3.50000	5.50	1.12

multiple regression analysis based on the DoE. The quadratic models were significant for both replies, according to the ANOVA findings for responses R1 and R2, which are displayed in Table 3. This suggests a nonlinear graphical relationship between the critical procedure variables and the responses. Both of the critical procedure variables' main effects and quadratic effects were discovered to be significant for both responses. Figure 4A and B shows the examination of response surface plots, which revealed quadratic contour lines in the 2D response surface plots for both R1 and R2, further demonstrating the quadratic link between the crucial procedure variables and the responses. These results lend support to the DoE-based response surface methodology for improving and comprehending the relationships between the important procedure factors and the replies R1 and R2.

R-squared, adjusted R-squared, and anticipated R-squared values were all over 0.9, according to multiple regression analysis. The adjusted and predicted R-squared values differed by less than 0.2, demonstrating the best-fit model for forecasting the intended responses. Response surface contour plots have been developed to visualize the multidimensional interactions between the critical procedure variables (A and B) and the responses (R1 and R2). These graphs were superimposed to help in navigating Method Operable Design Region (MODR) according to the Analytical Target Profile (ATP) of the RP-HPLC method and getting the desired outcomes. Figure 4C shows the plots of the overlay response surfaces.

Results of method validation study

For each fixed-dose combination (FDC) of THH and PIO ($n=3$), the chromatograms of the blank, standard, and sample solutions were superimposed to assess the specificity of the technique. Other than the peaks associated with the respective medications in the FDCs of THH and PIO, no other peaks were seen. This demonstrated that the formulations of THH and PIO did not contain any additives that would have interfered with the targeted chromatographic study of the medications of interest. The calibration curve procedure was carried out five times for the linearity study, and the linearity was evaluated by plotting the mean peak areas ($n=5$) against the corresponding concentrations of each drug. The peak areas of THH and PIO showed linearity over the concentration ranges of the 40–200 µg/mL and 30–150 µg/mL, respectively. According to Fig. 5, the correlation coefficient for the two drugs was higher than 0.999. The repeatability of sample injections ($n=7$) had a % RSD (Relative Standard Deviation) of 0.75% for THH and 0.83% for PIO, respectively. This showed how the technique for

Table 3 ANOVA (Analysis of variance) for study of main effects, two-way interactions and quadratic effects of high-risk CPVs (Critical Procedure Variables) on selected critical responses R1 and R2 using central composite design and MINITAB 18 software

Source	Degree of freedom	Adjusted SS	Adjusted MS	*F*-Value	*P*-Value	Level of significance
ANOVA for Response – R1						
Model	5	52.3728	10.4746	34.18	0.000	Significant
Linear	2	48.5472	24.2736	79.21	0.000	Significant
CPV—A	1	48.4942	48.4942	158.24	0.000	Significant
CPV—B	1	0.0530	0.0530	0.17	0.690	Non-significant
Square	2	3.8225	1.9113	6.24	0.028	Significant
CPV—A*CPV—A	1	3.6088	3.6088	11.78	0.011	Significant
CPV—B*CPV—B	1	0.4987	0.4987	1.63	0.243	Non-significant
2-Way Interaction	1	0.0030	0.0030	0.01	0.924	Non-significant
CPV—A*CPV—B	1	0.0030	0.0030	0.01	0.924	Non-significant
ANOVA for Response – R2						
Model	5	3.15345	0.63069	53.55	0.000	Significant
Linear	2	1.69232	0.84616	71.84	0.000	Significant
CPV—A	1	0.00000	0.00000	0.00	0.994	Non-significant
CPV—B	1	1.69232	1.69232	143.69	0.000	Significant
Square	2	1.46112	0.73056	62.03	0.000	Significant
CPV—A*CPV—A	1	0.01973	0.01973	1.67	0.237	Non-significant
CPV—B*CPV—B	1	1.46082	1.46082	124.03	0.000	Significant
2-Way Interaction	1	0.00000	0.00000	0.00	1.000	Non-significant
CPV—A*CPV—B	1	0.00000	0.00000	0.00	1.000	Non-significant

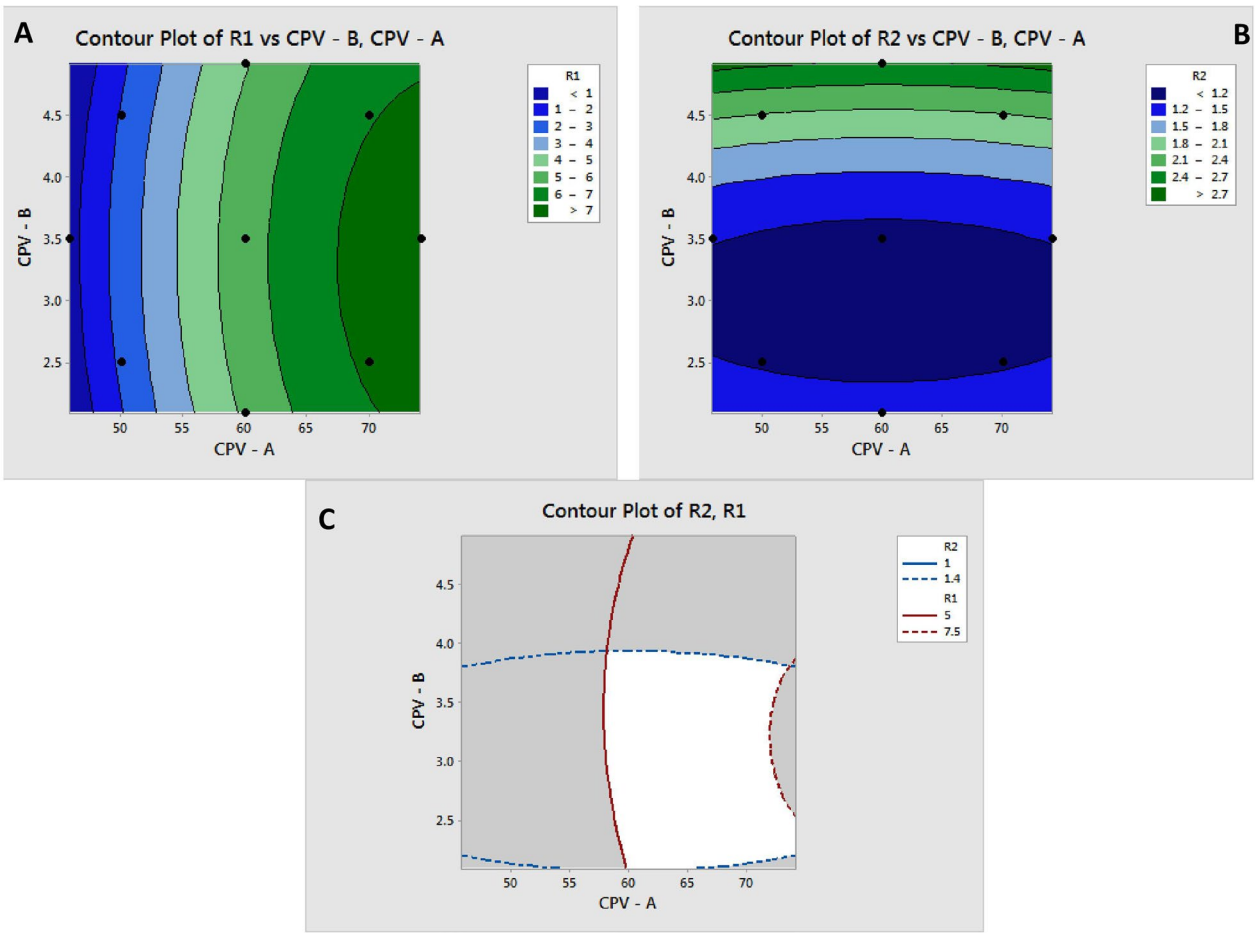


Fig. 4 Response surface modeling using a central composite design and Minitab 18 software **A** 2D contour plot showing multidimensional interactions between high-risk CPVs with response R1 **B** 2D contour plot showing multidimensional interactions between high-risk CPVs with response R2 **C** Overlaid contour plots showing analytical design space for the development of targeted RP-HPLC method as per analytical target profile

injecting samples was precise and consistent, delivering accurate and repeatable findings.

For the repeatability of sample measurement ($n=7$), the relative standard deviation (RSD) was found to be 0.45% for THH and 0.38% for PIO, respectively. Regarding intra-day precision ($n=3$), the% RSD ranged for THH from 0.87 to 0.98% and for PIO from 0.59 to 0.92%. For inter-day precision ($n=3$), the percentage RSD ranged for THH from 0.92 to 1.12% and for PIO from 0.77 to 1.04%. These findings show how the devised method for sample measurements at various time points is precise as well as trustworthy. The ICH Q2 (R1) guideline's standard addition method was used to conduct the accuracy study. The recovered percentages for the additional THH and PIO were found to be between 98 and 102%, showing that the method's estimation was accurate. The limit of quantitation (LOQ) was found to be 15 µg/mL for THH and 9 µg/mL for PIO ($n=3$), whereas the limit of detection (LOD) for THH and PIO was found to be 5 µg/mL and 3 µg/mL, respectively. For THH and PIO, respectively, these numbers represent the lower limits of detection and quantitation. To evaluate the method's robustness, robustness studies ($n=3$) were carried out by changing the mobile phase, flow rate, column oven temperature, and wavelength. The method's robustness in simultaneously estimating THH and PIO was determined to be less than 2.0% RSD for these variables. Table 4 gives a complete overview of the method's performance and validity for the simultaneous estimation of THH and PIO. It summarizes the validation parameters (Table 5).

Discussion

There are no published RP-HPLC methods for simultaneously estimating THH and PIO in their fixed-dose combinations (FDCs). Earlier RP-HPLC techniques used hazardous organic solvents like acetonitrile and methanol

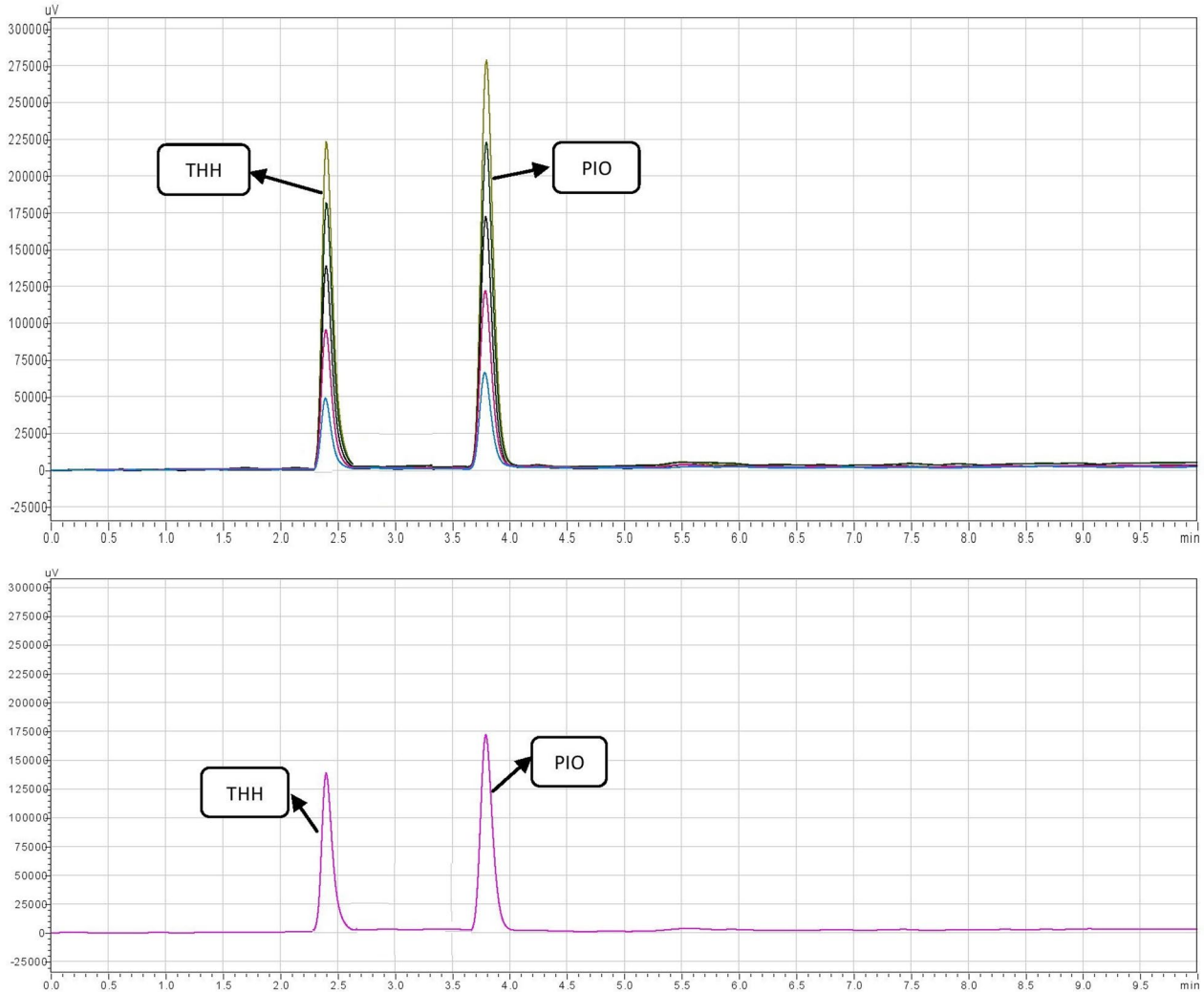


Fig. 5 Targeted RP-HPLC analysis of anti-diabetic drugs **A** Chromatogram showing linearity of THH (40–200 µg/mL) and PIO (30–150 µg/mL) **B** Chromatogram showing the peak of THH and PIO at the retention time of 2.71 and 3.75 min, respectively

to estimate PIO and THH separately in their prescription dose forms. These solvents endanger the environment, animal life, and human health. It is essential to eliminate, swap out, or minimize the use of these potentially harmful solvents in the RP-HPLC analysis of THH and PIO following the principles of green analytical chemistry (GAC) and the only recently developed idea of white analytical chemistry (WAC).

Ethanol, a more sustainable and environmentally friendly organic solvent, was used for sample preparation and mobile phase composition in the simultaneous estimation of PIO and THH to adhere to the principles of GAC and WAC. Compared to acetonitrile and methanol, ethanol is less harmful and volatile. It is a natural resource-based biodegradable solvent. As a result, the organic waste produced by chromatographic analysis using ethanol would not endanger aquatic life or humans.

The cost-effective RP-HPLC analysis of PIO and THH is further supported by the fact that ethanol is more affordable than acetonitrile and methanol. To maintain regulatory compliance with the ICH Q14 criteria and reduce waste generation during sample analysis, the Analytical Quality by Design (AQbD) approach was used in addition to adhering to the WAC concept. Analytical quality risk assessment and Design of Experiments (DoE) principles were used in this strategy.

The developed RP-HPLC method for the simultaneous measurement of THH and PIO offers a green and sustainable analytical solution that complies with regulatory requirements, minimizes waste generation, and lessens environmental effects by incorporating GAC, WAC, and the AQbD approach.

Table 4 Summary of validation parameters for simultaneous estimation of THH and PIO

Validation Parameters	THH	PIO
Linearity study (n = 5)		
Linearity range (µg/mL)	40–200 µg/mL	30–150 µg/mL
Regression line equation	1754 x – 17,654	4232 x -134.56
Correlation coefficient	0.9994	0.9996
Precision study (% RSD)		
Repeatability sample injection (n = 7)	0.75	0.83
Repeatability of sample measurement (n = 7)	0.45	0.38
Intra-day variation	0.87–0.98	0.59–0.92
Inter-day variation	0.92–1.12	0.77–1.04
Accuracy study (%) (n = 3)		
% recovery	99.32–99.45	98.12–99.32
LOD & LOQ (n = 5)		
Limit of detection (LOD)	5 µg/mL	3 µg/mL
Limit of quantitation (LOQ)	15 µg/mL	9 µg/mL
Robustness study (%RSD) (n = 3)		
Mobile phase variation	0.73–0.92	0.89–1.03
Wavelength variation	0.65–1.12	0.77–1.05
Flow rate variation	0.62–1.05	0.83–1.03
Column temperature variation	0.81–1.14	0.94–1.21
Specificity study by PDA detector (correlation-coefficient)		
Peak start (s)	0.99923	0.99934
Peak apex (a)	0.99928	0.99944
Peak end (m)	0.99956	0.99933

Implementation of analytical quality by design approach

The simultaneous determination of PIO and THH using a targeted RP-HPLC method was accomplished using the Analytical Quality by Design (AQbD) methodology. To enable chromatographic separation of the drugs following the system suitability testing criteria listed in IP 2018, the Analytical Target Profile (ATP) was developed. Its goal was to provide a method that was economical, reliable, and environmentally friendly. This led to the identification of critical method variables such as resolutions, tailing factors, retention factors, and the number of theoretical plates.

The development of the targeted RP-HPLC method was discovered to be dependent on the resolution between the peaks of THH and PIO (Response R1) and the tailing factor for the peak of THH (Response R2). The volume ratio of ethanol to water (Critical method variable A), pH of the mobile phase (Critical method variable B), flow rate (Critical method variable C), column oven temperature (Critical method variable D), and detection wavelength (Critical method variable E) for the desired responses R1 and R2 were also identified as potential critical method variables. Figure 2 contains a list of potential procedure variables, responses, and the analytical target profile.

The method development procedure for the RP-HPLC technique was optimized to simultaneously estimate PIO and THH while taking into account critical variables and responses for successful chromatographic separation by using the AQbD approach, which guides method development by particular analytical aims.

An analytical quality risk assessment was performed on the potential procedure variables to determine how they would affect the chosen responses (R1 and R2). The risk priority number (RPN) ranking and filtering method through the Plackett–Burman design was used to conduct this evaluation. Critical procedure variables A and B were determined to be high-risk based on the analysis; thus, they were further investigated for the RP-HPLC method's optimization using DoE-based response surface methodology. This strategy attempted to boost the method's overall performance and dependability by enhancing the identified critical procedural variables.

Using DoE-based response surface methodology, the high-risk critical procedural variables A and B were further examined for their substantial main effects, two-way interactions, and quadratic effects on responses R1 and R2. A central composite design was implemented using the Minitab 18 software to carry out this process. The volume ratio of ethanol to water (critical process

Table 5 Comparison and assessment of proposed and published RP-HPLC methods using RGB (Red, Green and Blue) model and principles of white chemistry for estimation of THH and PIO

Type of RP-HPLC method	Chromatographic condition	RGB model-based assessment	White analytical chemistry-based assessment
RP-HPLC method for estimation of THH (15)	The chromatographic analysis of THH was carried out using Kromasil C18 (250 mm, 4.6 mm, 5.0 µm) analytical column and acetonitrile, water and trifluoroacetic acid (60: 1940: 2.0, v/v) as mobile phase B and acetonitrile and trifluoroacetic acid (2000: 2.0, v/v). Acetonitrile was used as diluent. The method generated waste of more than 100 mL	**Red model** – The validation of method complied for ICH Q2 (R1) guideline **Green model** – Acetonitrile is class 2 solvent and its PDE limit is 410 ppm. Acetonitrile is toxic solvent and its total analytical hazards value is 28.6. The method generated organic waste of more than 100 mL **Blue model –** Acetonitrile is costly organic solvent	The proposed method has good validation efficiency but only estimate THH The method cannot be considered as green analytical method The method requires costly organic solvent such as acetonitrile
RP-HPLC method for estimation of PIO (18)	The RP-HPLC analysis of PIO was carried out using C18 (250 mm, 4.6 mm, 5.0 µm) column as stationary phase and acetonitrile: 10 mM dihydrogen phosphate buffer of pH 6.0 (50:50, v/v) as mobile phase. Acetonitrile was used as diluent and method generated organic waste of more than 100 mL	**Red model** – The validation of method complied for ICH Q2 (R1) guideline **Green model** – Acetonitrile is class 2 solvent and its PDE limit is 410 ppm. Acetonitrile is toxic solvent and its total analytical hazards value is 28.6. The method generated organic waste of more than 100 mL **Blue model –** Acetonitrile is costly organic solvent	The proposed method has good validation efficiency but only estimate PIO The method cannot be considered as green analytical method The method requires costly organic solvent such as acetonitrile
Proposed RP-HPLC method for simultaneous estimation of THH and PIO	The chromatographic analysis of PIO and THH was carried out using Shim-pack C18 ((250 mm, 4.6 mm, 5.0 µm) analytical column as stationary phase and ethanol/water of pH 3.5 (60:40, v/v) as mobile phase. Ethanol was used as diluent with water. The method generated organic waste of more than 100 mL but consisting of safe organic solvent	**Red model** – The validation of method complied for ICH Q2 (R1) guideline. The method development includes implementation of AQbD approach **Green model** – Ethanol is class 3 solvent and its PDE limit more than 5000 ppm. Ethanol is safe solvent and its total analytical hazards value is only 2.6. The method generated organic waste of more than 100 mL **Blue model –** Ethanol is very economical organic solvent	The proposed method has good validation efficiency and applied for simultaneous estimation of THH and PIO The method can be considered as green analytical method The method requires economical organic solvent such as ethanol

variable A) should be between 60:40 and 70:30%v/v, and the pH of the water (critical procedure variable B) should be adjusted to be between 2.5 and 3.5 using glacial acetic acid, it was found through optimization. Finally, by employing ethanol/water (65+35%) at pH 3.0, the final chromatographic separation of THH and PIO was accomplished. Figure 5 illustrates the retention times for the THH and PIO peaks, which were determined to be 2.41 and 3.75 min, respectively. Notably, for the chromatographic peaks of THH and PIO, the resolution, tailing factors, retention factors, and the number of theoretical plates all fulfilled IP 2018 criteria for system suitability testing ($n=7$). The following mathematical models (Eqs. 1 and 2) were used to forecast the responses and navigate the method operable design region for the optimization of the RP-HPLC process. These models were crucial in helping to improve the process and get the desired results.

$$\begin{aligned}\text{Response} - \text{R1} = &- 38.7 + 1.120\text{CPV} - A + 1.96\text{CPV} \\ &- B - 0.00720\text{CPV} - A * \text{CPV} \\ &- A - 0.268\text{CPV} - B * \text{CPV} \\ &- B - 0.0027\text{CPV} - A * \text{CPV} - B\end{aligned} \quad (1)$$

$$\begin{aligned}\text{Response} - \text{R2} = 7.04 &- 0.0639\text{CPV} - A - 2.748\text{CPV} \\ &- B + 0.000532\text{CPV} - A * \text{CPV} - A \\ &+ 0.4583\text{CPV} - B * \text{CPV} - B \\ &+ 0.00000\text{CPV} - A * \text{CPV} - B\end{aligned} \quad (2)$$

Red model-based assessment for validation efficiency

According to the ICH Q2 (R1) recommendations, the Red model-based assessment (R1 to R4) was used to evaluate the effectiveness of the validation of published and proposed RP-HPLC techniques. When calculating THH and PIO separately in their pharmaceutical dose forms, the published RP-HPLC methods showed a flawless score of 80 out of 100 for all four principles. Due to its wider scope and application, the proposed method, which uses ethanol as the solvent and the analytical quality by design approach, was able to simultaneously estimate THH and PIO in their Fixed-Dose Combination (FDC) and earned an additional 20 points. As a result, the proposed method received an impressive validation assessment score from the Red model of 100 out of 100.

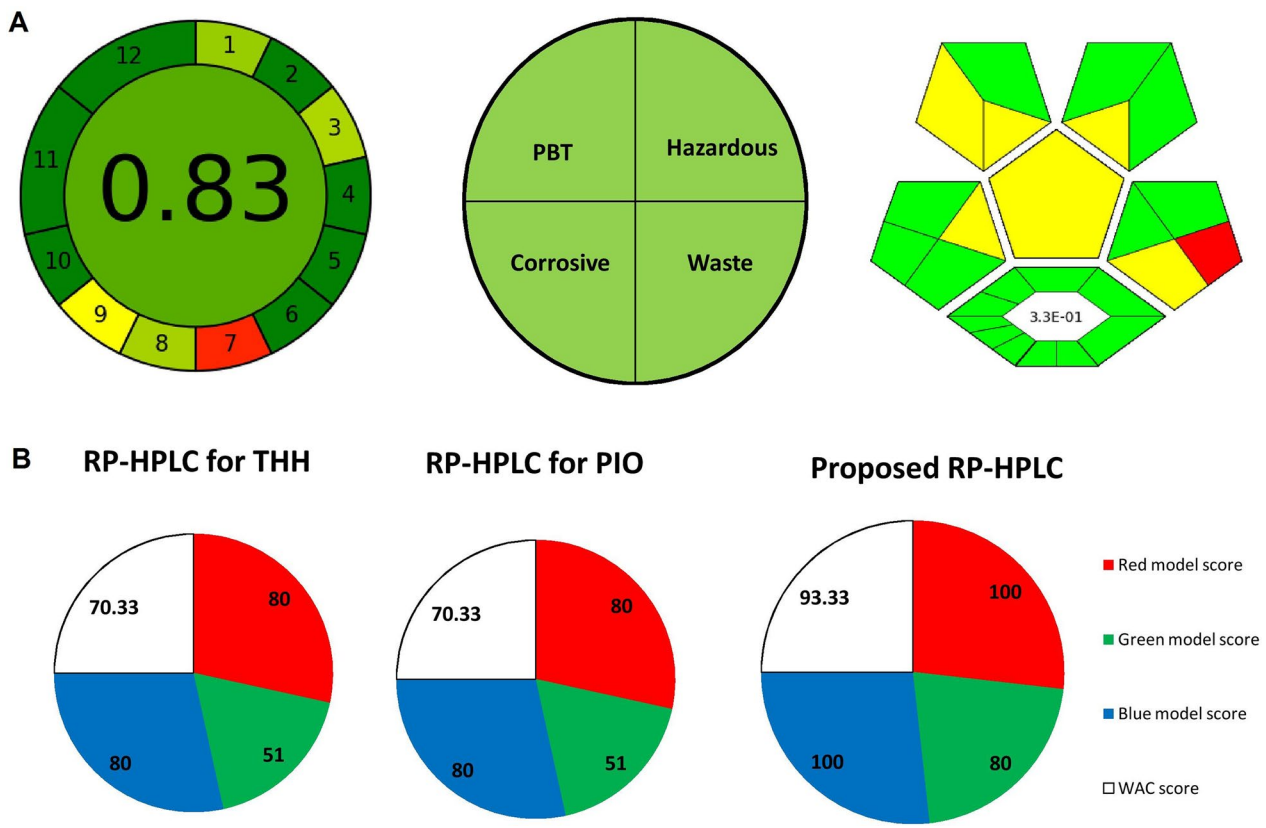


Fig. 6 **A** Green profile assessment of proposed method for estimation of PIO and THH using AGREE, NEMI and GAPI method **B** White analytical chemistry-based assessment and comparison of developed and published RP-HPLC methods for estimation of PIO and THH using RGB model

Green model-based assessment for procedure greenness

The Green model-based assessment was carried out to evaluate the environmental impact of the published and suggested RP-HPLC methods for the simultaneous determination of THH and PIO. The four core GAC principles (G1 to G4) were applied in this evaluation, which also made use of a variety of GAC tools, including the AGREE calculator, NEMI standards, Eco-scale assessment (ESA), and the complex GAPI programme. The procedure greenness profile of the published RP-HPLC procedures has been found to require the use of hazardous organic solvents (acetonitrile and methanol) and produce more than 300 mL of organic waste, suggesting serious environmental risks. As a result, these techniques scored 51 out of 100 in the evaluation based on the Green model. As opposed to acetonitrile and methanol, the suggested RP-HPLC estimation of THH and PIO in FDCs used ethanol, a less volatile, biodegradable, and environmentally benign solvent. Additionally, it produced only 100 mL of organic waste, thus lessening its impact on the environment. As a result, the suggested strategy received an evaluation of 80 out of 100 using the Green model. Figure 6 compares the complex GAPI software-based pictograms, the AGREE calculation, and the NEMI scale evaluation for the proposed and published RP-HPLC analyses of FDCs containing PIO and THH. These visual representations provide a thorough picture of how each strategy affects the environment.

Table 6 Assessment of published and proposed RP-HPLC methods for estimation of THH and PIO using principles of white analytical chemistry, RGB model, NEMI, ESA, AGREE and GAPI tools

Principles of white analytical chemistry	Published RP-HPLC analysis of THH	Published RP-HPLC analysis of PIO	Proposed RP-HPLC analysis of PIO and THH
Red model-based assessment			
R1 – Scope and application	Applied only for analysis of THH	Applied only for analysis of PIO	Applied for analysis of PIO and THH both
R2 – LOD and LOQ	Method was sensitive for estimation of THH	Method was sensitive for estimation of PIO	Method was sensitive for estimation of PIO and THH both
R3 – Precision	Method was precise for estimation of THH	Method was precise for estimation of PIO	Method was precise for estimation of PIO and THH both
R4 – Accuracy	Method was accurate for estimation of THH	Method was accurate for estimation of PIO	Method was accurate for estimation of THH and PIO both
Red-model score	80	80	100
Green model-based assessment			
AGREE score	0.51	0.51	0.83
NEMI assessment	Acetonitrile is hazardous, corrosive and toxic organic solvent	Acetonitrile is hazardous, corrosive and toxic organic solvent	Ethanol is safe organic solvent and less hazardous to the environment
Eco-scale assessment	Total penalty points for method were found to be 12	Total penalty points for method were found to be 12	Total penalty points for method were found to be 6.0
GAPI assessment	E-factor was found to be 0.50	E-factor was found to be 0.50	E-factor was found to be 0.33
Green model score	51	51	80
Blue model-based assessment			
B1 – Cost efficiency	Only THH sample was analyzed	Only PIO sample was analyzed	Both PIO and THH sample was analyzed
B2 – Time efficiency	THH sample was analyzed with run time of 10 min	PIO sample was analyzed with run time of 10 min	Both THH and PIO sample was analyzed with 5.0 min run time
B3 – Requirements	Require less than 3 steps for analysis	Require less than 3 steps for analysis	Require less than 3 steps for analysis
B4 – Operational simplicity	Skilled person requires for analysis	Skilled person requires for analysis	Skilled person requires for analysis
Blue model score	80	80	100
White analytical chemistry (WAC)-based assessment			
Average of Red, Green and Blue model scores	Average of (80 + 51 + 80) = 70.33	Average of (80 + 51 + 80) = 70.33	Average of (100 + 80 + 100) = 93.33
WAC status	Acceptable white	Acceptable white	Excellent white

THH Teneligliptin hydrobromide hydrate, *PIO* Pioglitazone hydrochloride, *AGREE* Analytical GREEnness, *GAPI* Green analytical procedure index, *NEMI* National environmental method index, WAC score: Poor white (0 to 25), Average white (26 to 50), Acceptable white (51 to 75), Excellent white (76 to 100)

Blue model-based assessment for cost and time efficiency

The Blue model-based assessment was used to compare the published and proposed approaches for the estimation of THH and PIO for cost, time, and user-friendliness. For each FDC of THH and PIO, distinct chromatographic applications as well as pricey organic solvents (acetonitrile and methanol) were required per the stated procedures. The proposed approach, in contrast, used a single chromatographic condition and a more cost-effective solvent to estimate both substances simultaneously. As a result, the suggested approach needed less money, time, and resources. Additionally, the reduced chromatographic conditions of the suggested approach were thought to make it more user-friendly. As a result, the Blue model-based assessment gave the proposed method a score of 100 (plus an extra 20 points) out of 100 for cost efficiency, time efficiency, and user-friendliness.

Principles of white analytical chemistry

The colors red, green, and blue are blended to generate the color white. Similarly to this, the published and suggested approaches' WAC (White Analytical Chemistry) scores were calculated by averaging the results from the Red, Green, and Blue models (see Table 6 for more information). In their FDC, the published RP-HPLC techniques simultaneously estimated THH and PIO, earning a WAC score of 70.33 out of 100. For the same analysis, the proposed RP-HPLC method, in contrast, received a remarkable WAC score of 93.33 out of 100. This suggests that the suggested procedure performed exceptionally well while analyzing the samples. Figure 6 compares the WAC scores and RGB models for the proposed and published RP-HPLC procedures.

Conclusions

To provide a reliable, eco-friendly, cost-effective, user-friendly, and quick RP-HPLC method for the simultaneous measurement of THH and PIO, White Analytical Chemistry (WAC) and Analytical Quality by Design (AQbD) approaches were coupled. For the specified analysis, the devised method used a green organic solvent and a single chromatographic condition, making it user- and environmentally friendly. The method is also shown to be quicker and more affordable for sample analysis when compared to the published RP-HPLC method in terms of analysis time, resource use, and cost. To guarantee the accuracy, precision, sensitivity, robustness, and specificity for the simultaneous estimate of THH and PIO, the AQbD technique was introduced into the method development process. Surprisingly, the approach that was devised produced excellent findings, demonstrating its remarkable performance for the analysis of THH and PIO. Given these characteristics, the developed method has a lot of potential as a routine analysis and quality control tool for FDCs including PIO and THH that is affordable, eco-friendly, user-friendly, and quick. Its scope of application encompasses small- and large-scale pharmaceutical companies.

Abbreviations

RP-HPLC	Reversed phase high-performance liquid chromatographic
AGREE	Analytical Greenness
ESA	Eco-scale assessment
GAPI	Green analytical procedure index
NEMI	National environmental method index
RGB	Red green blue
PDE	Permissible daily exposure
WAC	White analytical chemistry
IP	Indian Pharmacopoeia
AQRM	Analytical Quality Risk Management
DoE	Design of Experiments
AQbD	Analytical Quality by Design
CMPAs	Critical Method Performance Attributes
CPPs	Critical Procedure Parameters
DPP-4	Dipeptidyl Peptidase-4
THH	Teneligliptin hydrobromide hydrate
PIO	Pioglitazone hydrochloride
RPN	Risk priority number

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Author contributions

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Declarations

Ethics approval and consent to participate

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Consent for publication

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Competing interests

The authors of the manuscript already declared that they do not have any conflicts of interest for the publication of the manuscript.

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