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Red, green, and blue model assessment and AQbD approach to HPTLC method for concomitant analysis of metformin, pioglitazone, and teneligliptin

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Abstract

Background The CDSCO of India has authorized a combination of metformin hydrochloride, teneligliptin hydrochloride, and pioglitazone hydrochloride for the treatment of insulin-independent diabetes. For the purpose of estimating metformin, teneligliptin, and pioglitazone combinations as well as individual commercial formulations, there are a plethora of publicly accessible chromatographic techniques. More importantly, the development of these chromatographic procedures has included the use of chemical solvents that are dangerous to both animals and the environment.

Objectives However, to date, there has been no documented chromatographic technique that can concomitantly estimate various commercial formulations of drugs under study employing a uniform chromatographic condition and environmentally friendly solvents. In order to concomitantly estimate drugs under study utilizing unified chromatographic conditions, a green HPTLC method was developed.

Method The AQbD approach was used to carry out the method development. To determine the most important method parameters and response variables, the analytical risk assessment was conducted using the risk priority number ranking and screening approach. Critical method parameters and response variables were modeled using the response surface modeling approach, which relies on the central composite design. Optimal ranges for the intended method operable design region were determined, and control strategy was framed. The chromatographic separation was carried out on preparative TLC plate precoated with silica gel G-60 F_{254} using 1.0%W/V ammonium acetate in ethanol: water: triethylamine (6.5:0.4:0.6, V/V) as mobile phase. The detection of the anti-diabetic drugs under study was carried out at 267 nm wavelength.

Results The linearity of metformin, teneligliptin, and pioglitazone was found to be 5000–25000 ng/band, 200–1000 ng/band, and 150–750 ng/band, respectively. The %RSD for robustness and precision study was found to be less than 2.0%. The %recovery of method was found to be 98–102%. The assay results were shown to be in compliance with respective labeled claims of anti-diabetic medications when the suggested method was used for concurrent analysis of several formulations and combinations of drugs under study.

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Conclusion The suggested technique was evaluated utilizing red–green–blue model scoring tools. The suggested technique was determined to be precise, accurate, rapid, cost-effective, and easy to apply for the estimation of drugs under study.

Keywords Metformin hydrochloride, Teneligliptin hydrobromide hydrate, Pioglitazone hydrochloride, Analytical quality by design, Response surface modeling

Background

Insulin-independent diabetes has become increasingly widespread in modern society due to people's stress levels and poor dietary habits. For insulin-independent diabetes, there are a plethora of METH (metformin hydrochloride), TENH (teneligliptin hydrobromide hydrate), and PIOH (pioglitazone hydrochloride) formulations and combinations on the market. Recently, CDSCO (Central Drug for Standard Control Organization) of India authorized the use of combination of METH, TENH, and PIOH to treat insulin-independent diabetes. First-line medication for the treatment of type II diabetes is METH, a biguanide antihyperglycemic drug. Because it reduces type II diabetes blood glucose concentrations without producing hypoglycemia, METH is regarded as an antihyperglycemic medication [1]. The novel diabetic drug TENH efficiently controls blood sugar levels with little hypoglycemia risk [2]. The CDSCO has given the green signal to the oral anti-diabetic PIOH, which is a member of the thiazolidinedione medication family [3]. Figure S1 in the supplemental file shows the chemical structures of METH, TENH, and PIOH.

The literature review revealed that RP-HPLC techniques have seen increased usage in the pharmaceutical industry for routine and quality control analysis of marketed formulations including METH, TENH, and PIOH. There is a plethora of RP-HPLC techniques described in the literature for the analysis of METH, TENH, and PIOH in both their individual and combination commercial forms. Unfortunately, the analysis of these anti-diabetic drugs had to be done using hazardous organic solvents as acetonitrile, methanol, tetrahydrofuran, etc., by means of these RP-HPLC procedures. Not only that, but also the RP-HPLC procedures produced a great deal of organic waste, which posed an even greater threat to the lives of aquatic animals and the environment [4–15]. The HPTLC approach produces less organic waste and uses fewer organic solvents than the RP-HPLC method for analyzing pharmaceuticals. Thus, there are a plethora of HPTLC techniques that have been documented in the literature for the purpose of analyzing commercially available METH, TENH, and PIOH formulations. Toluene, 1, 4-dioxane, chloroform, methanol, etc., were necessary for these procedures, but they are very harmful to aquatic life and the environment [16–24].

The newly acknowledged WAC (white analytical chemistry) approach recommends that when conducting chromatographic analysis on medication samples, the use of harmful organic solvents shall be minimized, substituted, or recycled. On top of that, the WAC approach encourages scientists to develop chromatographic procedures that are easy to use, quick, economic, accurate, precise, sensitive, and straightforward. In order to provide risk-based analytical methodologies that provide robust, accurate, and precise drug analysis findings, the ICH (International Council for Harmonization) Q14 guideline was recently made public. Using quality risk management and DOE principles, the risk-based method development will be carried out via the application of the QbD (quality by design) approach [25]. Implementing the WAC approach to develop chromatographic methods that are robust, precise, and accurate is made easier with this guiding approach. There have been a plethora of chromatographic techniques reported in the literature that advocate for a WAC and QbD hybrid approach [26–30].

To ensure the safety of aquatic animals and the environment, it is recommended to replace organic solvents like acetonitrile, toluene, 1, 4-dioxane, chloroform, methanol, etc. with low-toxic organic solvents, as per the ICH Q3C (R8) guidelines and solvents selection guides published by different pharmaceutical industries. As per the ICH Q3C (R8) criteria, class 3 organic solvents are considered to be less damaging to the environment and to be low-toxic [31, 32]. In order to simultaneously analyze several commercially available formulations of METH, TENH, and PIOH utilizing class 3 solvents, a WAC-driven green HPTLC method was developed. Following the ICH Q14 standards, the HPTLC method was developed using the QbD approach. The RPN ranking and screening approach was used to identify important MPs (Method Parameters) and RVs (Response Variables) in the analytical quality risk assessment. The visual link between the selected key MPs and RVs was examined using response surface modeling by CCD (Central Composite Design). The statistical analysis was conducted using the trial version of the DE (Design Expert) 10 software. By overlaying the response surface plots, the MODR (Method Operable Design Region) was traversed to achieve optimal chromatographic conditions. The validation approach followed the most current recommendations of the ICH Q2

(R2) standards. The validation efficiency, cost efficiency, simplicity, and user-friendliness of the suggested method were evaluated using the following model scoring tools: AGREE (Analytical Greenness), GAPI (Green Analytical Procedure Index), BAGI (Blue Applicability Grade Index), and RGB (Red, Green, and Blue).

Methods

List of used instruments and software

Anchrom Enterprises Private Limited of Mumbai, India, supplied the HPTLC instrument equipped with scanner, and auto-spotter used for the chromatographic analysis of METH, TENH, and PIOH. We used a digital electronic balance to weigh the standard and samples of METH, TENH, and PIOH. The METH, TENH, and PIOH samples were dissolved and extracted using a sonicator water-bath. The HPTLC method was optimized, and response surface modeling was carried out using the DE-10 software (Trial version). An analytical GREEnness calculator and GAPI software were used to conduct the greenness evaluation of the HPTLC technique. The present HPTLC method was evaluated utilizing the online BAGI software to determine the applicability grade index score.

List of used materials and reagents

Reputed Pharmaceutical Companies of Gujarat, India, provided complimentary samples of METH, TENH, and PIOH with 99.9% purity of each, which were the Active Pharmaceutical Ingredients. The stationary phase employed for the chromatographic separation of METH, TENH, and PIOH was Silica Gel G-60 F_{254} precoated on aluminum plate. Chromatographic analysis used organic solvents of AR grade quality with %purity of 99.9 each, including ethanol and triethylamine, in the preparation of METH, TENH, and PIOH samples. We bought the METH, TENH, and PIOH-containing commercial formulations from local medical stores of Surat, Gujarat, India.

Preparation of combined standard solution of METH, TENH, and PIOH

A portion of 1.0 mL was taken from each of the standard stock solutions of METH (5 mg/mL), TENH (200 µg/mL), and PIOH (150 mg/mL), transferred to a 10-mL volumetric flask, and then diluted with ethanol until it reached the mark. The final concentrations of METH, TENH, and PIOH in the final combined working standard solution were 500 µg/mL, 20 µg/mL, and 15 µg/mL equally. In a similar vein, combined working standard solutions of METH, TENH, and PIOH with concentrations of 500–2500 µg/mL, 20–100 µg/mL, and 15–75 µg/mL, respectively, were prepared using the appropriate ethanolic dilutions.

Implementation of QbD approach

In order to identify the MPs and RVs that may be crucial, exploratory experiments were conducted. Each MP and RV was assigned a severity, occurrence, and detectability score to determine how crucial they were in developing the targeted method for chromatographic analysis of METH, TENH, and PIOH. In order to analyze the risks associated with MVs and RVs, the RPN ranking and screening approach was used. Using CCD and DE-10 software, the identified MVs and RVs were forwarded for response surface modeling. Critical RVs were measured during the experimental trials recommended by the software. Using the DE-10 program, we ran ANOVA (analysis of variance), Pareto charts, multiple regression, and response surface contour plots to draw conclusions about the data. To optimize the critical MPs and get appropriate RVs, the MODR was traversed by superimposing response surface contour plots. A control strategy was developed for the purpose of optimizing the HPTLC method's life cycle management and conducting chromatographic analyses using METH, TENH, and PIOH.

Optimized chromatographic procedure and MODR

Using a mobile phase consisting of 1%W/V ammonium acetate in ethanol/water/triethylamine (6.5:0.4:0.6, v/v), METH, TENH, and PIOH were analyzed chromatographically on pre-activated preparative silica gel G-60 F_{254} plates. Chromatogram development was carried out in a twin trough glass chamber with saturation duration of fifteen minutes. The auto-spotter equipment was used to spot the 8.0 mm bands of METH, TENH, and PIOH on the TLC plate. The TLC plate's band was positioned 15 mm from the plate's edges and base. The bands of METH, TENH, and PIOH were scanned using a scanner instrument at a migration distance of 75 mm. Using absorbance–reflectance mode at a wavelength of 267 nm, the bands of METH, TENH, and PIOH were detected.

Calibration curve of METH, TENH, and PIOH

The optimized chromatographic conditions were used to spot 10 µL of each combined working standard solution of METH, TENH, and PIOH on the same pre-activated TLC plate. The concentrations of METH, TENH, and PIOH were 500–2500 µg/mL, 20–100 µg/mL, and 15–75 µg/mL, respectively. Using the optimized chromatographic procedure as in the preceding section, the developed TLC plate was scanned at a wavelength of 267 nm after drying. The peak area and Rf value for each anti-diabetic drug under study were then calculated. An anti-diabetic drug concentration versus peak area of

METH, TENH, and PIOH calibration curve was constructed. For the METH, TENH, and PIOH calibration curves, the regression-line equation and correlation coefficient were calculated.

Method validation for estimation of METH, TENH, and PIOH

Following the recommendations made in the most current revisions to the ICH Q2 (R2) standards, the established HPTLC method was validated for METH, TENH, and PIOH estimations using criteria such as specificity, linearity, accuracy, precision, LOD, LOQ, and robustness. The method's specificity was investigated by comparing the absorbance–reflectance spectra and Rf values of standard METH, TENH, and PIOH with those of sample METH, TENH, and PIOH. We examined the calibration curve of average peak area versus respective concentration of anti-diabetic drug for correlation coefficient and regression-line equation, and we repeated the procedure for the calibration curve five times to ensure linearity. Repeatability of sample application and measurement, intra-day variance, and inter-day variation in data of the calibration curve were the aspects examined in the precision analysis of the method. For METH, TENH, and PIOH, the percentage of relative standard deviation (RSD) was calculated for every accuracy study. A standard addition technique was used to examine the method's accuracy. A pre-analysis sample of anti-diabetic medications had three concentrations of the standard METH, TENH, and PIOH added to it: 80%, 100%, and 120%. In order to examine the accuracy of the sample preparation, the percentage recoveries of added METH, TENH, and PIOH were calculated. We have estimated the LOD and LOQ for METH, TENH, and PIOH using the equation described in ICH Q2 (R2) guideline. Small purposeful adjustments in mobile phase composition, saturation duration, and detection wavelength were applied to the developed method for estimation of METH, TENH, and PIOH in order to estimate the %RSD for the robustness study.

Concomitant assay of multiple formulations of METH, TENH, and PIOH

Multiple pharmaceutical dosage forms and combinations of METH, TENH, and PIOH were concomitantly assayed utilizing the suggested HPTLC method under a single chromatographic condition. To conduct the sample test, 100 mL of ethanol was mixed with tablet powder that was equal to one unit dosage of anti-diabetic medications. A Whatman filter was used to filter the sample solution after it had been sonicated for 15 min. A portion of 10 µL was spread on the same pre-activated TLC plate with standard METH, TENH, and PIOH after the filtrate from each sample was suitably diluted with ethanol. The optimal chromatographic analytical conditions for METH, TENH, and PIOH were followed for developing, drying, and scanning the TLC plate at a 267 nm wavelength. Using the regression-line equation of the corresponding anti-diabetic drug, the amounts of METH, TENH, and PIOH in the tablet dose form were calculated.

Results

Analytical risk assessment for method development

Preliminary experiments and prior knowledge of HPTLC method were used to identify the possibly essential MPs and RVs. Using Minitab-18 software, a fishbone diagram was created depicting the identified MPs and RVs. The categories used were Method, Material, Man power, Mother nature, Machine, and Measurement (See Figure S1 in supplementary file). Applying the RPN rating and screening approach, we examined the outcomes of the preliminary experiments conducted for each MP. Each MP and RV's RPN score was determined by multiplying the occurrence (O), severity (S), and detectability (D) scores. Each MP and RV received an O score based on the contribution it made to the development of a specified method. For little effect on method development, an O score of 2.0 was given, and vice versa, an O score of 10.0 was offered for high effect. Also, each MP and RV was given a S score based on the influence they had throughout the development of the present method. In terms of the D score of the MP and RV, a low score of 02 indicates easy impact detection during method development, while a high score of 10 indicates poor detection. To determine which MPs and RVs may be crucial for the method's development, we computed their RPN scores (Refer to Table S1 in the supplemental file for details). In terms of ammonium acetate percentage weight by volume (MP-X1), ethanol volume (MP-X2), and the volume ratio of triethylamine to ethanol (MP-X3), an RPN score of 200 was achieved. In a similar vein, RV-Y1 (resolution between METH and TENH peaks), RV-Y2 (tailing factor for MFH peak), and RV-Y3 (Rf value for PIOH peak) all obtained RPN scores of 200. Furthermore, the mother nature MPs, including temperature and relative humidity, also achieved an RPN score of 200; nevertheless, these MPs proved to be somewhat challenging to manage during the method development process. Therefore, in an air-conditioned laboratory, the chromatographic separation of METH, TENH, and PIOH was performed at a temperature of 25 ± 2 °C and a relative humidity of 35 ± 5%. The RPN score for the remaining MVs and RVs was 8.0. So, it was thought that MP-X1, MP-X2, and MP-X3 may be crucial for the development of the HPTLC approach. To optimize the HPTLC method, the RV-Y1, RV-Y2, and RV-Y3 were determined to be the

most important RVs. Figure 1 shows the ranked RPN scores of MPs and RVs graphically.

QTAP and critical RVs

In this study, the QTAP was to develop a green HPTLC method that could concomitantly analyze various marketed formulations of METH, TENH, and PIOH using class 3 organic solvents. After conducting an analytical risk assessment, three critical RVs were identified: RV-Y1 (resolution between peaks of METH and TENH), RV-Y2 (tailing factor of peak of METH), and RV-Y3 (Rf value of the peak of PIOH). Therefore, these RVs were deemed critical responses for optimizing the method in order to implement the QbD approach.

Response surface modeling by CCD

After analytical risk assessment using RPN ranking and screening approach, the selected key MPs and RVs were submitted for response surface modeling and optimization. The CCD was employed for linking of MP-X1, MP-X2 and MP-X3 with RV–Y1, Y2 and Y3 utilizing DE-10 software. The software was used to create design metrics based on the critical MPs, which have three levels: low: -1, medium: 0, and high: +1. For more information, refer to Table S2 in the supplementary file. The DE-10 software was used to statistically analyze the recorded RVs (Y1, Y2, and Y3) after the suggested experimental trials were carried out. The p-values for the direct effect and quadratic effects for MP-X1, MP-X2, and MP-X3 were determined to be less than 0.05, according to the ANOVA data shown in Table 1. Therefore, these MPs were determined to play a crucial role in the advancement of the HPTLC method. The response surface plot analysis (refer to Fig. 2) revealed a nonlinear graphical relationship between critical RVs (Y1, Y2 and Y3) and MPs (MP—X1, X2 and X3). For RVs (Y1, Y2, and Y3), the software's multiple regression analysis revealed adjusted R^2 and predicted R^2 values greater than 0.95, suggesting that the mathematical models predicted were the most suitable for RV prediction. The RVs (Y1, Y2, and Y3) were predicted using the following mathematical models (refer to Eqs. 1, 2, and 3):

$$RV - Y1 = 1.83 - 0.87 * (MP - X1) - 0.23 * (MP - X2) + 0.0034 * (MP - X3) \quad (1)$$

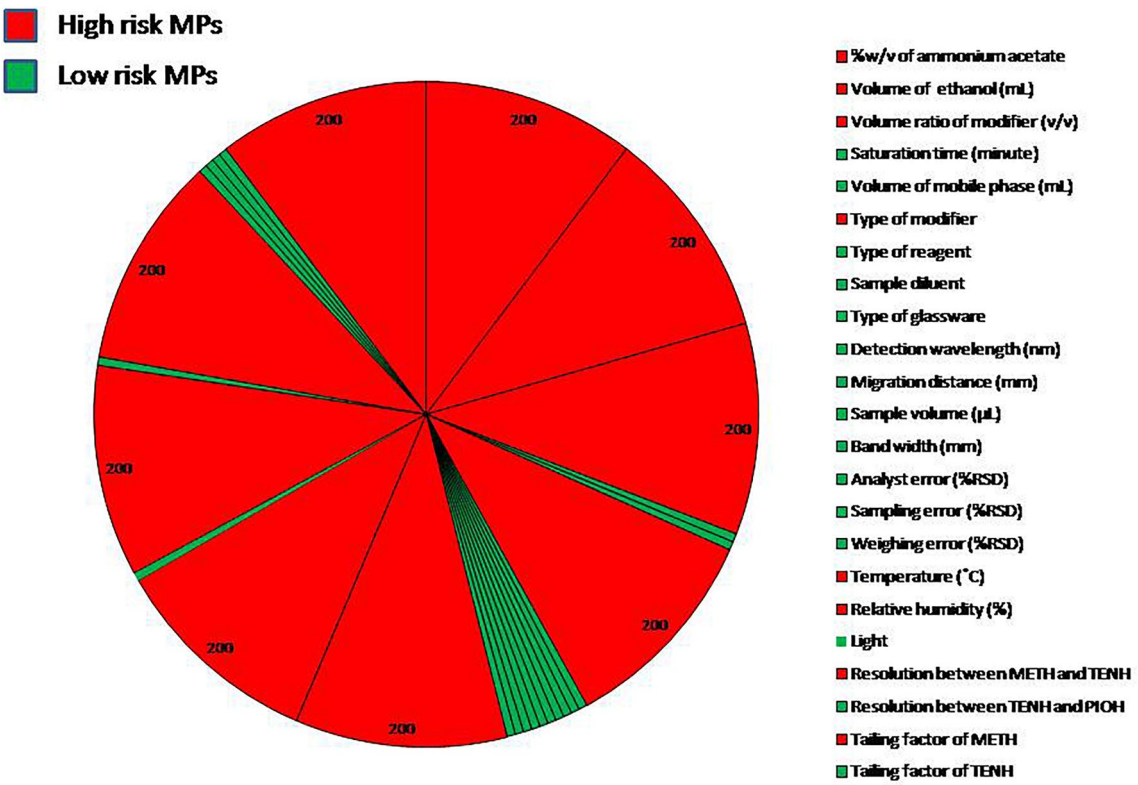

Fig. 1 Pie chart showing analytical risk assessment using RPN ranking and screening method for development of targeted HPTLC method for concomitant assay of multiple formulations of METH, TENH, and PIOH

Table 1 ANOVA for response variables RV-Y1, RV-Y2 and RV-Y3 for study significant main effects, interactions and quadratic effects of critical method parameters MP-X1, MP-X2 and MP-X3) using CCD and DE-10 software

Effects of MPs	Sum of squares	df	Mean square	F value	p-value Prob > F	Level of significance
Analysis of variance table [Partial sum of squares—Type III]: RV—Y1						
Model	11.14	3	3.71	109.76	< 0.0001	Significant
MP—X1	10.40	1	10.40	307.51	< 0.0001	Significant
MP—X2	0.74	1	0.74	21.78	0.0003	Significant
MP—X3	1.605E-004	1	1.605E-004	4.745E-003	0.9460	Not significant
Analysis of variance table [Partial sum of squares—Type III]: RV—Y2						
Model	4.79	9	0.53	45.33	< 0.0001	Significant
A-MP—X1	4.43	1	4.43	376.68	< 0.0001	Significant
B-MP—X2	3.752E-003	1	3.752E-003	0.32	0.5858	Not significant
C-MP—X3	8.307E-003	1	8.307E-003	0.71	0.4222	Not significant
AB	6.050E-003	1	6.050E-003	0.51	0.4912	Not significant
AC	7.200E-003	1	7.200E-003	0.61	0.4538	Not significant
BC	0.011	1	0.011	0.96	0.3534	Not significant
A^2	0.33	1	0.33	27.76	0.0005	Significant
B^2	0.013	1	0.013	1.10	0.3206	Not significant
C^2	0.021	1	0.021	1.83	0.2092	Not significant
Analysis of variance table [Partial sum of squares—Type III]: RV—Y3						
Model	0.34	3	0.11	229.32	< 0.0001	Significant
A-MP—X1	2.106E-004	1	2.106E-004	0.43	0.5242	Not significant
B-MP—X2	0.000	1	0.000	0.000	1.0000	Not significant
C-MP—X3	0.34	1	0.34	687.55	< 0.0001	Significant

$$\begin{aligned} RV - Y2 = {} & +1.42 + 0.57 * (MP - X1) \\ & - 0.017 * (MP - X2) \\ & - 0.025 * (MP - X3) \\ & - 0.02 * (MP - X1 * MP - X2) \\ & - 0.030 * (MPX1 * MP - X3) \\ & + 0.038 * (MPX2 * MPX3) \\ & + 0.15 * (MP - X1)^2 \\ & + 0.031 * (MP - X2)^2 \\ & + 0.040 * (MP - X3)^2 \end{aligned} \quad (2)$$

to be optimal for MP-X1 was 1.0–2.0%W/V. A volume of 6.0–7.0 mL of ethanol in the mobile phase was determined to be the optimal range for MP-X2. The range of volume ratios for water to triethylamine in the mobile phase composition that was ultimately determined to be optimal for MP-X3 was 0.1:0.9 V/V to 0.5:0.5 V/V. Experiments on recommended optimum combinations of MPs (MP-X1, X2, and X3) were conducted to compare the predicted RVs with actual RVs. This was done for the purpose of model validation. There was less than a 5% discrepancy between the anticipated and observed RVs, suggesting that the proposed mathematical models were

$$RV - Y3 = +0.72 - 0.0039 * (MP - X1) + 0.0001 * (MP - X2) + 0.16 * (MP - X3) \quad (3)$$

MODR and control strategy

While navigating MODR, the DE-10 software superimposed the response surface plots to get the desired RVs (Y1—greater than 1.5, Y2—less than 1.5, and Y3—less than 0.80). Figure 3 shows the three MODRs in yellow for various combinations of MPs (X1, X2, and X3) that may be used to generate the appropriate RVs. The range of ammonium acetate in ethanol that was determined optimal for RV prediction and targeted concurrent chromatographic analysis of METH, TENH, and PIOH. The desirability plot shown in Figure S3 indicated that the desirability value was found to be close to one for prediction of the RV-Y1, Y2 and Y3 using software suggested mathematical models. The chromatograms of METH, TENH, and PIOH exhibited peaks at Rf values of 0.35, 0.51, and 0.72 correspondingly, as shown in Fig. 4A, corresponding to the experimental trials.

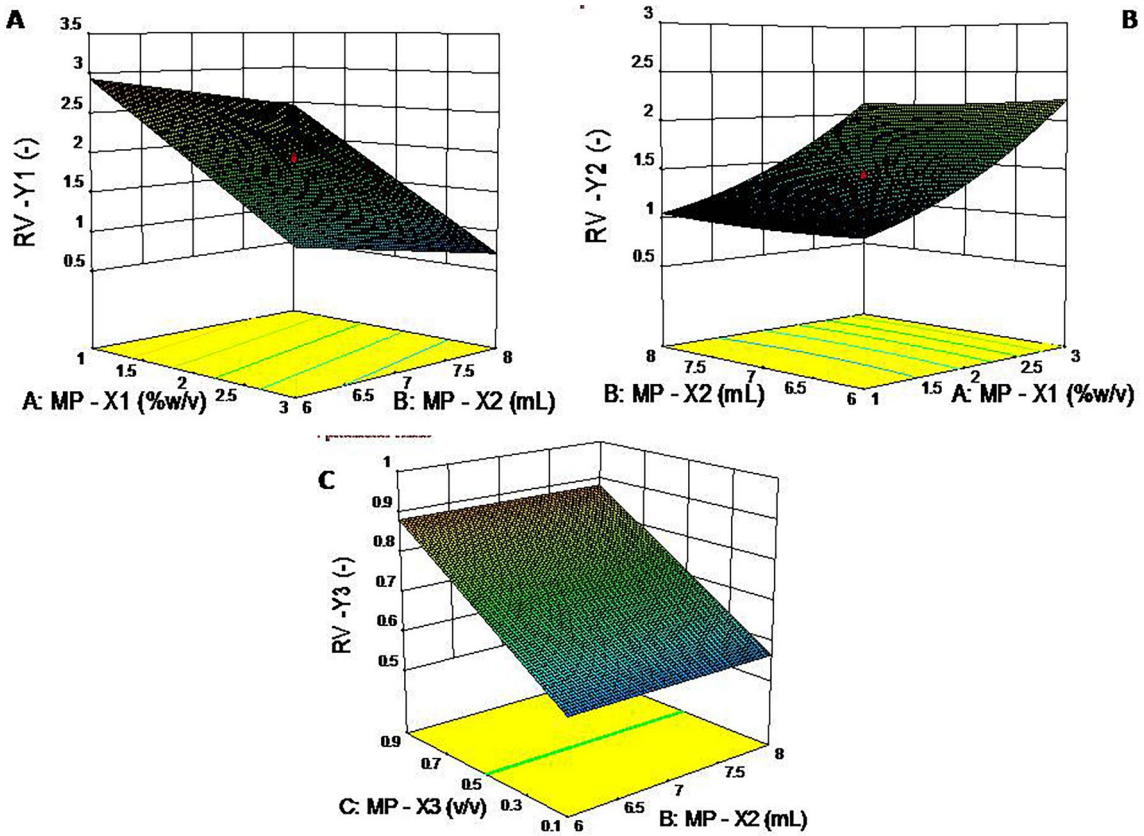

Fig. 2 3D response surface contour plots showing multidimensional interactions of critical MPs (MP—X1, X2 and X3) with **A** RV—Y1 **B** RV—Y2 and **C** RV—Y3

Results of method validation as per ICH Q2 (R2)

Stability of working standard solution

The combined working standard solution of the METH, TENH, and PIOH was stored for 24 h at 25° ± 2 °C temperature. The peak area of the each drug after 24 h storage was compared with the respective peak area of the drug at initial time point. The %variation (n = 3) in peak area was calculated for evaluating stability of the combined working standard solution of METH, TENH, and PIOH. The %variation in peak area was found to be less than 2.0 which indicated the combined working standard solution of the METH, TENH, and PIOH was found to be stable for 24 h at 25 ± 2 °C.

Specificity study

The peak Rf values for METH, TENH, and PIOH in the sample were found to be identical to those in the standard. Comparison of the peak purity spectra of these compounds in the sample and the standards revealed a strong correlation at the beginning, middle, and end of the peaks, with a correlation coefficient greater than 0.999. This finding validated the peak purity of these compounds in the standard and the sample.

Linearity study

Over the concentration range of 5000–25000 ng/band, 200–1000 ng/band, and 150–750 ng/band, respectively, the mean peak areas of TENH, PIOH, and METH shown a significant degree of linear relationship. Every linearity curve has a correlation coefficient greater than 0.995. As shown in Fig. 4-B, the linearity of METH, TENH, and PIOH is shown by the 3D chromatogram.

Precision study

For the estimate of METH, TENH, and PIOH, the %RSD for repeatability of sample measurement and application was determined to be less than 2.0%. Upon examination, it was discovered that the %RSD for intra-day and inter-day precision in METH, TENH, and PIOH was below 2.0. Precision study results demonstrated that the suggested method precisely estimated METH, TENH, and PIOH.

Accuracy study

For these anti-diabetic medications, the %recoveries for increased amounts of METH, TENH, and PIOH in pre-analyzed samples ranged from 98 to 102%, proving that

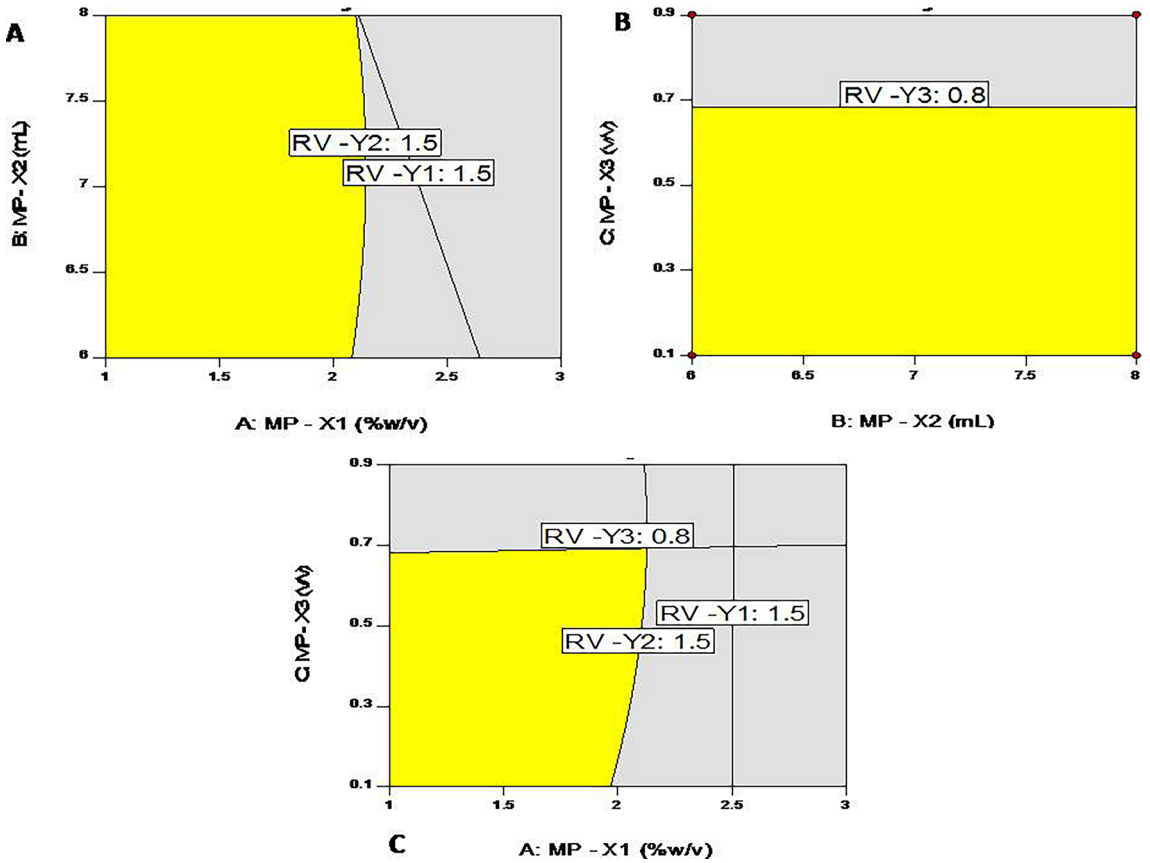

Fig. 3 Derived MODRs (in yellow shades) from overlaid response contour plots for optimization of critical MPs (X1, X2, and X3) to get desired **A** RV—Y1, **B** RV—Y2, and **C** RV—Y3

the proposed method was accurate for synchronous estimation of these three anti-diabetic drugs.

LOD and LOQ

The LOD (3.3* standard deviation of y-intercept/mean slope) and LOQ (10* standard deviation of y-intercept/mean slope) was calculated using equations mentioned in ICH Q2 (R2) guideline. The LOD value of 177.28 ng/band for METH, 8.13 ng/band for TENH, and 5.79 ng/band for PIOH was determined. The LOQ value of 537.22 ng/band for METH, 24.65 ng/band for TENH, and 17.54 ng/band for PIOH was also determined using equations.

Robustness study

Estimation of METH, TENH, and PIOH all had %RSD values below 2.0%, indicating that the mobile phase composition was deliberately kept very consistent. It was also discovered that the %RSD for change in saturation time and detection wavelength for METH, TENH, and PIOH estimate was less than 2.0%. Table 2 displays the validation parameters summary.

Results of concomitant assay of marketed tablet formulations

When assayed in a single tablet dose form, METH, TENH, and PIOH all achieved concentrations between 98 and 102% of their anti-diabetic medicine label claims. In the combination tablet dosage forms, the percentage assay of METH, TENH, and PIOH ranged from 98 to 102% of the respective stated claim of the anti-diabetic medicine. When taken as a pill combination, METH, TENH, and PIOH were determined to have an assay value between 98 and 102% of the stated claim. Peaks at Rf 0.35, 0.51, and 0.72 were seen in the chromatograms of METH, TENH, and PIOH, respectively, derived from the sample. Aside from the METH, TENH, and PIOH peaks, the sample's chromatogram did not show any other peaks. These findings demonstrated that the METH, TENH, and PIOH tablet additives and excipients did not affect the estimate of the aforementioned anti-diabetic medications. Table 3 displays the findings of the experiment in summary. The statistical comparison of the assay results of published [37] and proposed method was carried out using student's paired t test. The t-critical value

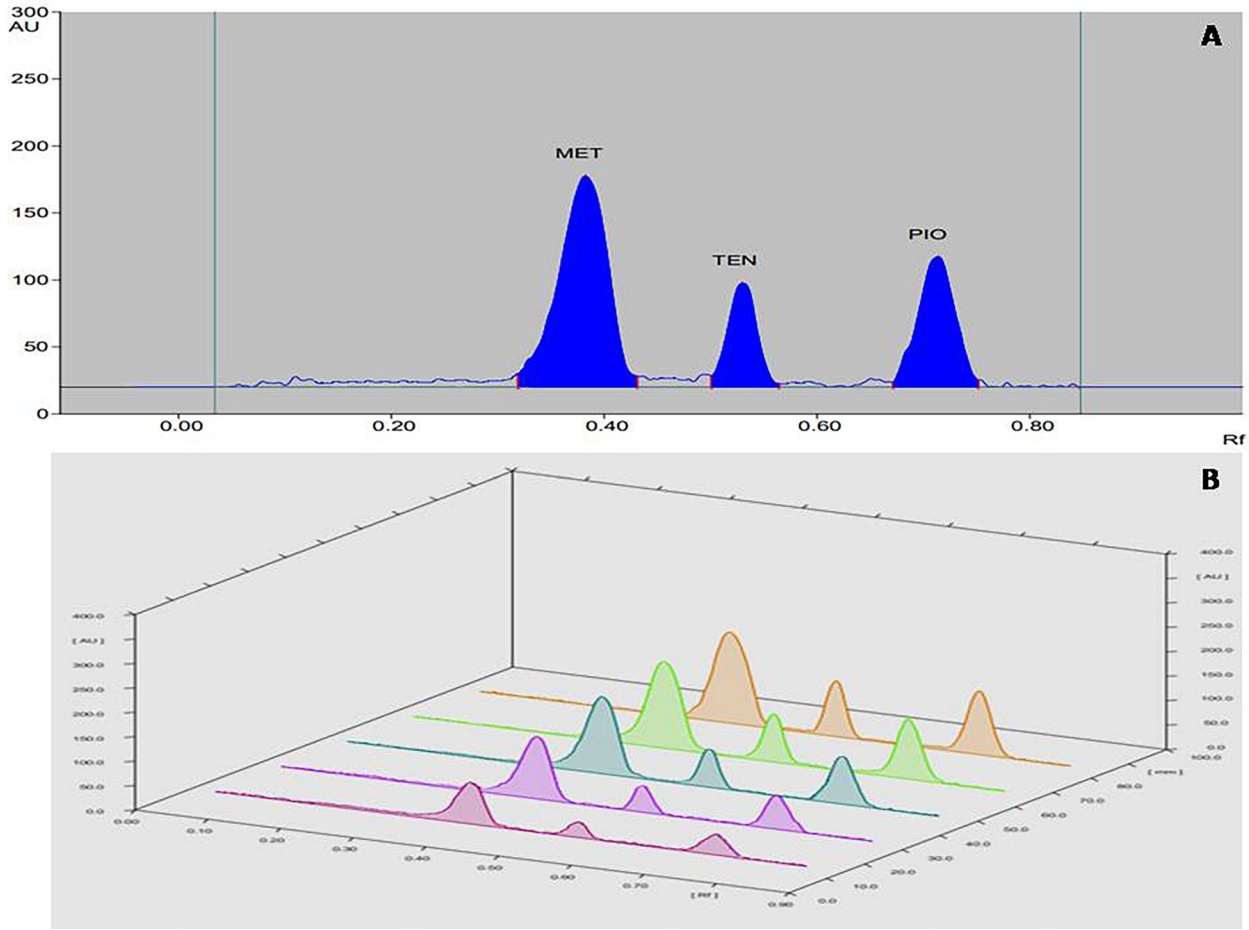

Fig. 4 **A** Chromatogram showing peaks of standard METH, TENH, and PIOH at Rf values of 0.35, 0.51, and 0.72, respectively **B** chromatogram showing peaks of METH, TENH, and PIOH at Rf values of 0.35, 0.52, and 0.71, respectively

of the proposed (1.982) and published method (1.961) was found to be less than t-table value (2.912) for confidence interval of 95%. There was no statistical difference observed between assay results of published and proposed method for the simultaneous estimation of the anti-diabetic drugs under study.

Discussion

The literature reports various chromatographic methods for estimating pharmaceutical dosage forms and combinations of METH, TENH, and PIOH, including RP-HPLC and HPTLC methods. As part of the mobile phase and as a diluent, the described chromatographic technique made use of organic solvents that were known to be neurotoxic and teratogenic, including 1, 4-dioxane, chloroform, toluene, acetonitrile, and methanol. Furthermore, each pharmaceutical dosage form and combination of METH, TENH, and PIOH needed its own unique chromatographic conditions for analysis, according to the reported results. Therefore, a great deal of organic waste has been generated, which is detrimental to both human and animal lives, as well as the environment, as a result of the concurrent estimation of various tablet dosage forms and combinations using a published chromatographic methods. To ensure the safety of both humans and the environment, recent concepts in GAC and WAC propose reducing, recycling, or replacing these harmful organic solvents. There was currently no published chromatographic method that could concomitantly estimate METH, TENH, and PIOH under a single chromatographic condition, which would greatly benefit analysis in terms of time, cost, and resources. In order to concomitantly estimate METH, TENH, and PIOH in all of their commercially available dosage forms and combinations, a WAC-driven HPTLC method was developed. This method makes use of environmentally friendly and low-toxic organic solvents. Following the recommendations made in the most recent ICH Q14 guideline, the suggested HPTLC method was developed using an analytical

Table 2 Method validation for targeted HPTLC method as per ICH Q2 (R2) guidelines

Sr. no.	Parameters	Drugs		
		METH	PIOH	TENH
1	Linearity range (ng/band)	5000–25000	150–750	200–1000
2	Regression equation	$y = 0.3309x + 1193.9$	$y = 6.0276x + 49.158$	$y = 2.8633x + 189.53$
3	Correlation coefficient (R^2)	0.9986	0.9991	0.9980
4	Precision (%RSD)			
	Repeatability of sample application	0.64	0.75	0.67
	Repeatability of sample measurement	0.62	0.73	0.64
	Intra-day precision	0.36–0.69	0.30–0.69	0.42–0.80
	Inter-day precision	0.52–0.86	0.68–1.26	0.51–1.63
5	% Recovery	98.30–99.56	98.91–100.63	98.93–100.63
6	LOD (ng/band) using equation DL = 3.3 * (SD/slope)	177.28	5.79	8.13
7	LOQ (ng/band) using equation DL = 10 * (SD/slope)	537.22	17.54	24.65
8	Robustness(%RSD)			
	Change in mobile phase volume	0.58–0.80	0.57–1.50	0.64–1.58
	Change in saturation time	0.29–0.89	0.31–0.66	0.47–0.99
	Change in wavelength	0.42–0.53	0.49–0.82	0.42–1.0

Table 3 Results for concomitant assay of multiple tablet combinations of METH, TENH, and PIOH

Types of tablet formulations	Labeled claim of METH, TENH and PIOH in mg	Amount found ± standard deviation (n = 3)	%Assay of the anti-diabetic drug found
METH tablet	500 mg (METH)	494.55 ± 0.77	98.91
TENH tablet	20 mg (TENH)	19.78 ± 0.25	98.90
PIOH tablet	15 mg (PIOH)	14.71 ± 0.23	98.06
Combined tablet of METH and PIOH	500 mg (METH)	490.15 ± 0.67	98.03
	15 mg (PIOH)	14.96 ± 0.31	99.73
Combined tablet of METH and TENH	500 mg (METH)	495.32 ± 0.59	99.06
	20 mg (TENH)	19.92 ± 0.28	99.60
Combined tablet of PIOH and TENH	20 mg (TENH)	19.81 ± 0.29	99.05
	15 mg (PIOH)	14.91 ± 0.22	99.40
Combined tablet of METH, TENH and PIOH	500 mg (METH)	492.31 ± 0.65	98.46
	20 mg (TENH)	19.75 ± 0.25	98.75
	15 mg (PIOH)	14.83 ± 0.24	98.86

QbD approach based on the concepts of analytical risk assessment and DOE.

R-model scoring for validation efficiency

The whiteness and greenness profile of the proposed and published method for the estimation of anti-diabetic drugs under study was assessed by following guidelines described in recently published literature [33–36]. The redness profile evaluation of the suggested and published [37] method was done utilizing the red model score system. The analytical method evaluates the red model scoring system based on its accuracy, precision, LOD, LOQ, scope, and applicability. Estimations of METH, TENH, and PIOH were determined to be accurate and precise using the suggested approach. Nanogram levels were determined for the limits of detection and quantitation (LOD and LOQ) for METH, TENH, and PIOH. For the published chromatographic approach to be used for estimating each combination of anti-diabetic medications, a separate validation step was necessary. The RP-HPLC method published in the literature for concomitant analysis of the anti-diabetic

drugs under study required lots of toxic organic solvents and also generated lots of toxic organic waste at end of analysis [37]. In contrast, the suggested method only needed single chromatographic condition to estimate METH, TENH, and PIOH combinations using low-toxic solvents and also generated less organic waste at the end of sample analysis. Multiple tablets and combinations of METH, TENH, and PIOH were analyzed simultaneously utilizing the suggested and published [37] method approach under unified chromatographic conditions. The published methods approach was stability indicating for concomitant analysis of the anti-diabetic drugs under study. Hence, a red model score of 80 and 80 out of 100 was therefore given to the suggested and published approach for estimating METH, TENH, and PIOH.

G-model scoring for greenness profile

Using the AGREE and GAPI programs, we evaluated the suggested method's greenness profile for METH, TENH, and PIOH estimation by referring published literature [38–40]. Twelve principles of green analytical chemistry were used to evaluate the analytical method using the AGREE calculator. In order to simultaneously estimate METH, TENH, and PIOH, the RP-HPLC was reported and necessitated the use of hazardous class 2 organic solvents such as methanol and acetonitrile [37]. The reported HPTLC methods were required toxic class 2 organic solvents such as chloroform, toluene, and 1,4-dioxane for concomitant estimation of anti-diabetic drugs under study. On top of that, in order to simultaneously estimate the METH, TENH, and PIOH, the chromatographic methods that were published would have produced a great deal of harmful organic waste. These harmful organic solvents should have been substituted with green solvents to ensure the safety of humans,

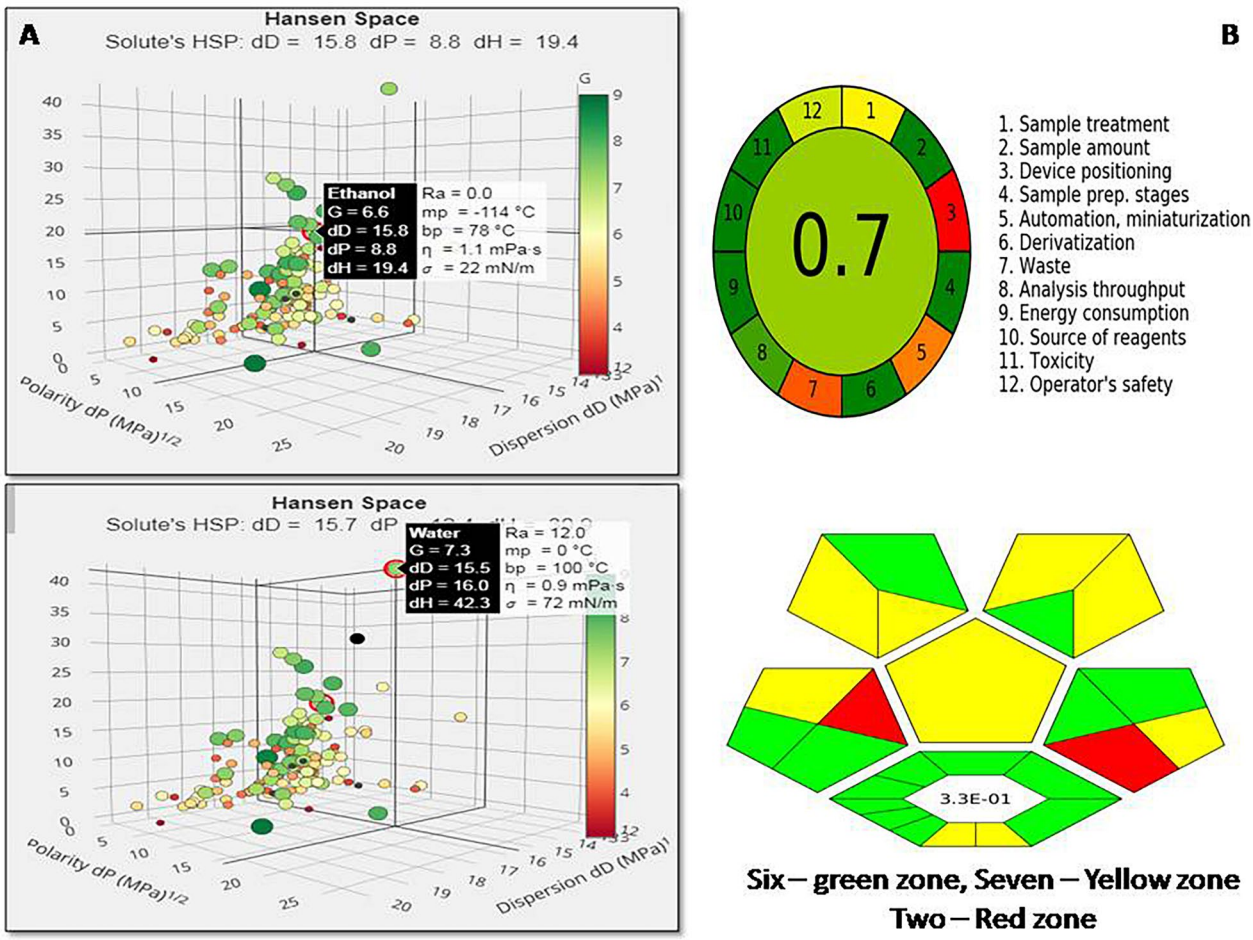

Fig. 5 **A** Green solvent selection graph showing greenness score of water, ethanol and ethyl acetate **B** Greenness profile assessment of proposed HPTLC method using AGREE and GAPI tools

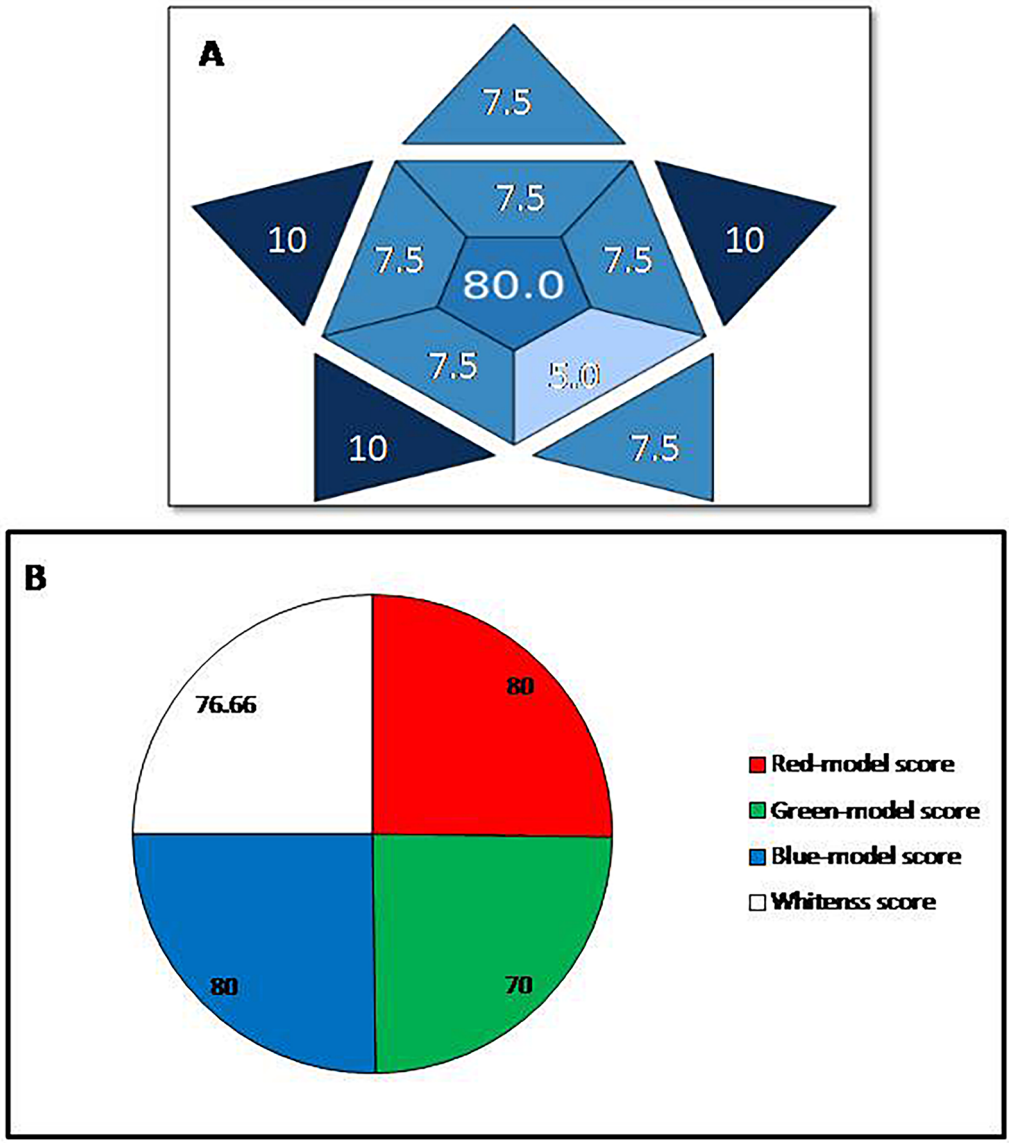

Fig. 6 **A** Blueness profile assessment of proposed HPTLC method using BAGI tool **B** assessment and comparison of RGB model scores of proposed HPTLC method for concomitant estimation of METH, TENH and PIOH

animals, and the environment, as stated in the green solvent selection guides produced by various pharmaceutical companies. The green solvent selection tool suggested class 3 low-toxic organic solvents such as triethylamine and ethanol were used for chromatographic analysis. These organic solvents are environmentally friendly, in the suggested procedure (refer to Fig. 5-A). In proposed method, triethylamine was included as modifier, and its volume was less than 1.0 mL. With only one set of chromatographic parameters needed for the simultaneous estimation of METH, TENH, and PIOH, the suggested method reduced organic waste as well. Accordingly, the AGREE calculator gave the published [37] and suggested method a score of 0.60 and 0.70 out of 1.0. See Fig. 5-B for an example of a pictogram produced by GAPI software; two red zones, seven yellow zones, and six green zones were obtained for the proposed HPTLC method. Therefore, the published [37] and suggested method for estimating METH, TENH, and PIOH was given 65 and 70 out of 100 green model score.

B-model scoring using BAGI tool

We evaluated the blueness profile of the suggested and published [37] method approach to METH, TENH, and PIOH estimate using the BAGI tool. The BAGI tool was used to evaluate the approach based on its ease of

Table 4 Redness, blueness, whiteness, and greenness assessment of targeted HPTLC method using RGB model scoring system, BAGI tool, and AGREE calculator

Principles and criteria of white and green analytical chemistry	Redness, greenness, blueness and whiteness profile assessment of proposed method	RGB model scoring out of 100
Redness profile assessment using red model scoring system		
R1—Scope and applications	The proposed method was applied for assay of multiple pharmaceutical dosage forms METH, TENH and PIOH using single chromatographic condition	The method was found to be sensitive, accurate, robust and precise as per ICH Q2 (R2) guideline. Additionally, it requires single chromatographic condition for analysis of multiple formulations. Hence, score of 80 out of 100 was allotted
R2—Linearity, LOD and LOQ	The proposed method was found to be validated for linearity as per ICH Q2 (R2) guideline. The LOD and LOQ of the method were found to be at nanogram level estimation of anti-diabetic drugs	
R3—Accuracy	The %recoveries of METH, TENH and PIOH were found to be in the range of 98–102%	
R4—Precision and robustness	The %RSD for precision study of the proposed method was found to be less than 2.0%. The proposed method was developed using design of experiments approach for robust results	
Greenness profile assessment using AGREE tool		
G1—Environment impact	The developed method requires ethanol as primary solvents which are less harmful to the environment. It also included triethylamine as component of mobile phase but volume was less than 1.0 mL. While published chromatographic method required toxic organic solvents such as 1, 4-dioxane, chloroform, toluene, acetonitrile and methanol which are harmful to the environment	The proposed method requires environment friendly and safe organic solvents. The proposed method consumes less energy and generates less organic waste. The AGREE score of method was found to be 0.70 out of 1.0. Hence, green model score of 70 out of 100 was allotted to the proposed method
G2—Human and animal health impact	The required organic solvents in proposed method are class 3 organic solvents which are less toxic to the human and aquatic animal lives. While required organic solvents in published chromatographic methods are Class 1 and 2 solvents which are carcinogenic, neurotoxic and teratogenic to the human and aquatic animal lives	
G3—Energy consumption	The single chromatographic conditions required for analysis of multiple pharmaceutical dosage forms of anti-diabetic drugs. Hence, proposed method consumes less energy than published chromatographic method	
G4—Waste generation	The multiple dosage forms analysis of anti-diabetic drugs requires single chromatographic condition with organic waste up to 10 mL only	

Table 4 (continued)

Principles and criteria of white and green analytical chemistry	Redness, greenness, blueness and whiteness profile assessment of proposed method	RGB model scoring out of 100
Blueness profile assessment using BAGI tool		
B1—Time efficiency	Less time required than published chromatographic condition for analysis of multiple pharmaceutical dosage forms of anti-diabetic drugs	The proposed method was found to be cost-effective, rapid, simple and user-friendly. Using BAGI tool, the blue model score of 80 out of 100 was allotted to the proposed method
B2—Cost of analysis	Less cost for estimation of anti-diabetic drugs than published chromatographic methods as its requires single chromatographic condition	
B3—Simplicity of steps	Requires less than three steps for analysis	
B4—User friendliness	The HPTLC is more user-friendly method	
Whiteness profile assessment		
Average of RGB model score	Average of RGB model = (80 + 70 + 80)/3 = 76.66	The white model score of 76.66 was allotted to the proposed method
Whiteness status	If score of white model is above 75 than the method can be considered as excellent white	Hence, the proposed method was found to be excellent white

analysis, user-friendliness, cost-effectiveness, and time efficiency. In contrast to the published chromatographic method [37], which necessitated chromatographic condition using costly HPLC grade solvents such as methanol and acetonitrile for METH, TENH, and PIOH synchronous estimation, the suggested method only needed a single chromatographic condition with AR grade economical solvents such as ethyl acetate, ethanol, and triethylamine. The published RP-HPLC method required lot of time, costly organic solvents, and skilled analysis for handling of instruments [37]. The suggested method needed less time for analysis, economical organic solvents and less skill for handling of instruments. The results showed that the suggested approach to simultaneous estimate of several commercially available formulations and combinations of METH, TENH, and PIOH was more efficient, less expensive, easier, and more user-friendly. The published RP-HPLC method was allotted score of 70 out of 100 using BAGI tool for the simultaneous estimation of the anti-diabetic drugs under study. According to Fig. 6-A, the suggested method for estimating METH, TENH, and PIOH achieved a score of 80 out of 100 when tested using the BAGI tool.

Whiteness profile assessment using WAC approach

After averaging the method's red, green, and blue model scores, we were able to determine the analytical method's whiteness profile using guidance given in literature [41]. The suggested and published [37] method has an average RGB model score of 76.66 and 71.66 out of 100 for METH, TENH, and PIOH estimation, which is the WAC score. The analytical approach may be regarded as an excellent white method for drug sample estimation if the whiteness profile score is more than 75. Therefore, the suggested method worked excellent white for the simultaneous determination of METH, TENH, and PIOH. The results of the RGM model score are summarized in Table 4 and Fig. 6-B.

Conclusions

The HPTLC method was developed and validated for the concomitant estimate of numerous marketed formulations and combinations of METH, TENH, and PIOH using a harmonized approach of WAC, GAC, and analytical QbD. It is robust, inexpensive, green, and quick HPTLC method for the estimation of anti-diabetic drugs under study. The analytical QbD method was implemented in accordance with the most current recommendations made public in ICH Q14, drawing on concepts from analytical risk management and experimental design. In order to estimate several METH, TENH, and PIOH formulations concomitantly, the suggested method only needed a single validation operation. According to the most current version of the ICH Q2 (R2) guidelines, the suggested HPTLC method for estimating METH, TENH, and PIOH was found to be accurate, precise, robust, sensitive, and specific. For the concomitant determination of METH, TENH, and PIOH, the suggested HPTLC method has included environmentally friendly solvents like ethanol and water in lieu of harmful organic solvents such as 1, 4-dioxane, chloroform, toluene, methanol, and acetonitrile. Reduced organic waste means the suggested process is safe for human, animals, and the environment. A variety of tools and software were used to evaluate the redness, greenness, blueness, and whiteness profiles of the proposed method approach according to the principles of GAC and WAC. For the concomitant estimate of METH, TENH, and PIOH, the suggested HPTLC method was determined to be excellent green and white. Consequently, the suggested HPTLC method may be used as a cost-effective, environmentally friendly, and strong analytical instrument for quality control and routine analysis of various METH, TENH, and PIOH formulations and combinations, thereby saving time, cost, and resources.

Abbreviations

METH	Metformin Hydrochloride
TENH	Teneligliptin Hydrobromide Hydrate
PIOH	Pioglitazone Hydrochloride
ICH	International Council for Harmonization
CDSCO	Central Drug Standard Control Organization
CCD	Central Composite Design
DE	Design Expert
AGREE	Analytical Greenness
GAPI	Green Analytical Procedure Index
BAGI	Blue Applicability Green Index
RGB	Red, Green and Blue
HPTLC	High-Performance Thin-Layer Chromatography
HPLC	High-Pressure Liquid Chromatography
DOE	Design of Experiments
WAC	White Analytical Chemistry
GAC	Green Analytical Chemistry
QbD	Quality by Design
MODR	Method Operable Design Range
MP	Method Parameter
RV	Response Variable
ANOVA	Analysis of Variance
LOD	Limit of Detection
LOQ	Limit of Quantitation
RSD	Relative Standard Deviation
QTAP	Quality Target Analytical Profile

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Author contributions

Pintu Prajapati involved in drafting, conceptualization, supervision, and review. Pooja Patel involved in methodology, project administration, and writing. Dhrumi Naik involved in methodology and formal analysis. Anzarul Haque involved in software, drafting of manuscript, and review. Shailesh Shah involved in supervision and review support.

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Declarations

Ethics approval and consent to participate
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Competing interest
The authors of the manuscript have already declared that they do not have any competing interest for publication of the manuscript.

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