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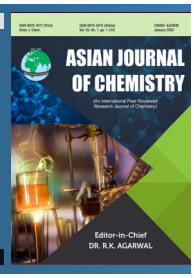

Simultaneous Estimation of Repaglinide and Voglibose in Newly Approved Fixed-Dose Combination by using UFLC: Application to ICH Q14 Concept and Comparative Method Greenness Assessment

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A validated liquid chromatographic method was developed to estimate repaglinide and voglibose present in a newly approved fixed dose combination. Critical method variables such as organic proportion, pH and flow of the mobile phase were identified. The identified variables were investigated using chemometrics and effect on analyte retention and resolution was studied. The optimized chromatographic condition utilized acetonitrile: 0.01 M KH_2PO_4 buffer pH 3.4 (using orthophosphoric acid) (79:21%, v/v) flowing at a flow rate of 1.2 mL/min. Diode array detection was carried out at 244 nm.The new analytical method was validated as per ICH regulations and found to be linear, accurate, precise and sensitive. Both drugs were recovered to the optimum (> 99%) level. Further, method greenness was compared by using multiple tools. This developed method based on ICH Q14 concept is scientifically sound and can be applied for quality control of both the anti-diabetic drugs in their combined formulation.

Keywords: Box-Behnken design, Chemometrics, Repaglinide, Robustness, Voglibose.

INTRODUCTION

Diabetes mellitus is a major metabolic disorder of present times that accounts for a significant portion of deaths around the world every year [1,2]. Reasonable control over the glycemic index of blood can further save a person from several associated diseases and health complications [3,4]. In certain instances, it was noticed over time that single-drug therapy is of a limited benefit than compared to following a combination therapy for diabetes [5-9]. These combinations of two or more anti-diabetic drugs are found to control blood glucose levels and thereby improve the health condition of the patient community [10,11]. Recently, a new fixed-dose combination of two potent anti-diabetic drugs is approved by drug regulatory agencies for the treating Type 2 diabetes mellitus [12].

The first drug is repaglinide, which is a metiglinide class of drug, chemically known as 2-ethoxy-4-[2-[[(1*S*)-3-methyl-1-(2-piperidin-1-ylphenyl)butyl]amino]-2-oxoethyl]benzoic acid (Fig. 1a) [13]. Repaglinide is an effective medication option in its single component dosage form along with an appropriate diet and exercise regime [14-17]. The second drug voglibose is a potent α-glucosidase inhibitor, chemically known as (1S,2S,3R,4S,5S)-5-(1,3-dihydroxypropan-2-ylamino)-1-(hydroxymethyl)cyclohexane-1,2,3,4-tetrol (Fig. 1b) [18]. Voglibose is a highly effective medication for decreasing the postprandial blood glucose levels [19-22]. However, it is for the first time that repaglinide and voglibose are combined into a single fixed-dose combination dosage form. Understanding the need of the hour, the analytical community seeks a simple, reliable, robust, yet scientifically sound and efficient analytical method for routine quality control of this new fixed-dose combination.

Upon literature review, only two HPLC methods are reported for both drugs in their combined dosage forms [23,24]. However, to the best of our knowledge and available literature, no other analytical methods are available for simultaneously quantified repaglinide and voglibose in the newly approved combined formulation.

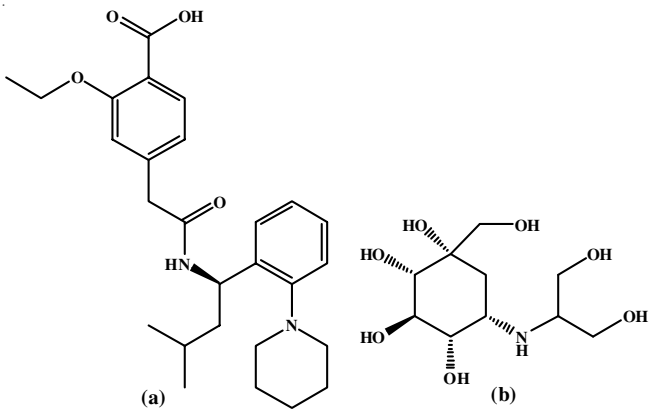

Fig. 1. Chemical structure of (a) repaglinide and (b) voglibose

Recently, International Council for Harmonization (ICH) of Technical Requirements for Pharmaceuticals for Human Use based Q14 concept urges to use a risk assessment followed by chemometrics based designed experiments to disseminate high-quality scientific information to fortify the knowledge pool of analytical scientists [25]. The risk assessment is an integral and often overlooked component of an analytical method [26]. As identifying the actual risky method variables and optimizing them for developing a robust analytical method is of critical importance, analysts can implement guidance of ICH Q 9 and undertake suitable risk assessment exercise as per the analytical target profile (ATP) [27,28]. Systematized optimization of a reliable LC method to quantify repaglinide and voglibose in simultaneously in their new fixed dose combination using analytical procedure development (APD) principles is the prime intent of the current method.

However, in the last half a decade, analysts have been interested in developing green and sustainable analytical methods [29,30]. The concept of green analytical chemistry (GAC) is such a guideline that efficiently sets a framework for assessing method greenness using various tools such as National Environmental Methods Index (NEMI), Green Analytical Procedure Index (GAPI) and Analytical GREEnness Metrics (AGREE) approach, *etc.* [31-34]. The first one is a simple pictogram representation that qualitatively identifies the methods green nature. The other two are more comprehensive and provide both easily understandable pictogram and greenness scores. Hence in the present study, the GAPI and AGREE tools has been applied for this purpose.

The present study is centred on the systematized investigation of chromatographic method variables and optimizes those using chemometrics tools. Afterward, the optimized liquid chromatographic method is validated and it selectively quantifies the two anti-diabetic agents that are combined for the first time into a combined tablet dosage form. Also, a comparison of the green scores achieved by the reported and current method is presented to establish the green nature of the method.

EXPERIMENTAL

Repaglinide and voglibose were kindly provided by Roland Institute of Pharmaceutical Sciences, Berhampur, India. HPLC quality acetonitrile, orthophosphoric acid, potassium dihydrogen phosphate were procured from Merck, Mumbai, India. The new fixed-dose combination of repaglinide (1 mg) and voglibose (0.3 mg) was purchased from the local pharmaceutical market for analysis.

Chromatographic instrumentation and separation conditions: For chromatographic separation, binary gradient UFLC (ultrafast liquid chromatography) pumps (Prominence Series, Shimadzu, Japan) equipped with a photodiode array detector (DAD) and manual injection port of capacity 20 µL was employed. A Shim-Pack C-18 column (250 mm × 4.6 mm i.d., 5 µm) was used. A pH meter (Eutech, India), ultrasonicator (GT-Sonics, China) and TKA HPLC water unit (Thermo- Fisher, Germany) were also used during the study. Design Expert (Stat-Ease, Inc., Minneapolis, USA) software was used for chemometrics based designed experiment modeling and optimization data analysis.

Preparation of standard and sample solutions: The standard solutions of repaglinide and voglibose (each 1000 µg/mL) were prepared by dissolving 10 mg of the standard drugs in the mobile phase within 10 mL volumetric flasks. These stock solutions were stored at 4 ºC and further utilized to prepare mixed standard solutions.

Twenty tablets were weighed and ground to a finely powdered state. Tablet powder equivalent to 1 mg of repaglinide and 0.3 mg of voglibose was transferred to 10 mL volumetric flask containing 5 mL of mobile phase. The contents were ultrasonicated for 30 min before final dilution up to 10 mL using the mobile phase. After final dilution (100 µg/mL of repaglinide and 30 µg/mL of voglibose), the suitable aliquots of the sample were used to prepare test solutions within the studied concentration range of both the drugs.

Chemometrics based optimization of LC condition: The chemometrics based optimization involved a systematic workflow, which is based on the prior knowledge on method development and efficient risk assessment exercise. In present study, the ATP was to separate repaglinide and voglibose efficiently while quantifying them with adequate accuracy and precision (Table-1). The fact of obtaining a higher resolution between analyte peaks was considered vital, as it provides greater opportunity of being utilized as a basis for the future impurity and bioanalytical based studies. Several experimental variables originating from various origins such as men, material, milieu, measurement, method and the machine were evaluated using failure modes and effect analysis (FMEA). The variables were ranked according to their risk priority number (RPN) using eqn. 1:

$$\text{Risk prioriy number (RPN)} = \text{Severity (S)} \times \text{Occurence (O)} \times \text{Detectability (D)} \quad (1)$$

The variables with scores more than 250 were then named as critical method variables (CMVs) for chemometric based robustness-cum-method optimization studies. A chemometrics based designed experiment approach was employed using a Box-Behnken design (BBD) for the CMVs. A total of 17 experiments were generated at three levels for three CMVs using the Design-Expert software. The influence of CMVs on critical analytical attributes (CAAs) such as analyte retention

TABLE-1
ANALYTICAL TARGET PROFILE AND CRITICAL ANALYTICAL ATTRIBUTES
FOR THE SIMULTANEOUS LC METHOD FOR RGN AND VGS

Components of ATP	Target	Reason
Analyte	Repaglinide (RGN) and voglibose (VGS)	No simultaneous analytical methods are available.
Sample Type	API/Tablets	As per the requirements of patients.
Method	UFLC	Appropriate for routine quality control of analytes.
Instrumentation	Binary pump UFLC coupled with DAD Detector	Accurate mixing of the mobile phase, lower pressure levels and DAD identifies co-eluting peaks.
Method Goal	Optimum separation and robust simultaneous assay of RGN and VGS with acceptable accuracy and precision	Reliable estimation of both analytes throughout the product life-cycle.
Preparation of Standards	Weighing, standard dilution	Accurately prepared dilutions produce better calibration curves.
Preparation of Samples	Preliminary processing, weighing, ultrasonication, volumetric dilution, membrane filtration	Ensures suitable dilution of sample solution as well as the absence of extraneous matter.
Critical analytical attributes	**Target**	**Reason**
Retention time (Rt)	Optimum	Rt is an essential identification criterion in liquid chromatography and variations in *Rt* indicate a problem with method performance.
Resolution (Rs)	High	Maximal Rs is always desired, which provides accurate quantification as well as serves as a basis for future applications of the method for more separation in complex sample mixtures.

(Rt) and resolution (Rs) between the two peaks was monitored during the study. The Rt and Rs were considered as the responses to be observed during designed experiments. Afterward, the obtained data were analyzed to optimize and establish a design space (DS) for obtaining the robust performance of the method.

Validation study: Validation parameters *viz.* linearity, detection and quantitation limit, accuracy, precision, solution stability and system suitability limits were verified to ensure method aptness. These studies were complied with the ICH directives [35].

Method linearity was assessed based on the average peak areas determined from the triplicate injections of the mixed standard solutions of repaglinide and voglibose. The studied concentrations include 1.0, 2.5, 5.0, 10, 15, 20 and 25 µg/mL of both the analytes. The averaged responses were plotted (*y*-axis) against the respective concentrations (*x*-axis). Correlation coefficients were also determined with desirable ideal R^2 closer to 1. When an analyte generates a signal-to-noise ratio of 3:1, the corresponding concentration is designated as a limit of detection (LOD). However, when the ratio approaches a value of 10:1 and can be quantified by the developed method, the corresponding concentrations are known as the limit of quantitation (LOQ).

Method accurateness was confirmed by considering the % recovery of standard spiked drugs from the fixed sample solution at three concentration levels (80, 100 and 120% level of 100% concentration).Intraday (repeatability) and interday (intermediate) precision were determined in hexaplicate determination of a fixed concentration (10 µg/mL of both the drugs) of solutions over the same and different days. Additionally, instrument precision was determined by employing six injections of the same concentration solution. For both of the above studies, mean responses, standard deviation (SD) and % relative standard deviation (%RSD) were also calculated to check the suitability of the obtained results.

The solution stability of the analytes was performed at room temperature and refrigeration conditions. The analyte solution was placed on a benchtop for 48 h and the room temperature stability was assessed after completion of time. Also, another analyte solution was stored under refrigeration at 4 ºC for up to 7 days, followed by analysis by the developed method. In both cases, triplicate injections were performed and mean recoveries were calculated.

System suitability of LC methods is effectively tested and reported by analysts, which indeed establishes method performance [36]. Retention time (Rt), resolution (Rs), theoretical plates (N) and tailing factor (T) are the suitability parameters that were determined for the purpose [37]. Eqns. 2 and 3 reveal the lower and higher limits employed for deriving the SST limits.

$$\left\{\overline{Z} - t\alpha, n-1.\left(\frac{s}{\sqrt{n}}\right)\right\} \quad (2)$$

$$\left\{\overline{Z} + t\alpha, n-1.\left(\frac{s}{\sqrt{n}}\right)\right\} \quad (3)$$

where, $\overline{Z}$ = average of three observations, $t\alpha,n\text{-}1 = t_{critical\ (\alpha = 0.05,\ n\text{-}1 = degrees\ of\ freedom)}$, s = SD of three observations and $\sqrt{n}$ = square root of the number of observations.

Method greenness assessment: The GAPI approach evaluates the various aspects such as sample collection, preservation, transport, storage, method type, sample preparation, reagents and compounds and instrumentation requirements of an analytical method. Five pentagons in this pictogram represent a specific category of the method and an appropriate colour (red/yellow/green) indicates the green nature of the method. Therefore, this pictogram provides a more practical overview of the analytical greenness of a method.

The 12 holistic principles of GAC are the pillars of the AGREE approach and utilize a software based analysis that provides scores ranging from 0 to 1. A score closest to unity

exhibits the optimum method greenness. These parameters are sampling procedure, amount of sample, the position of the analytical device, steps of sample preparation, degree of automation, derivatization agents, amount of waste, the number of analytes determined per run, most energy-intensive technique, renewable reagents, toxic reagents and various unavoidable threats.

RESULTS AND DISCUSSION

Prior knowledge and method development approach: The initial investigations revealed that both drugs (repaglinide and voglibose) have better solubility in acetonitrile comparative to other organic solvents. Hence, acetonitrile was selected as the appropriate organic phase for the mobile phase. The pH of mobile phase plays a critical role in the separation of analytes on the reversed stationary phase. A phosphate buffer system using KH_2PO_4 formed the aqueous portion of the mobile phase. A 0.01 M phosphate buffer was studied at various pH values such as 3.0, 3.5, 4.0 and 4.5. The PDA spectra the repaglinide shows three absorption peaks at 207, 243 and 293 nm and voglibose shows an absorption maximum at 206 nm. However, when overlaid (not shown in figure) the spectra were found to show a common absorbance at 244 nm. Further, 244 nm was selected as the common detection point for both analytes. It was observed that in most of the case, either the peak shape was not good or drug peaks were not detected, except pH 3.5, where very symmetric peaks were obtained. The initial trials with different mobile phase ratios and flow rate construed that acetonitrile: 0.01 M KH_2PO_4 buffer pH 3.5 flowing at a flow rate of 1.3 mL/min when detected at 244 nm, is suitable for further systematic investigations employing chemometrics approaches.

Designed experiments based robustness-cum-method optimization: The preliminary scrutiny of method variables using prior method development knowledge, fish-bone diagram and modern FMEA approach (Table-2) unearthed that acetonitrile %, pH and the flow rate are the riskiest variables that demand an investigation to optimize a robust and reliable LC method for repaglinide and voglibose. Seventeen designed experiments (Table-3) were performed and obtained data were analyzed using a quadratic polynomial model. During this phase analysis of variance (ANOVA), model F-value and adequate precision were evaluated (Table-4) and found satisfactory for further investigations. The model was found befitting for the purpose as the R^2 values were acceptable (values > 0.9). The polynomial equations for responses such as Rt and Rs were framed as shown in eqn. 4:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 AB + \beta_5 AC + \beta_6 BC + \beta_7 A^2 + \beta_8 B^2 + \beta_9 C^2 \quad (4)$$

where Y = response (s) obtained, A = acetonitrile %, B = pH and C = flow rate (mL/min).

The obtained coefficient is enlisted in Table-4, which indicates the effect of CMVs on the CAAs. The synergistic and antagonistic effects are denoted by '+ve' and '-ve' signs, respectively. The interpretation of model graphs such as perturbation chart, three-dimensional response and two-dimensional contour plots surfaces confirmed the preliminary assessment. Curved and intersecting factor lines in the perturbation charts (not shown in figure) confirmed interaction amongst the studied CMVs. The three-dimensional response surfaces (Fig. 2a-i) allowed a detailed interpretation of the influences, interactions and helped understanding the method behaviour to the variations in CMVs.

In Fig. 2a, a curvilinear decrease in Rt of repaglinide was observed when there was an increase in acetonitrile %. However, pH didn't contribute to the decline in Rt as the responses were unaffected over the studied range. A similar response (Fig. 2b) to that of Fig. 2a was obtained in case of acetonitrile % and flow rate, where a curvilinear decrease in Rt of repaglinide

TABLE-2
IDENTIFICATION OF CRITICAL METHOD VARIABLES USING FMEA

Source	Mode of failure	Effect	S	O	D	RPN[a]
Method	Acetonitrile (%)	Multiple	8	6	8	384
	pH	Multiple	8	7	6	336
	Flow rate	Multiple	8	7	5	280
	Stationary Phase	Extended retention	6	5	5	150
Material	Standard Purity	Extraneous peaks	7	6	5	210
	Sample Purity	Extraneous peaks	6	5	4	120
	Solvent grade	Extraneous peaks	5	4	4	80
	Reagent Purity	Extraneous peaks	3	3	4	24
Milieu	Temperature	Variable resolution	4	3	3	36
	Humidity	Inaccurate weighing	4	3	2	24
	Photosensitivity	Analyte degradation	3	3	3	27
Measurement	Peak integration	Varied response	3	3	3	27
	Sampling rate	Varied potency	3	2	2	12
	Peak purity	co-eluting peaks	2	3	3	18
	Glassware error	Wrong dilution factor	2	2	2	8
Men	Mislabelling	Faulty peaks	4	3	2	24
	Dilution error	Incorrect potency	3	2	3	18
	Calculation error	Incorrect purity	3	2	2	12
Machine	UFLC integrity	Poor performance	2	3	2	12
	Sonicator integrity	Varied pressure	2	2	2	8
	Analytical balance integrity	Incorrect potency	2	2	2	8

[a]RPN = Risk priotiy number = S × O × D where S = severity, O = occurrence and D = detectability.

TABLE-3
CRITICAL METHOD VARIABLES AND THEIR LEVELS FOR CHEMOMETRICS BASED OPTIMIZATION STUDY

Run No.	Acetonitrile (%)	pH	Flow rate (mL/min)
1	0	-1	-1
2	0	-1	+1
3	-1	-1	0
4	+1	-1	0
5	-1	0	-1
6	-1	0	+1
7	0	0	0
8	0	0	0
9	0	0	0
10	0	0	0
11	0	0	0
12	+1	0	-1
13	+1	0	+1
14	-1	+1	0
15	0	+1	-1
16	0	+1	+1
17	+1	+1	0
Coded levels investigated			
Low (-1)	78%	3.2	1.2 mL/min
Nominal (0)	80%	3.5	1.3 mL/min
High (+1)	82%	3.8	1.4 mL/min

TABLE-4
RESULT OF ANOVA AND COEFFICIENTS OBTAINED FOR THE QUADRATIC POLYNOMIAL EQUATIONS

Parameter	Retention time (Rt)		Resolution (Rs)
	Repaglinide	Voglibose	
ANOVA (P = 0.05)	< 0.0001	< 0.0001	0.0093
Model F-value	152.44	98.51	6.90
Adequate precision	40.895	29.32	9.445
Coefficients	Retention time (Rt)		Resolution (Rs)
	Repaglinide	Voglibose	
β_0	+1189.51	+90.15	-341.18
β_1	-24.6	-1.43	+26.57
β_2	-68.84	-6.98	-222.50
β_3	-58.76	-25.68	-385.46
β_4	-0.01	-0.04	+0.03
β_5	-1.47	-0.16	+3.8
β_6	+5.23	+1.12	+9.65
β_7	+0.16	+0.01	-0.2
β_8	+8.69	+1.31	+28.84
β_9	+57.62	+12.38	+17.21

reached to a "static minimum" at high levels of acetonitrile proportion and mobile phase flow rate. A slight decline in Rt of repaglinide with a perfect "static-minimum" was obtained at mid-to-high levels of both pH and flow rate (Fig. 2c).

A curvilinear increase in Rt of voglibose was obtained (Fig. 2d), which initially (low-to-mid levels of acetonitrile % and pH) behaves as a "static-minimum" but later on gives an increase in Rt of voglibose. However, with an increase in acetonitrile %, the Rt was found to increase slightly. The Rt of voglibose decreased significantly with an increased flow rate in a curvilinear pattern (Fig. 2e). Acetonitrile % was found non-influential along with flow rate. A larger "static-minimum" was obtained (Fig. 2f) when the flow rate increased from low-to-high values, whereas with increasing pH and lower flow lead to higher Rt values for voglibose.

A typical "toffee" shaped response surface was obtained for Rs considering acetonitrile % and pH as the CMVs (Fig. 2g). The surface indicated a complex interaction amongst the CMVs. Initially, a "rising-ridge" was obtained with high Rs values at a low-level of pH and acetonitrile %. However, with the gradual increase in acetonitrile %, Rs decreased slightly. At mid-levels of acetonitrile % and pH, a significant decrease in Rs was observed. Also, it depicted that at low-levels of acetonitrile % with increasing levels of pH, the Rs was better and remained unaffected. Further, the response decreased gradually at a higher level of acetonitrile proportion with mid-to-high levels of pH. The Rs was found to reduce in a curvilinear pattern with an increasing ratio of acetonitrile over all the studied levels of flow rate (Fig. 2h). The flow of the mobile phase had no significant influence on Rs. A "rising-ridge" was obtained (Fig. 2i) at low-levels of pH throughout all the studied levels of flow rate. A curvilinear decrease in Rs was observed with increasing levels of pH and at mid-levels of pH, a minimal Rs was found. However, with an increase in pH, the Rs again increased slightly. The authors obtained a comparable analysis from the two-dimensional contour plot (not shown in figure) study, the top view of the three-dimensional response surface. The plots corroborated with the respective three-dimensional response surfaces and confirm the interpretation and scientific information derived from response surface mapping. So, it can be established that all three CMVs had minor to moderate influence on Rt of the analytes, whereas acetonitrile % and pH were highly influential on Rs between the two drug peaks.

Based on the above observations, the final optimization was conducted using the numerical and graphical mode to obtain desirability values closest to 1. The overall goal of the optimization was to get a robust DS region to achieve the desired Rt of both analytes along with maximal Rs between the respective peaks. The overlay plot (Fig. 3) depicts the robust DS region, which can be used for obtaining the desired method performance. The optimized solution proposed that 79% acetonitrile, pH 3.4 and 1.2 mL/min flow of the mobile phase can provide adequate retention as well as resolution amongst the peaks for repaglinide and voglibose. Typical chromatograms (Fig. 4a-d) of standard drugs individually and in combined solution and commercial fixed-dose combination tablet dosage form are in confirmation with the optimized chromatographic conditions.In the optimized chromatographic conditions, repaglinide and voglibose show Rt of 13.472 and 3.836 min, respectively. An excellent Rs value of 30 was obtained between both the peaks. During a simultaneous estimation procedure larger Rs values are always desirable as it provides ample opportunity for future application of the method for estimation of related substances, degradation products and bioanalytical studies, *etc.* Apart from the above two CAAs, the theoretical plate count (N) was also found to be more than 15500 and 4000 for repaglinide and voglibose, respectively. The obtained peaks were relatively symmetric with peak tailing values within 1.25 (1.05 for repaglinide and 1.2 for voglibose, respectively) for both the analytes.

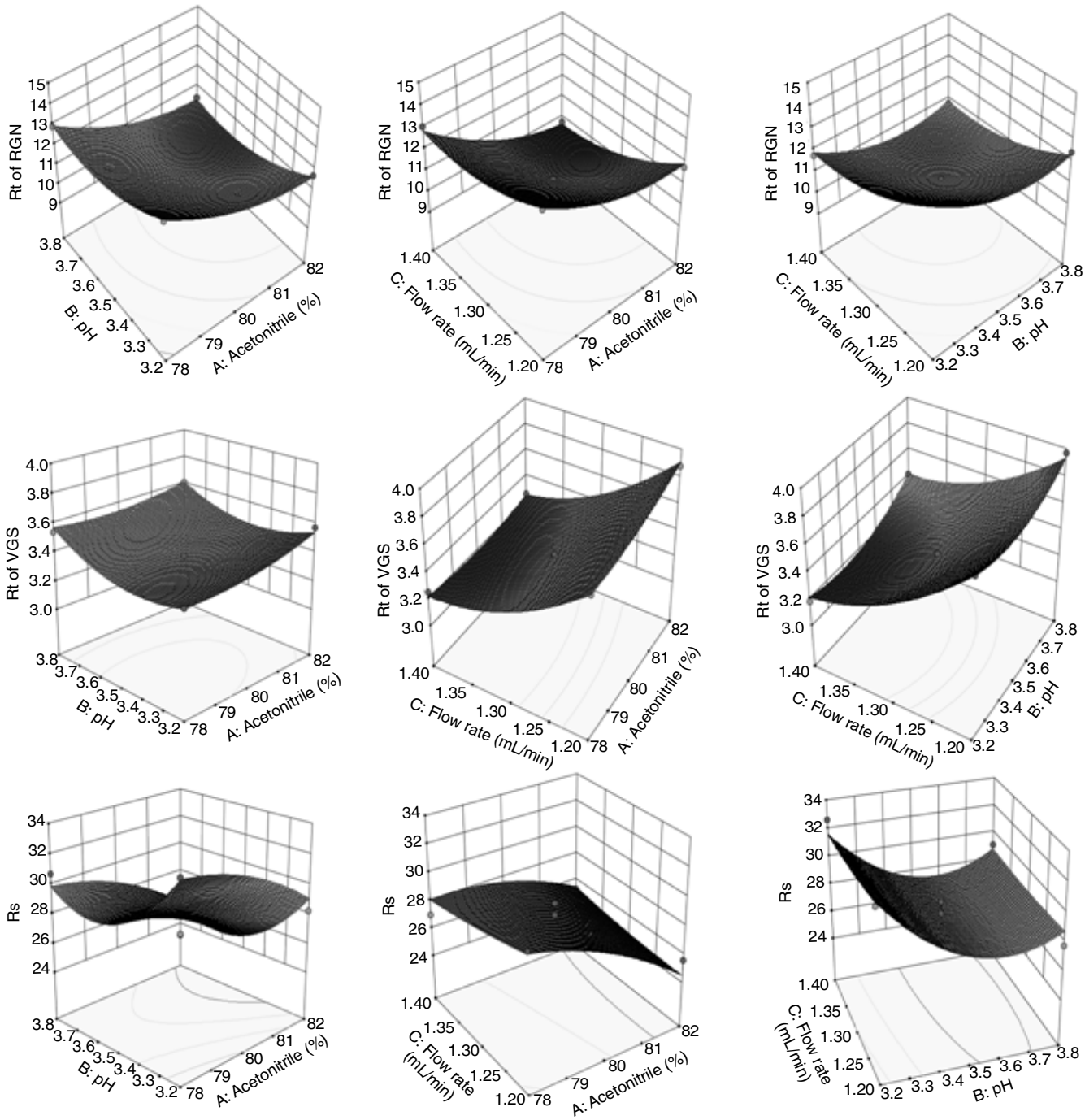

Fig. 2. Three-dimensional response surfaces obtained for CAAs (a-c) Rt of repaglinide, (d-f): Rt of voglibose and (g-i): Rs using designed experiments

Validation summary: Both drugs were analyzed in triplicate over seven concentrations (1.0-25 µg/mL) and the linearity was well accompanied by regression coefficients closest to 1 (Table-5). An ICH Q2(R1) oriented S/N ratio based visual evaluation was employed for establishing the LOD and LOQ. The lowest concentrations of both analytes were evalu-ated for confirming these limits and the results presented in Table-5 established the method sensitivity.

As shown in Table-5, excellent recovery of both analytes (> 99%) at all the three levels assured method accuracy and indicated the absence of matrix effects. Similarly results of method preciseness displayed in Table-5 suggest reliability and reproducibility of the method as the %RSD values are within 1%.

Both analyte's stability was good (up to 48 h at room temperature and up to a week at 4 ºC) with average recoveries over 98% (Table-5). The results imply no significant decrease in peak area during the storage period. The results presented in Table-6 prove the system suitability and complies with regulatory requirements. The results also assure the aptness of the developed method for application throughout the analytical method life-cycle.

TABLE-5
RESULTS OF METHOD VALIDATION AND LC BASED SIMULTANEOUS QUANTIFICATION OF RGN AND VGS

Parameter	Repaglinide	Voglibose
Beer's range (µg/mL)	1.0-25	1.0-25
Regression equation	Y = 49565x + 18280.29	Y = 242398.1x – 36681.22
Correlation coefficient (R^2)	0.999	0.999
LOD (µg/mL)	0.4	0.25
LOQ (µg/mL)	1.0	1.0
Accuracy (% recovery[a], %RSD)		
80%	100, 0.29	100.43,0.38
100%[b]	100.03, 0.25	100,0.57
120%	100.06, 0.13	99.74,0.31
Precision (% RSD[c])		
Repeatability	0.02	0.08
Intermediate	0.05	0.48
Instrument	0.04	0.08
Stability (Mean[d] ± SD)		
At room temperature	99 ± 0.1	98.66 ± 0.33
At 4 °C	100.43 ± 0.3	100.22 ± 0.38
Analysis of commercial tablets[f]		
(Mean ± SD)	100.26 ± 0.50	100.32 ± 0.86

[a]% recovery = Average of three determinations at each level; [b]100% level = 10 µg/mL of RGN and 3 µg/mL of VGS of the sample solutionand the three final concentrations after standard addition method are 18, 20 and 22 µg/mL of RGN and 5.4, 6 and 6.6 µg/mL of VGS, respectively; [c]%R.S.D. is the relative standard deviation; [d]Mean = Average of three determinations at 10 µg/mL of RGN and 3 µg/mL of VGS; [e]S.D. = Standard deviation; [f]Commercial tablets have = 1mg of RGN and 0.3 mg of VGS.

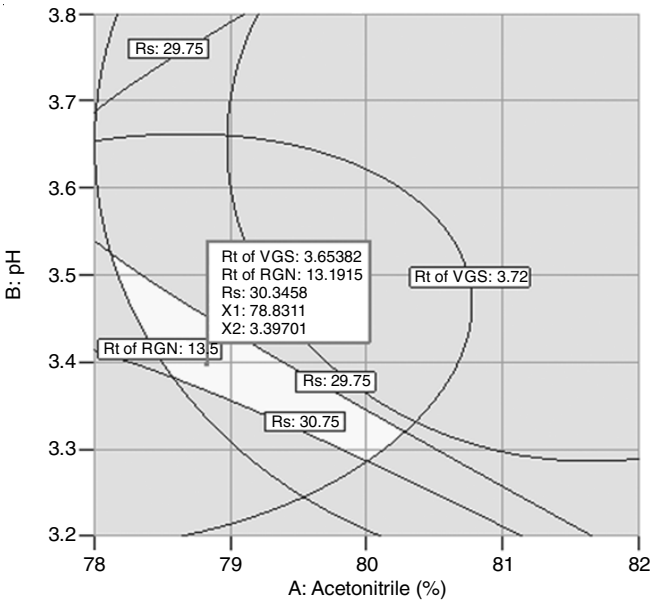

Fig. 3. Design space obtained for the newly developed LC method

A recently approved fixed-dose combination of repaglinide and voglibose was estimated for analyte content using the present methodology. The results (average recovery >100%) shown in Table-5 vouches for selectivity (Fig. 4d) of the present chromatographic method for routine estimation of repaglinide and voglibose in combined tablets.

Method greenness: A comparison of results obtained for the GAPI and AGREE assessment was performed for the two recently reported HPLC and the current UFLC method. Fig. 5e represents the GAPI pictograms where the UFLC method was found to score maximum (five) green scores compared to the reported ones (Fig. 5a,c). In addition, a matching pictogram and overall greenness score (0.33) (Fig. 5f) were obtained for the current method that illustrates the least number of red shades (four) and a maximum of five moderate to highly green shades than the reported methods (Fig. 5b,d). An overall, comparison of the results from both approaches infers that the current UFLC method is greener than the other two HPLC methods and can be used for routine quality control purposes.

Conclusion

The authors employed holistic principles of ICH Q14 to develop a validated and reliable LC procedure to simultaneously estimate repaglinide and voglibose in a new combined dosage form. Three risky critical method variables (CMVs)

TABLE-6
RESULTS OF THE SYSTEM SUITABILITY TEST

Run	Retention time (Rt)		Resolution (Rs)	Theoretical plates (N)		Tailing factor (T)	
	Repaglinide	Voglibose		Repaglinide	Voglibose	Repaglinide	Voglibose
1	13.933	3.744	30.397	15652.52	4433.635	1.049	1.169
2	13.41	3.558	27.031	15671.39	4211.412	1.065	1.208
3	12.925	3.477	30.707	16697.08	4173.984	1.052	1.174
Mean	13.422	3.593	29.37	16007	4273.01	1.05	1.18
SD	0.5041	0.1368	2.038	597.7	140.35	0.0085	0.0212
(n)	3	3	3	3	3	3	3
SST-limits	13.422 – 2.92 (0.5041/√3) = 12.572	3.593 – 2.92 (0.1368/√3) = 3.362	29.37 – 2.92 (2.038/√3) = 25.934	16007 – 2.92 (597.7/√3) = 15770.38	4273.01 – 2.92 (140.35/√3) = 4036.392	1.05 + 2.92 (0.0085/√3) = 1.064	1.18 + 2.92 (0.0212/√3) = 1.215

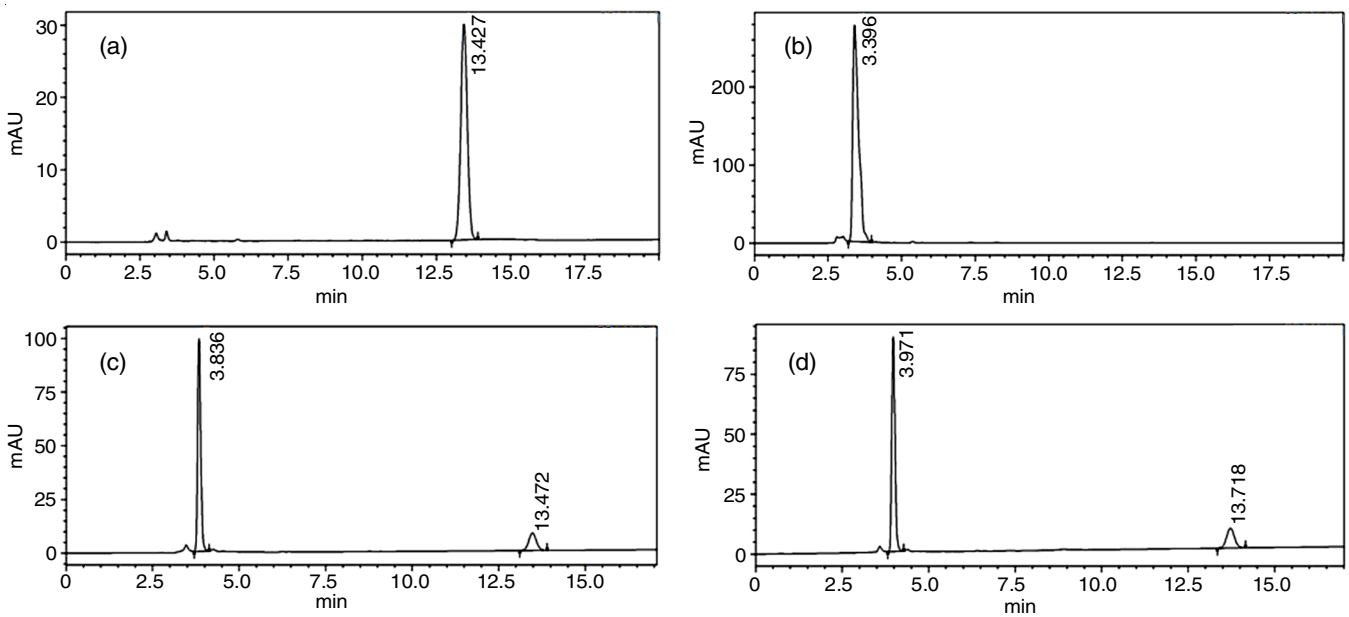

Fig. 4. Typical chromatograms of (a) repaglinide standard drug, (b) voglibose standard drug, (c) repaglinide and voglibose standard drugs in mixed solution and (d) repaglinide and voglibose in their new fixed-dose combination

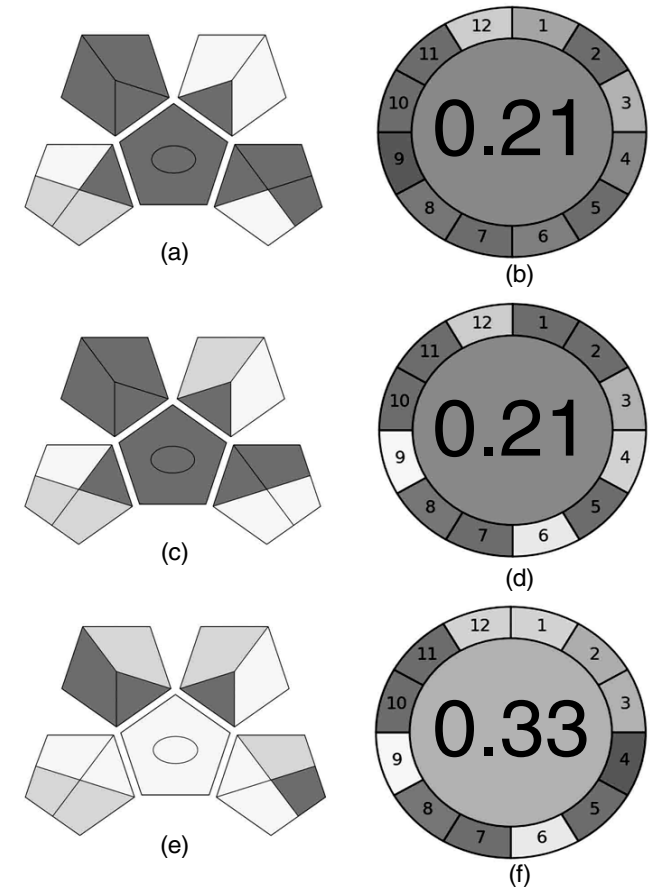

Fig. 5. Typical GAPI and AGREE pictograms obtained for the reported and current method: (a,b) Patel *et al.* [23], (c,d) Khusbu *et al.* [24] and (e,f) current method

were identified employing risk assessment tools such as to cause and effect oriented fish-bone diagram and FMEA study. During the chemometrics based method optimization, it was observed that resolution (Rs) was significantly affected by the acetonitrile % and pH. Accordingly, the CMVs were optimized to achieve maximal separation between the analyte peaks, which can be best utilized in the future for impurity and bio-analytical based studies. The statistical validation of designed experiment based model data revealed interrelationship and interactions amongst organic proportion, pH and flow of mobile phase. The typically curved response surfaces corroborated the complex interaction amongst the CMVs. Further, a method operable robust design space (DS) is established to obtain pre-determined performance during the life-cycle of the method. This approach has several unique advantages over traditional practices, such as a cost-effective way to obtain maximal scientific information and better method understanding, which ultimately expedites the method development phase. The robust nature of the method was practically studied during the pre-optimization phase and served the method's goal. Also, the method is linear, accurate, precise, sensitive as well as selective. Furthermore, the SST limits promised method aptness for everyday use in quality control laboratories. The dual GAPI and AGREE approach-based greenness assessment supports better overall green nature of the current method. The present analytical method estimated repaglinide and voglibose efficiently from their combined tablets and can be applied to obtain sustainable analytical performance.

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CONFLICT OF INTEREST

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

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