RESEARCH PAPER

Failure Mode Critical Effect Analysis and Design of Experiment Based Robust and Stability Indicating Method Development and Validation for Simultaneous Estimation of Antidiabetic Drugs in Tablets Formulation.

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ABSTRACT
Failure Mode Critical Effect Analysis and Design of Experiment Based Robust and Stability Indicating Method Development and Validation for Simultaneous Estimation of Metformin Hydrochloride, Dapagliflozin Propanediol Monohydrate and Teneligliptin Hydrobromide Hydrate in Tablets Formulation. Using the Ishikawa diagram for method development, failure modes were determined based on experimental data and prior knowledge. By assigning a risk priority number and criticality rank based on initial experimental trials, the criticality of the failure mode was evaluated. Design of experiment (DoE)-based central composite design screening design was used to analyze the impact of the identified key failure modes. Optimum condition was achieved on Zorbax C18 (300*4.6 mm*3 µm), the mobile phase consisting phosphate buffer (pH 4.75): methanol in the ratio of 75:25 with, the flow rate of 1 ml/min, injection volume 50 µL, detection wavelength 222.2 nm. The method's robustness, ruggedness, accuracy, precision, and linearity were all confirmed. The validation study's findings demonstrated that the recommended single method allowed for the analysis of medicines in the presence of their degradation products, which are created under various stress conditions. The described method can also be used to test the stability of antidiabetic medicines in tablet dose form.

KEY WORDS
Analytical Quality by Design, RPLC Method Development, Forced Degradation Study, Metformin Hydrochloride, Dapagliflozin Propanediol Monohydrate and Teneligliptin Hydrobromide Hydrate

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INTRODUCTION:

Diabetes Mellitus (DM) refers to a metabolic disease caused by a malfunction in either insulin secretion, insulin action, or both. Chronic hyperglycaemia and abnormalities in the metabolism of proteins, fats, and carbohydrates result from insulin insufficiency. The most prevalent endocrine condition, diabetes mellitus (DM) is predicted to affect over 200 million individuals globally by 2010 and 300 million by 2025 [1]. Diabetes-specific consequences include retinopathy, nephropathy, and neuropathy [2]. The main purposes of drugs are to treat symptoms and save lives. The prevention of long-term diabetes problems and the enhancement of longevity through the removal of numerous risk factors are secondary goals. While food and lifestyle changes are thought to be the cornerstone for the treatment and maintenance of type 2 diabetes, insulin replacement therapy is the cornerstone for individuals with type diabetes. Biguanides and sulfonylureas are two

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examples of hypoglycaemic medications that can be used to treat diabetes [3].

Metformin hydrochloride (MET) is biguanide hypoglycaemic agent used in non-insulin-dependent diabetes mellitus. Chemically it is 1, 1-dimethylbiguanide hydrochloride [4]. Metformin's main effects include lowering the amount of glucose produced by the liver, increasing insulin-mediated glucose utilization in peripheral tissues, decreasing the amount of glucose absorbed in the small intestine, and lowering the concentrations of plasma free fatty acids. This reduces the availability of substrate for gluconeogenesis, which lowers blood glucose levels in type 2 diabetes without causing overt hypoglycaemia [5].

Teneligliptin hydrobromide hydrate (TNL) newly developed oral dipeptidyl peptidase 4 inhibitor is prescribed to treat type 2 diabetes [6]. Chemically it is {(2S,4S)-4- [4-(3-methyl-1-phenyl-1H- pyrazol-5-yl) piperazin-1-yl] pyrrolidin-2-yl} (1,3-thiazolidin-3-yl) methanone hemipentahydrobromide hydrate [7]. Even in individuals with end-stage renal disease, oral teneligliptin once daily, either alone or in combination with metformin, glimepiride, or pioglitazone, improved glycaemic control and was generally well tolerated [8].

An effective, reversible, and specific sodium-glucose cotransporter-2 inhibitor, dapagliflozin is recommended globally to treat type 2 diabetes (T2D) [9]. The chemical name of Dapagliflozin propanediol monohydrate (DAPA) is (2S)-propane-1,2-diol (2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl}-6(hydroxymethyl)oxane-3,4,5-triol hydrate [10].

Analytical Quality by Design (AQbD) is an analytical application of the Quality by Design (QbD) idea. It allows mobility for the analytical process inside the Method Operable Design Range (MODR). Unlike existing methods, the analytical method created using Analytical Quality by Design (AQbD) in the method development process minimizes the amount of out-of-Trend (OOT) and out-of-Specification (OOS) discoveries due to the method's robustness within the region [11-12]. The analytical target profile (ATP), critical method parameters or variables, and critical method attributes (CMAs) or responses are all included. Significant factors can be identified and optimized by statistical analysis using screening and response-surface experimental designs [13].

Since the establishment of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), developing this approach has been explicitly required. Stability-indicating techniques can provide information about how the drug's quality varies with changes in environmental variables including pH, humidity, temperature, light, etc. Additionally, when developing new formulations, SIM helps a formulator choose suitable vehicles [14-15]

Numerous analytical techniques, such as UV spectrophotometric techniques for estimating DAPA and TNL, are documented for the quantification of these medications [16-17], for MET and DAPA [18]. Stability Indicating Reverse Phase High Performance Liquid Chromatography (RPLC) methods are also reported for these drugs with other one. Unlike for MET Stability indicating RPLC methods [19-21], for DAPA [22,23] and for TNL [24-25].

However, there is no method reported for simultaneous estimation MET, DAPA and TNL by RPLC method. Hence there is a need for sensitive RPLC method which is stability indicating for MET, DAPA and TNL. Stability studies were carried out by forcing the drug under variety of stress conditions like thermal, oxidative, light and hydrolysis (Acid and base), The developed RPLC method was validated as per ICH guidelines [26]. The aim of the present study was to develop a RPLC method for these drugs in tablet formulation and to perform the stability studies under various stress conditions by applying AQbD principles.

2 EXPERIMENTALS

2.1 Chemical and Reagents

MET (purity ≥99%), was supplied as a gift sample from Emcure pharmaceuticals. RMS scientific limited kindly donated DAPA (purity ≥98%) and TNL (purity ≥99%). Chemical reagents used during this research work including methanol (purity min 99.9 %), potassium dihydrogen ortho phosphate (purity ≥98%), acetonitrile (purity min 99.7 %), sodium hydroxide pellets (purity min 98.9 %), water (HPLC grade) were purchased from Merk science Pvt. Ltd.

2.2 Instrumentation and Chromatographic Condition

The current study used an HPLC Agilent 1100 for separation, which includes a Quaternary Pump (G1311A) and a photo diode array (PDA) detector. Clarity software was used for system controls, chromatographic data collecting, and processing. The stationary phase for separation was a Zorbax C18 (300*4.6 mm*3 µm). A Millipore glass vacuum filtration machine was used to filter the mobile phase via a 0.22-micron cellulose nitrate membrane. An ultrasonic bath sonicator (Analab Scientific Instruments, India) was used to sonicate the sample solution and mobile phase solution for ten minutes. To provide accurate pH management, pH measurements were carried out using a Systronics (micro controller-based pH system, model 362). The injected volume was 50 µL, the flow rate was set to 1 ml/min, the column oven temperature was 30 °C. The detection wavelength was 222.2 nm.

2.3 Method Development

2.3.1 Preparation of buffer and diluent

13.61 g of potassium dihydrogen ortho phosphate were dissolved in 1000 ml of HPLC-grade water to create phosphate buffer. With a addition of 0.1 M NaOH pH 4.75 was adjusted, the final mobile phase was filtered through a 0.45 µ nylon membrane filter and sonicated for three minutes in an ultra sonicator. Following sonication and degassing, a 50:50% v/v mixture of methanol and water was chosen as the diluent.

2.3.2 Preparation of standard solutions

A separate 100 mL volumetric flask was filled with precisely weighed 100 mg of MET, 20 mg of TNL, and 20 mg of DAPA. Diluent (50:50-methanol: water) was used to bring the volume up to the required level. (200 ppm of TNL and DAPA, 1000 ppm of MET). 25 ml of MET, 5 ml of TNL, and 2.5 ml of DAPA were pipetted out from mentioned solution into separate 50 ml volumetric flasks, and the volume was adjusted with diluent up to the mark (MET-500 ppm, TNL-20 ppm, DAPA-10 ppm).

2.3.3 Standard mixture solution preparation:

Take 25 ml MET from 1000 ppm stock solution, 5ml TNL and 2.5 ml DAPA from 200 ppm standard stock solution and transferred to 50 mL volumetric flask and volume made up to the mark with diluent. Resultant conc. of MET (500 µg/mL) + TNL (20 µg/mL) + DAPA (10 µg/mL).

2.3.4 AQbD approach

In accordance with International Conference on Harmonization (ICH) Q2(R1), Q8(R2), and Q14 criteria, the study used the Analytical QbD framework to create a reliable and repeatable RPLC method for estimation of MET, TNL, and DAPA in tablet formulations [27-29].

2.3.4.1 Analytical target profile and failure mode identified by preliminary trials

The goal of the analytical target profile (ATP) is to create an RPLC technique for the simultaneous estimate of MET, TNL, and DAPA in tablet formulations with resolution between peaks greater than two and tailing factors (T_f) of each peak within a range of 1-1.5. Using failure effect and critical effect analysis as a technique for quality risk management, the first step in creating an analytical process to accomplish ATP is identifying possible failure modes. Several failure modes were discovered by experiments and existing chromatographic expertise. Several mobile phases have been explored for drug separation. The Ishikawa diagram (Figure 1) was created by classifying the identified failure modes into categories (men, instrumental parameter, mobile phase, sample characteristics and preparation, material and column characteristics). Risk assessment was done by assigning a score to the failure mode's occurrence (O), severity (S), and detectability (D). Among all identified failure mode critical method parameters and critical process parameters like mobile phase composition, column temperature, flow rate, ambient temperature, pH of mobile phase, humidity, column age taken into consideration for quality risk assessment of failure mode. The probability of O and S very low (1), low (2), medium (3), high (4), and very high (5) was used to determine the scale for evaluating the failure mode. For D, score decided as per the certainty of failure mode measurement from 1 to 5. Risk priority number was calculated by multiplication of S × O × D (Figure 2, Table 1).

2.3.4.2 Critical failure mode analysis by central composite design

Only three out of the seven critical failure modes were determined to be critical by a statistical test conducted by the quality risk assessment of failure mode. DoE-based central composite design was used to further investigate the mobile phase's composition, flow rate, and pH for their failure mode effect study on resolution. A total of 17 trial runs, comprising 8 factorial, 6 axial, and 3 canter points, were recommended for the central composite design. In order to link the relationship between critical failures, modes (CQP), and resolution (CQA), all recommended experimental runs were carried out in the lab and the measured resolution was entered against the corresponding runs in design expert software (trial version 10). ANOVA testing and contour plots were used to examine the connection between failure modes and resolution (Table-2)

2.3.4.3 Construction and evaluation of the operable design region (MODR) strategy

A flexible multidimensional space where minimal variability is ensured by the interaction of independent elements is defined by the MODR. Numerical optimization was used to determine MODR, with predetermined bounds for each individual response.

The best option with the highest desire score was found using numerical optimization. Three confirmatory experimental runs were used to further validate the MODR, and the outcomes matched the values predicted by the model.

2.4 Method Validation

2.4.1 System Suitability Parameters

System suitability parameters were observed in order to assess system performance. Six duplicate injections of standard MET, DAPA, and TNL solutions were used to achieve this. Retention time, resolution, tailing factor, theoretical plates and peak area parameters were evaluated.

2.4.2 Specificity

Specificity of method was done to assure that there was no interference from other related components and that it was well resolved from them. Specificity was used to detect the analyte in the presence of components that might be expected to be present in the sample matrix. Peak purity analysis was performed for selectivity, and blank and placebo

were injected into the designed RPLC procedure to achieve specificity.

2.4.3 Linearity and Range

Linearity was carried out in range from 50 % to 150 % of standard by diluting standard stock solution for obtaining aliquots such that the final concentration of MET was in the range of 250-750 µg/mL, 5-15 µg/mL conc. range for DAPA and TNL was in the range of 10-30 µg/mL. 50µL of mixture was injected five times for each concentration level and calibration curve was constructed by plotting the peak area versus the drug concentration.

2.4.4 Accuracy and Precision

Accuracy was assessed by standard addition method for three different level of MET, DAPA and TNL. It was evaluated by spiking known amount of all drugs at 80%, 100% and 120% levels, three samples were analysed for each level. From the result analysing these concentrations, the mean percentage recovery, % RSD was calculated for each concentration.

Repeatability was performed by scanning one concentration of each drug six times without altering the chromatographic conditions, the RSD% of peak area measurement was computed. Six distinct independent solutions with the same concentration were prepared in order to test the sample application's repeatability. To conduct the intra-day precision study, the calibration curve technique was repeated three times in a single day, and the RSD% of peak area was computed. The calibration curve technique was repeated three days in a row for the inter-day precision research, and RSD% was computed for the fluctuation in the peak area of all three medications.

2.4.5 Limit of Detection and Limit of Quantification

The LOD and LOQ were separately determined from calibration curve. Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated using following equation:

$LOD = 3.3 \times (\sigma/S)$

$LOQ = 10 \times (\sigma/S)$

were,

σ = Standard deviation of y-intercepts of five calibration curves.

S = Mean slope of five calibration curves.

2.4.6 Assay of marketed formulation

Marketed formulation Zita DM-tablets was selected for assay performance. 20 tablets were weighed and powdered. Powder equivalent to 100 mg of MET (2 mg DAPA + 4 mg TNL) was added into 100 ml volumetric flask. About 60 ml of diluent added and sonicated for about 15 minutes, dilute up to the mark with diluent and mix. (1000 µg/ml MET + 20 µg/ml DAPA + 40 µg/ml TNL). Pipetted out 25 ml of above solution into 50 ml volumetric flask, dilute up to the mark with diluent. (500 µg/ml MET + 10 µg/ml DAPA + 20 µg/ml TNL). About 50 µl of above solution was injected three time for the assay analysis. Average % assay and %RSD were calculated.

2.5 Forced degradation studies

Forced degradation analysis were done on drug substance in which the standard drug substances were subjected to stress conditions such as acidic hydrolysis (1 N HCl, at 60°C for 5 hrs.), alkaline hydrolysis (1 N NaOH, at 60°C for 2 hrs.), oxidative hydrolysis (3 % H_2O_2 for 12 hrs.), for thermal degradation the solution was submitted to heating (80°C for 12 hrs.) and for photolytic degradation solution was exposed to UV Light for 12 hrs.

3 RESULT

3.1 Preliminary testing

Methanol: water and acetonitrile: water mixtures were found to produce effective resolution in the early stages of the method's development, although the quality of the peaks was degraded in both combinations with increased tailing. A range of buffers, including phosphate buffer, were evaluated at varying strengths. We chose phosphate buffer for our method optimization because it was found to generate consistent separation when compared to acetate buffer. One of the key elements in achieving successful separation is pH, thus we chose a range of pH after researching the literature and finding that phosphate buffer with a pH between 5 and 8 would work well. Methanol and phosphate buffer (pH 4.72) in the ratio of 25:75 was selected for chromatographic separation.

3.2 Chromatographic condition optimization via experimental design with chemometric assistance

For optimizing the different variables for efficient chromatographic separation and to study the interactions between them, central composite experimental design (CCD) opting three variables (proportion of methanol, pH of mobile phase and flow rate), at three level with 17 experiments. The effect of variables and the efficiency of model were verified by analysis of variance (ANOVA). The response factors were $T_{f\ of}$ MET, $T_{f\ of}$ DAPA, $T_{f\ of}$ TNL, Theoretical plates (TP) of peak of MET, TP of DAPA, TP of TNL, Retention time (R_t) of MET, R_t of DAPA, R_t of TNL. The response factors against the selected three variables are presented in Table 3. For all responses, model P-values <0.05 that implied the suggested linear and quadratic models were found to be significant for the development of the target RPLC method. The R-squared, adjusted R-squared, and anticipated R-squared values in the regression analysis of responses were all more than 0.9. The chosen models were the best-fit models for the prediction of response at any experimental condition in the experimental domain, as evidenced by the 4.0 difference between the adjusted R-squared value and the predicted R-squared value. The quadratic contour line for Responses 1–8 and

the linear line for Response 9 were displayed in the 2D contour plots. (Figure 3-10).

The following mathematical models were suggested by the software for the prediction of the response for getting desired results for the development of the target RPLC method as per ATP.

Response 1 (T_f of MET) = 1.23+0.0071*B-0.0036*C-0.0048*AB-0.0035*AC-0.0079*C^2

Response 2 (T_f of DAPA) = 1.23+0.0064*B-0.0065*AB -0.0074*C^2

Response 3 (T_f of TNL) = 1.36+0.0960*C+0.1629*AB

Response 4 (TP of MET) = 3422.14+539.98*B-147.25*AB-236.00*AC-169.00*BC+185.85*A^2+124.33*B^2-170.71*C^2

Response 5 (TP of DAPA) = 3751.44+446.04*B-340.88*AB-405.05*C^2

Response 6 (TP of TNL) = 3714.65+192.84*A+516.16*B-199.75*AC-267.94*C^2

Response 7 (R_t of MET) = 4.03+0.1096*A-0.1229*C-0.1419*B^2

Response 8 (R_t of DAPA) = 23.48+0.6707*A-0.5389*C-0.4635*AB-0.4300*C^2

Response 9 (Rt of TNL) = 30.41+0.7812*A+0.0845*B-0.3190*C+0.6114*AC-0.2794*BC+1.09*A^2+0.0173*B^2

3.2 Risk control and mitigation

In order to use MODR for controlled operating of high-risk method parameters for the construction of the target RPLC method in accordance with ATP, the recommended mathematical models for Responses 1–9 were utilized. According to the USP system suitability testing parameters for the RPLC technique, the MODR was guided to obtain the Response I, II, III >1.5, Response IV, V, and VI larger than 2000. In Figure 11, the recommended MODR is indicated in yellow. Responses were measured after the recommended experimental runs were carried out in the lab. When the measured and anticipated responses were compared, it was discovered that the percentage differences were less than 2%. As a result, the chosen models could accurately predict responses in the specified experimental domain.

3.3 Forced degradation study

The evaluation of the stability indicating property of the developed RPLC method under various stress conditions, such as alkaline, acidic, oxidative, thermal, and photolytic, suggested degradation of all pharmaceuticals in various contexts. Under all deterioration situations, MET shows the least amount of degradation. Acidic and hot environments caused the greatest degradation for all drugs (Figure 12-16, Table 4).

3.4 Method Validation

3.3.1 System suitability parameters

To evaluate the system's performance, system suitability studies were carried out. The system suitability investigation was carried out using six replicate injections of the standard solutions of MET, DAPA, and TNL. Peak area, theoretical plate count, tailing factor, resolution between peaks, and retention time were all evaluated.

3.3.2 Method specificity

To ensure that there were no interferences with the peaks of the target molecules, the specificity of the method was assessed. We used this technique to inject the blank, sample combination, and standard into the chromatographic parameters. At the retention time, it was discovered that none of the three analytes interfered (table 5)

3.3.3 Linearity and range

The linearity and range of the method were assessed for MET, DAPA, and TNL from 25% to 175% of the labelled concentration. A significant degree of linearity was indicated by all correlation coefficients being greater than 0.999. The regression equation and correlation coefficient for drugs are shown in Table 5.

3.3.4 Precision

The precision results, including repeatability, interday, and intraday, are shown in Table 5. The % RSD of peak regions for MET, DAPA, and TNL was less than 1, suggesting a high degree of system and technique precision for the developed approach.

3.3.5 Accuracy

Accuracy trials were conducted using the standard addition approach. The standard was added to the sample at three different concentration ranges (80%, 100%, and 120%) for each of the three drugs. The recovery study results, displayed in Table V, demonstrated the accuracy of the process and the lack of interference from the formulation's excipients.

3.3.6 Limit of detection (LOD) and limit of quantitation (LOQ)

For LOD and LOQ we adopted standard deviation method. The LOD and LOQ were separately determined from calibration curve. Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated using following equation:

$LOD = 3.3 \times (\sigma/S)$

$LOQ = 10 \times (\sigma/S)$

were,

σ = Standard deviation of y-intercepts of five calibration curves.

S = Mean slope of five calibration curves.

The results are presented in table 5.

3.3.7 Asay of marketed formulation

To perform assay of marketed formulation (Zeta DM, Glenmark) was used. For MET 99.624 average percent assay was found. 99.933 % and 99.900 % assay were found for DAPA and TNL respectively (table 5)

4 DISCUSSION

The initial chromatographic parameters for the development of a RPLC technique for MET, DAPA, and TNL together with their degradation products were chosen based on the RPLC methods of these medications in various matrices. After analysing the earlier research, we found that C18 was the favoured stationary phase. Early-stage tests also investigated various combinations of mobile phases including water: methanol, acetonitrile: water, phosphate buffer: acetonitrile, and phosphate buffer: methanol in several ratios. When phosphate buffer and methanol are employed in a 75:25 ratio, all drugs are separated with good peak symmetry, theoretical plates, and resolution. A Zorbax C18 (300*4.6 mm*3 µm) column was used to achieve the separation at a detection wavelength of 222.2 nm.

Experimental design is a deliberate approach to planning and defining various parts of experiments in order to reduce unwanted trails and unnecessary time. Experimental design, as opposed to trial and error, provides a systematic means of controlling various analytical procedure factors, producing superior findings while saving time. Recently, this strategy has been effectively used to create intricate analytical techniques involving several variables. In this work, an experimental design technique was used to regulate the mobile phase's pH, flow velocity, and percentage of methanol. The T_f of MET (TF 1), T_f of DAPA (TF 2), T_f of TNL (TF 3), Theoretical plates (TP 1) of peak of MET, TP of DAPA (TP 2), TP of TNL (TP 3), Retention time (R_t) of MET (RT 1), R_t of DAPA (RT 2), R_t of TNL (RT 3). were selected as response factors with 17 experiments. Here, the study marked the effect of variables and efficiency of CCD model. The ANOVA for quadratic model for TF 1 suggested P-value was 0.0009 and F-value was14.59, for TF 2 P value 0.0297, F value 4.52 and for TP 3 it was about 0.0437 and 3.37 respectively. For TP 1 P value was 0.0003 and F value was 21.97 and for TP 2 and 3 it was 0.0142, 5.94 and 0.0032, 9.92 respectively. For retention time of MET P value was 0.0467 and F value was 3.78, for RT 2 0.0317, 4.41 and for RT 3 it was about 0.0489 and 3.71. P value of all responses is less than 0.05 suggesting all models were significant.

A variety of extreme conditions, including acidic, basic, oxidative, thermal, and photolytic, were used to forcefully deteriorate a mixture of standard drugs. The proportion of degradation obtained to varied degrees indicates the stability of therapeutic compounds. Each degradant peak was also well defined. The degradation products that were produced were distinct from the principal peak, and there was no interference during the analyte's retention period. The method's dependability was demonstrated by the mass balance, which was within permitted boundaries. Consequently, the presented technique is robust, particular, and stability-indicating.

The developed method was validated as per ICH recommended guidelines. The method was validated for linearity, specificity, precision, repeatability, accuracy, LOD and LOQ. The method was found to be linear with R2 values for MET 0.9998, for DAPA 0.9992 and for TNL 0.9997. The repeatability showed %RSD of 0.66, 0.89 and 1.12 for MET, DAPA and TNL respectively. Recovery of 99.998 % (MET), 99.978 % (DAPA), 100.030 % (TNL) was found in 100 % spiking of standard. The method showed excellent precision values for interday and interday precision less than 2 % RSD were found. The LOD values for MET, DAPA and TNL are found to be 3.971 µg/ml, 0.652 µg/ml, 0.742 µg/ml. 12.035 µg/ml, 1.976 µg/ml and 2.249 µg/ml are the value found for LOQ of MET, DAPA and TNL respectively.

5. CONCLUSION

An effective, simple, accurate, and focused RPLC method was developed and validated for analysis of tablets comprising Metformin hydrochloride, Dapagliflozin propanediol monohydrate and Teneligliptin hydrobromide hydrate were made using an experimental design method. There are a number of advantages to optimizing the RPLC process through experimental design as opposed to a trial-and-error method, including fewer experiments, cheaper costs, and less use of pricey organic solvents. The new method showed exceptional resolution by effectively separating the main medication components from breakdown products. Additionally, the validation data showed that the method was linear, specific, precise, accurate, and selective. Thus, the recommended method might be used for routine quality control of these drugs in pharmaceutical dose forms.

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7 ABBREVIATIONS USED

RPLC: Reversed Phase High Performance Liquid Chromatography
MET: Metformin hydrochloride
DAPA: Dapagliflozin propanediol monohydrate
TNL: Teneligliptin hydrobromide hydrate
R_t: Retention Time
TP: Theoretical Plates
T_f: Tailing Factor
QBD: Quality by Design
AQbD: Analytical Quality by Design
ATP: Analytical target profile
CMAs: Critical method attributes
OOT: Out of Trend
OOS: Out of Specification

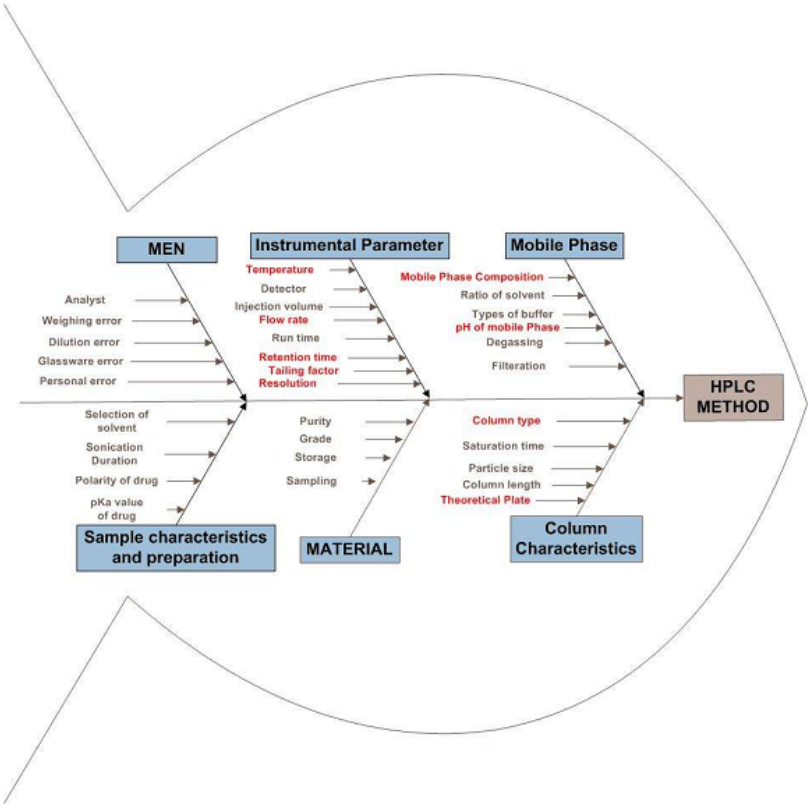

Figure:1 Ishikawa fish- bone diagram indicating the cause and sub-cause relationship(s) of potential process parameters affecting the method outcome(s)

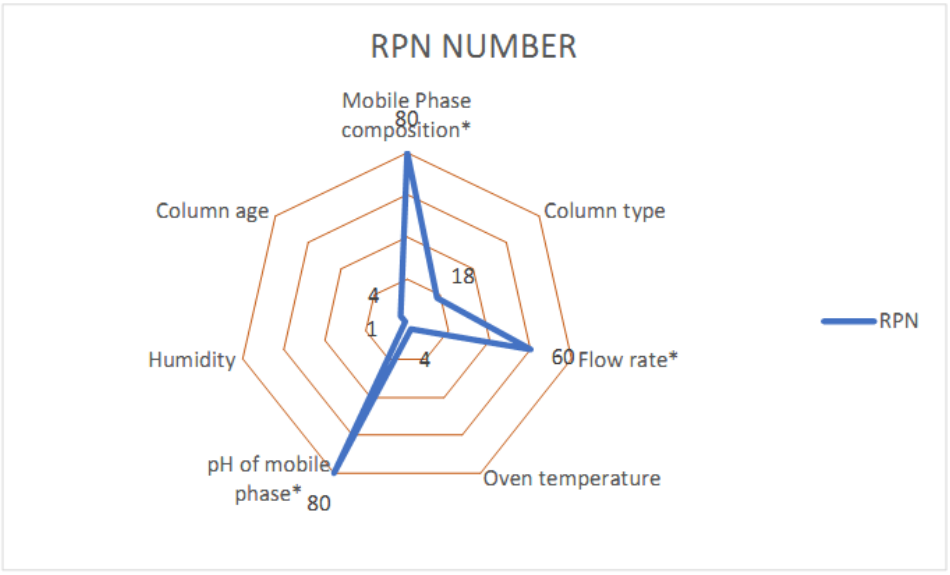

Figure:2 Failure Mode and Effects Analysis (FMEA) with RPN Number

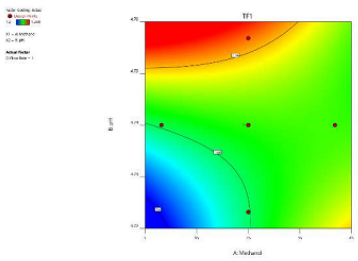

Fig: 3 Contour plot for response 1

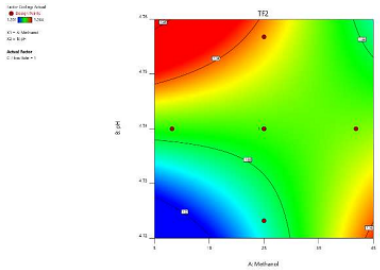

Fig: 4 Contour plot for response 2

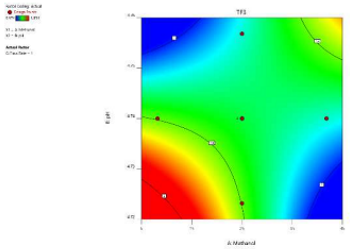

Fig: 5 Contour plot for response 3

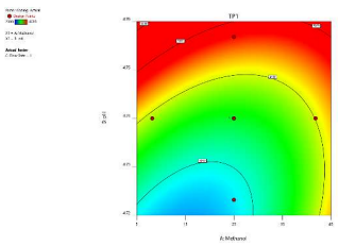

Fig: 6 Contour plot for response 4

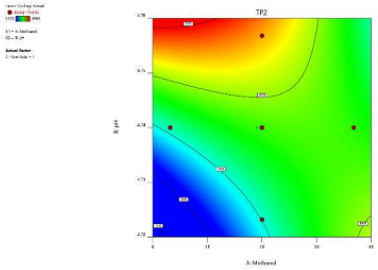

Fig: 7 Contour plot for response 5

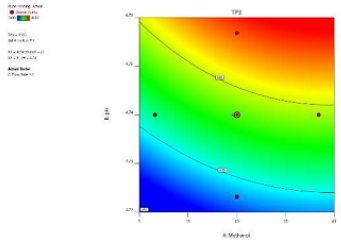

Fig: 8 Contour plot for response 6

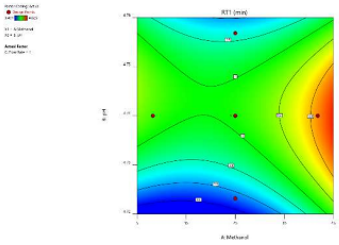

Fig: 9 Contour plot for response 7

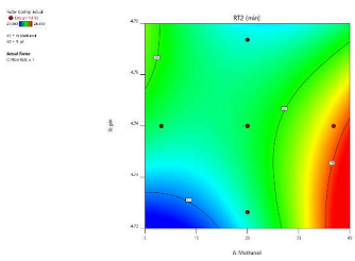

Fig: 10 Contour plot for response 8

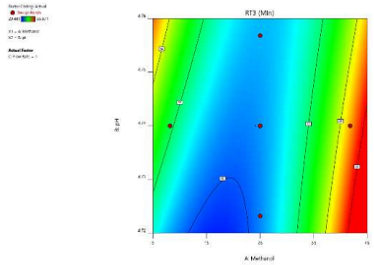

Fig: 11 Contour plot for response 9

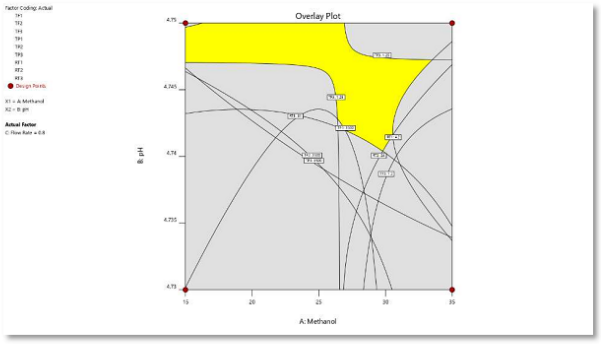

Fig: 12 Design space showing yellow shade desirability region for control of critical failure mode as per ATP.

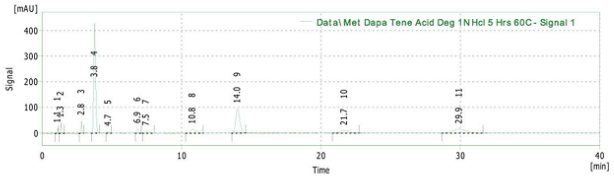

Fig: 13 Chromatogram of Acid Hydrolysis with 1 N HCL and refluxed for 5 hrs. 60 ºC

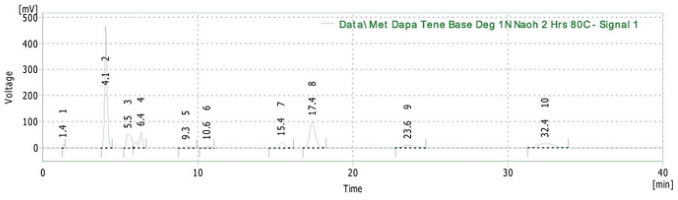

Fig: 14 Chromatogram of Base Hydrolysis with 0.1 N NaOH and refluxed for 2 hrs. 80 ˚C

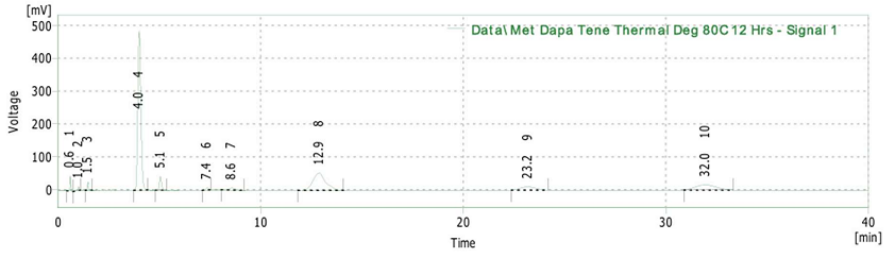

Fig: 15 Chromatogram of thermal stress at 80˚C for 12 hrs.

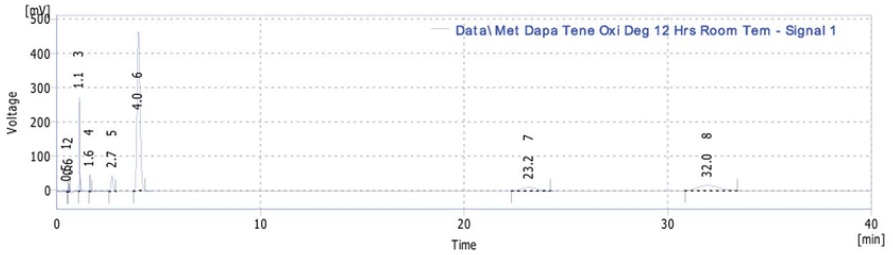

Fig: 16 Chromatogram of oxidative stress at 3 % H_2O_2 for 12 hrs. at Room Temperature

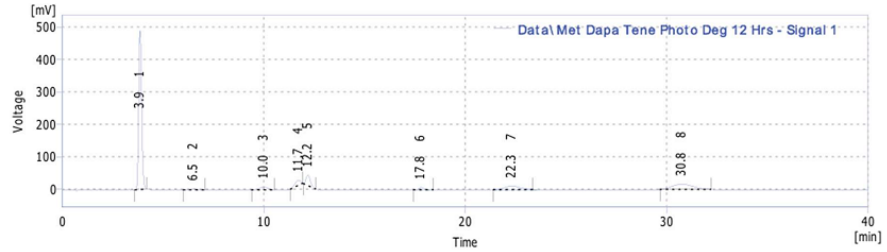

Fig: 17 Chromatogram of photolytic degradation by Exposing UV Light for 12 hrs.

Table 1: Failure Mode and Effects Analysis (FMEA) with RPN Number.

Sr. No	Critical Method Parameter (CMPs)/Critical Process Parameters (CPPs)	Failure Effect	O	S	D	RPN	Strategy
1	Mobile phase composition*	Change in peak symmetry and retention time	5	4	4	80	Optimized by DoE and control
2	Column type	Retention variation	3	2	3	18	Appropriate column should be used as per particle size of analyte
3	Flow rate*	Changes in peak resolution and elute time	5	4	3	60	Optimized by DoE and control
4	Ambient Temperature	Detection and resolution problem	1	2	2	4	Check the stability of drug and store it at suitable temperature.
5	pH of mobile phase*	Retention variation	4	5	4	80	Optimized by DoE and control
6	Humidity	Changes in weighing	1	1	1	1	Standard operating procedure to be followed to dry the sample
7	Column age	Reduce resolution, Peak broadening	1	2	2	4	Monitor Column use

Table 2: Dependant and Independent factor with its level for central composite design

Sr. No	Independent variable	Minimum (-1)	Maximum (+1)	Mean (0)
1	Proportion of Methanol	15	35	25
2	Flow Rate	0.8	1.2	1
3	pH of Mobile Phase	4.73	4.77	4.75
Dependent Factors				
1	Tailing Factor of MET (T_f 1)			
2	Tailing Factor of DAPA (T_f 2)			
3	Tailing Factor of TNL (T_f 3)			
4	Theoritical Plate of MET (TP 1)			
5	Theoritical Plate of DAPA (TP 2)			
6	Theoritical Plate of TNL (TP 3)			
7	Retention Time of MET (R_t 1)			
8	Retention Time of DAPA (R_t 2)			
9	Retention Time of TNL (R_t 3)			

Table 3: Design Metrics with Measured Responses for Response Surface Analysis of Critical Method Parameters (CQA) and Critical Process Attributes (CPAs) by DoE-Based Central Composite Design (CCD)

Run	Factor 1	Factor 2	Factor 3	Response 1 T_f1	Response 2 T_f2	Response 3 T_f3	Response 4 TP 1	Response 5 TP 2	Response 6 TP 3	Response 7 R_t1	Response 8 R_t2	Response 9 R_t3
1	35	4.73	1.2	1.212	1.218	1.517	2898	3100	2900	3.953	22.847	33.642
2	25	4.74	1	1.228	1.222	1.325	3399	3699	3792	4.101	23.42	30.567
3	25	4.74	1	1.222	1.226	1.326	3400	3744	3535	3.977	23.33	30.265
4	25	4.75682	1	1.249	1.244	1.24	4689	4988	4511	3.945	23.552	31.411
5	25	4.74	0.663641	1.21	1.212	1.316	3120	3165	3059	4.221	23.128	31.865
6	15	4.75	0.8	1.228	1.218	1.185	4215	3415	3535	4.133	23.128	31.865
7	15	4.73	1.2	1.207	1.201	1.654	3100	2356	2541	3.697	21.369	29.441
8	35	4.75	1.2	1.215	1.212	1.624	3548	3205	3606	3.875	22.397	30.858
9	15	4.73	0.8	1.207	1.204	1.522	2300	2235	2403	3.982	23.012	31.705
10	35	4.75	0.8	1.228	1.224	1.337	4368	3542	4680	3.993	23.076	31.794
11	25	4.74	1.33636	1.2	1.201	1.302	3000	2300	3000	3.826	22.115	30.469
12	41.8179	4.74	1	1.228	1.224	0.987	4125	3564	3716	4.625	26.359	35.871
13	25	4.74	1	1.229	1.227	1.329	3426	3785	3792	4.012	23.56	30.268
14	8.18207	4.74	1	1.222	1.232	1.321	4012	3411	3570	3.997	23.21	31.828
15	15	4.75	1.2	1.226	1.23	1.358	4125	3652	3570	3.689	22.267	30.679
16	25	4.72318	1	1.216	1.212	1.658	3100	2689	2874	3.401	22.987	30.215
17	35	4.73	0.8	1.229	1.227	0.974	3256	3898	3251	4.12	25.32	31.265

Table 4: Results of stress degradation of MET, DAPA and TNL

Stress Condition										
Drug	**Acid Hydrolysis (1 N HCL 5 hrs. 60 ˚C)**		**Base Hydrolysis (1 N NaOH 2 hrs. 60˚C)**		**Thermal Degradation (80˚ C for 12 hrs.)**		**Oxidative Degradation (3 % H2O2 for 12 hrs. at Room Temperature)**		**Photolytic Degradation (Exposure of UV Light for 12 hrs.)**	
	% Drug Assay	**% Degradation**	**% Drug Assay**	**% Degradation**	**% Drug Assay**	**% Degradation**	**% Drug Assay**	**% Degradation**	**% Drug Assay**	**% Degradation**
MET	93.176	6.824	95.031	4.969	92.419	7.581	94.711	5.289	97.440	2.560
Mass Balance	100		100		100		100		100	
DAPA	87.333	12.667	87.473	12.527	85.259	14.741	85.366	14.634	93.793	6.207
Mass Balance	100		100		100		100		100	
TNL	85.167	14.833	91.693	8.307	87.952	12.048	87.480	12.520	95.501	4.499
Mass Balance	100		100		100		100		100	

Table 5: Summary of validation parameter

Parameters		**MET**	**DAPA**	**TNL**
System Suitability Parameters	**Theoretical Plates (Mean±SD, % RSD)**	3385.40±9.476, 0.280	3576±18.900, 0.528	3898±51.703, 1.326
	Tailing Factor	1.28±0.012, 0.923	1.00±0.06, 0.458	1.00±0.009, 0.896
	Resolution	21.31±0.295, 1.385	5.00 ± 0.029, 0.594	
Specificity	No interference- Specific			
Linearity and Range		250-750 µg/ml	5-15 µg/ml	10-30 µg/ml
Correlation co-efficient (R^2)		0.9998	0.9992	0.9997
Precision (%RSD)	**Repeatability**	0.66	0.89	1.12
	Intraday precision	0.01-0.02	0.36-0.21	0.19-0.08
	Interday precision	0.54-0.04	0.20-0.22	0.13-0.75

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Accuracy (%recovery)	**80%**	99.938 ± 0.058	99.910 ± 0.155	100.078 ± 0.079
	100%	99.998 ± 0.009	99.978 ± 0.131	100.030 ± 0.075
	120%	99.996 ± 0.004	100.075 ± 0.067	99.998 ± 0.018
Limit of detection		3.971 µg/ml	0.652 µg/ml	0.742 µg/ml
Limit of quantitation		12.035 µg/ml	1.976 µg/ml	2.249 µg/ml
% Assay		99.624 ± 0.567	99.933 ± 0.160	99.900 ± 0.043