RESEARCH ARTICLE

Stability Indicating Method Development of Metformin, Teneligliptin and Pioglitazonein Marketed Formulation by HPLC

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ABSTRACT

Expansion and validation of a stability-indicating HPLC approach allowed one to simultaneously test metformin (MET), teneligliptin (TNG), and pioglitazone (PGL) in their commercially available pharmaceutical forms. Techniques were developed to detect active pharmaceutical ingredients (APIs) and any degradation byproducts in settings either acidic, alkaline, oxidative, or photolytic. To evaluate the stability of MET, TNG, and PGL and make sure the technique could extract the medications from their breakdown products, forced degradation studies were conceded out. The ICH guiding principle followed in validation of method. The outcomes demonstrated that the developed method could accurately and sensitively identify and measure the drugs and the products of their degradation.

Under stress, formed degradation products were excellently isolated and measured; therefore, method was found to be robust in several conditions. This stability-indicating HPLC methodology useful for repetitive quality control and stability analysis of MET, TNG, and PGL in pharmaceutical formulations.

Keywords: Metformin, Teneligliptin, Pioglitazone, Pharmaceutical formulations, Forced degradation, Validation.

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INTRODUCTION

For pharmaceutical compounds to be harmless, effective, and have a extensive shelf life, stability-indicating methodsnecessity be developed.[1]A validated analytical method that constantly separates API from its breakdown products in stressful circumstances is known as a stability-indicating technique.[2] These techniques are fundamental for evaluating stability of medications, tracing their deterioration, and promising that medication holds its effectiveness and safety over course of its shelf life in context of quality control and regulatory compliance.[3]Pioglitazone (PGL), metformin (MET), and teneligliptin (TNG) are often prescribed medicines for controlling of type 2 diabetes mellitus.[4]

Teneligliptin is a DPP-4 inhibitor, Pioglitazone is a thiazolidinedione, and MET is a biguanide mainly used for glycemic control[5,6]. Even though fact that these medicines have confirmedbeneficialefficiency, a number of variables, with temperature, dampness, light, and pH, can disturb how stable they are in pharmaceutical formulations, which may effect in degradation and production of hazardous byproducts.[7,8]

Thus, it is crucial to generate analytical methods that can recognizeAPI as well as byproducts of their breakdown. One of the most prevalent methods for investigating pharmaceutical compounds is HPLC, which is prominent for its high resolution, accuracy, and capacity to distinct intricate drug mixtures and degradation products.[9]

HPLC is a perfect method for stability-indicating studies as it can be used to ascertain and quantify API as well as products of their degradation.[10]stability of MET, TNG, and PGL under several stress circumstances has been the subject of many investigations. A stability-indicating HPLC method was developed to assess the hydrolytic, oxidative, and photolytic stability of metformin and sitagliptin phosphate.[11]

Similarly, a forced degradation study involving TNG was conducted to evaluate its stability under acidic, alkaline, oxidative, and thermal stress conditions.An HPLC method was produced to explore stability and degradation of glimepiride and metformin HCI in pharmaceutical formulations, under several circumstances[12].

Stability testing has been subject to stricter regulatory requirements in recent years. Guidelines from the International Conference on Harmonization (ICH) place a strong emphasis on the necessity of testing pharmaceutical products for stability, including the creation of stability-indicating techniques that can precisely determine how environmental influences affect drug formulations.[13] Stability studies are necessary to guarantee that the medication stays within predetermined bounds for the duration of its shelf life and that any degradation products produced do not surpass permissible limits, as per ICH Q1A(R2)[14]guiding principle.

Making a stability-indicating HPLC method that can assess PGL, TNG, and MET in their commercial formulations all at once is the goal of this research. The

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Table 1: Separation conditions

Variable	Condition
Column	
Particle Size	5µ
Bonded Phase	Octadecylsilane (C_{18})
Dimension	250mm x 4.60mm
Mobile Phase	
Acetonitrile	85
15mM KH_2PO_4	15
Flow rate	1.0 ml/min
Diluent	Acetonitrile
Temperature	Ambient
Sample Size	20 µl
Detection wavelength	254mm
Retention time	
Metformin	4.125 ± 0.3min
Teneligliptin	6.226 ± 0.3min
Pioglitazone	8.315 ± 0.3min

Table 2: Outcomes of linearity of Metformin, Teneligliptin and Pioglitazone

Parameter	MET	TNG	PGL
Concentration (µg/ml)	2-10	0.1-0.5	0.2-1.0
Slope (m)*	107.3	7172	4443
Correlation Coefficient (r^2)*	0.999	0.999	0.999
Intercept (c)*	- 48.19	19.167	54.063

*Significance of three repeat

Table 3: Outcomes of recovery study

% Level	% MEAN±SD*		
	MET	TNG	PGL
80%	99.24±0.481	97.40±0.981	97.85±1.213
100%	98.53±0.598	96.01±1.811	97.19±0.973
120%	99.15±0.284	97.75±0.902	98.51±0.760

*Significance of 3repeat and 5 concentrations.

method will undergo validation according to ICH criteria for factors such as specificity, linearity, accuracy, precision, in addition to robustness. Forced degradation experiments in oxidative, photolytic, alkaline, and acidic settings will also be used to evaluate stability of drugs.

MATERIAL AND METHODS

Material

In the study, KH_2PO_4 (Himedia) and Acetonitrile (Thomas Baker, HPLC grade) were used to prepare the buffer and mobile phase for HPLC analysis. OPA (Himedia, Analytical grade) was employed for derivatization. HPLC-grade water was utilized to avoid contamination. The pharmaceutical compounds Metformin, Teneligliptin, and Pioglitazone were received as gift samples from a pharmaceutical company and were used for method development in HPLC analysis.

Methods

Mobile phase Selection

In order to determine the MET, TNG and PGL were first investigated in a fixed dosage form with various mobile phases in varying ratios. The ideal mobile phase for analysis was found to be a 15:85v/v mixture of 15mM KH2PO4 and acetonitrile (pH 4 with OPA), after taking system suitability factors including RT, Tailing factor, number of theoretical plates, and HETP into account. A 0.45 m filter paper was used to remove particulate particles from the mobile phase before sonication was used to degas it. At 1.0 ml/min, the flow rate for the analysis was set.[15]

Diluent Selection

Acetonitrile was selected as the diluent following many testing, which demonstrated that the analyte's retention and resolution were unaffected by the sample preparation diluent and that it was also compatible with the mobile phase.[16]

Selection of separation variable

The separation variables are selected as per Table 1.

Preparation of standard stock solution, sub stock and working standards

The following steps were taken: 10 milligrammes of Metformin (MET), 5 millilitres of methanol, and 10 minutes of sonication after each drug was shifted to a 10 millilitre volumetric flask: dissolving Teligliptin (TNG), pioglitazone (PGL), and Metformin (MET). The stock solution (Stock-A) had 1000 µg/ml of each medication after the volume was reduced to 10 ml using acetonitrile. A concentration of 100 µg/ml was attained by diluting 1 ml of Stock-A with 10 ml of acetonitrile before being conveyed to separate 10 ml volumetric flasks for manufacture of the sub-stock solution (Stock-B). To make altered concentrations, 0.2, 0.4, 0.6, 0.8, and 1.0 ml of Stock-B were each put into a 10 ml volumetric flask and diluted by acetonitrile until final concentrations were 2 for MET, 4, 6, 8, and 10 µg/ml for acetonitrile. For TNG, the concentrations were 0.1, 0.2, 0.3, 0.4, and 0.5 µg/ml, and for PGL, they were 0.2, 0.4, 0.6, 0.8, and 1 µg/ml.[17]

Linearity and Calibration Graph

We created a range of dilutions for MET, TNG, and PGL to show that the analytical procedure was linear, with MET ranging from 2-10 µg/ml, TNG from 0.1-5 µg/ml, and PGL from 0.2-1 µg/ml. Chromatograms were taken at 270 nm following three injections and complete filtering of the solution through a 0.2 nm membrane filter. By creating a calibration graph that connected the concentration to the mean peak area, we were able to derive the regression equation.

System suitability parameters

The column was filled with mobile phase at 1 mL/min separation parameters were established. After the column was fully saturated, the working standard of 10 mg/ml MET, 0.5 mg/ml TNG, and 1.0 mg/ml PGL were each injected independently. Both the peak and column performance reports were included with each chromatogram.

Validation of HPLC method development

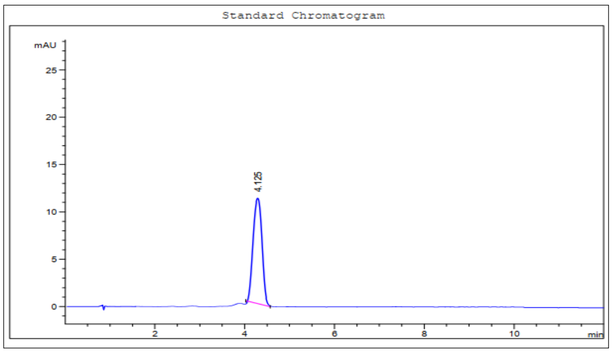

Figure 1: MET Chromatogram

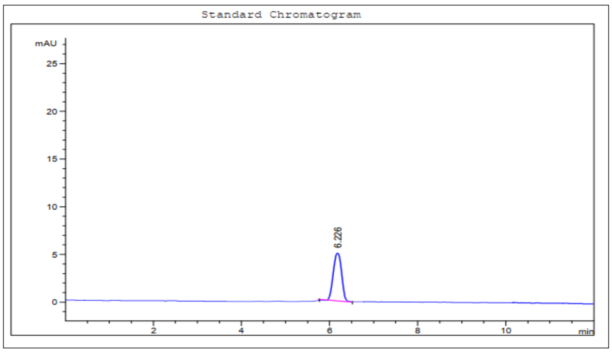

Figure 2: TNG Chromatogram

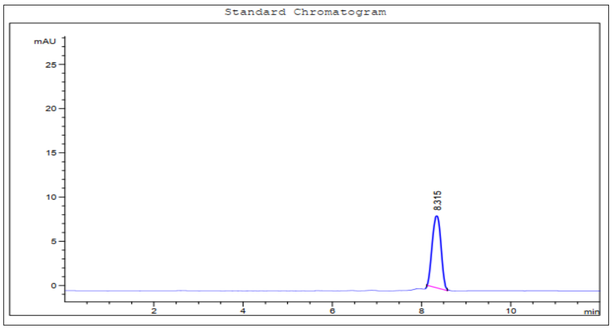

Figure 3: PGL Chromatogram

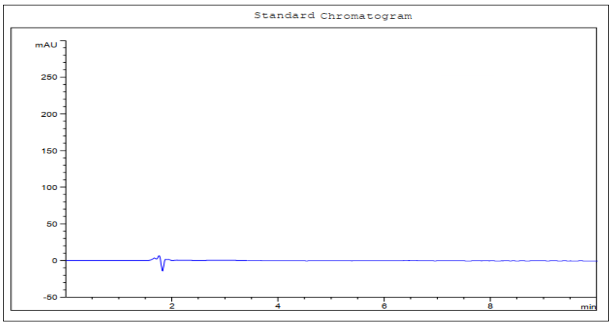

Figure 4: Blank Chromatogram

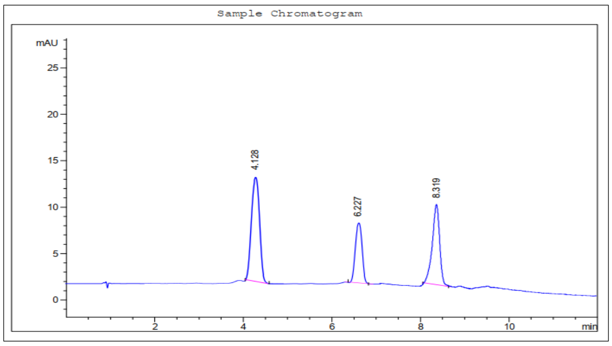

Figure 5: MET, TNG, PGL Chromatogram

Linearity

Drug response ratios were used to determine the linearity of both medications. The drug's response ratio is determined by dividing absorbance by conforming concentration. Relationship between concentration and response ratio was then plotted on a graph[18].

Specificity

The specificity of the approach was examined in order to identify analyte in existence of potential component

Accuracy

Recovery studies evaluated the suggested methods' accuracy at three different levels: 80%, 100%, and 120%. In order to conduct the recovery studies, preanalyzed tablet powder was mixed with a known quantity of standard MET, TNG, and PGL solution. The suggested techniques were then used to re-analyze the final solutions. To determine recovery of additional drug sample, entire examination process was repetitive. Three replicates of the five concentration levels were used to repeat this recovery analysis.

Precision

Repeatability, intermediate precision (amongst analysts and from day to day), and reproducibility were the three tiers of method precision that were investigated. Reproducibility was attained by conducting five separate analyses of the identical drug concentration. Five distinct drug concentrations were analyzed every day for three days of the week.

Detection Limit and Quantitation Limit

Slope of linearity curve and response SD were used to compute LOD and LOQ of developed technique.

Analysis of tablets formulation

A dosage of 10 mg of MET, which is equal to 2 mg of PGL and 1.5 mg of TNG, was administered using a 10 ml volumetric flask following the weighing and milling of the tablet powder into a fine powder. After adding 5 ml of acetonitrile to the flask, the medication in the tablet powder was dissolved by subjecting it to sonication for approximately 10 minutes. This was followed by the addition of acetonitrile to achieve the desired volume. A 0.45µ membrane filter was used to pass the filtrate through after the sonication process. The final medicine concentrations were brought within the working range by further diluting the filtrate with methanol after collection. To find the concentrations, we used the calibration curve approach and averaged the areas of the final dilutions. The procedure underwent five rounds of refinement.

Forced degradation studies

The method's stability signifying probable was tested by subjecting drug powder to forced degradation trials. An HPLC system furnishedby a UV detector was utilised for investigation. There was an injection of 20 µl for every forced degradation sample.

Acid degradation

The three medications were combined with 50 ml of 0.1 M HCl solution in a 50 ml round-bottom flask. For eight

Table 4: Outcomes of precision

Parameter	% MEAN±SD*		
	MET	TNG	PGL
Repeatability	99.33±0.029	95.73±0.009	95.95±0.013
Intermediate precision			
Analyst-to-Analyst	98.96±0.026	96.11±0.005	96.73±0.007
Day to day precision	99.35±0.017	96.11±0.009	96.04±0.010
Reproducibility	99.34±0.015	95.66±0.006	96.93±0.010

* Value of five replicate and five concentrations

Table 5: LOD and LOQ of MET, TNG and PGL

Name	LOQ (µg/ml)	LOD (µg/ml)
MET	1.50	0.50
TNG	0.09	0.03
PGL	0.48	0.15

hours at 80ºC, all of the components were mixed together while stirring continuously. After removal, samples were diluted to a concentration of 10 µg/ml before being analysed through HPLC. The percentage of deterioration was calculated using the drug calibration curve.

Alkaline hydrolysis

Fifty millilitres of 0.1 M NaOH solution in 50 ml round-bottom flask that already contained ten milligrammes of each medicine. After combining all of the ingredients, they were left to stir continuously at 80ºC for eight hours. We used the drug calibration curve to determine the percentage of degradation after extracting and diluting the samples to 10 µg/ml, and then we ran them via HPLC.

Oxidative degradation

Each of the three drugs was added to a 50 ml round-bottom flask along with 50 ml of a 3% hydrogen peroxide solution. Ingredients were combined well and swirled continuously for 24 hours at room temperature. Drug calibration curve was used to calculate % of degradation after samples were diluted to 10µg/ml and then exposed to HPLC.

Thermal degradation

Ten milligrammes of each drug was cooked at 50 degrees Celsius in a petri dish for four weeks. After diluting the samples to 10µg/ml and exposing them to HPLC, the drug calibration curve was utilised to determine the degradation percentage [19].

RESULTS AND DISCUSSION

Linearity of method was evaluated by constructing calibration curves for MET, TNG, and PGL (Table 2). For all three drugs, correlation coefficients (r^2) were 0.999,representative an admirable linear relationship among concentration and response within respective tested concentration ranges. The concentration ranges for MET (2-10 µg/ml), TNG (0.1-0.5 µg/ml), and PGL (0.2-1.0 µg/ml) were selected based on their typical pharmaceutical dosages. The slopes for MET, TNG, and PGL were 107.3, 7172, and 4443, respectively. The high slope for TNG indicates that the method is more sensitive for TNG compared to MET and PGL. Theintercepts for MET (-48.19), TNG (19.167), and PGL (54.063) are close to zero, further confirming accuracy and precision of technique (Figure 1, Figure 2).

Recovery studies were achieved at three changed spiking levels: 80%, 100%, and 120% (Table 3). The recovery percentages for MET, TNG, and PGL ranged from 97% to 99%, demonstrating that the method is highly accurate. At the 80% level, MET showed a recovery of 99.24%, TNG had 97.40%, and PGL showed 97.85%. Similarly, at the 100% level, MET recovered 98.53%, TNG 96.01%, and PGL 97.19%, and at the 120% level, MET recovered 99.15%, TNG 97.75%,and PGL 98.51%. The low standard deviations (SD) at all levels further confirm the precision and dependability of method.

Precision was evaluated finished intermediate precision, repeatability, and reproducibility (Table 4). For repeatability, the % recovery for MET, TNG, and PGL was 99.33%, 95.73%, and 95.95%, respectively, with very low SD values, indicating high precision. The intermediate precision data showed that day-to-day variations were also minimal, with MET, TNG, and PGL showing recovery values of 99.35%, 96.11%, and 96.04%, respectively. For the analyst-to-analyst variation, the recovery values were 98.96% for MET, 96.11% for TNG, and 96.73% for PGL. The overall reproducibility results were similarly consistent, supporting the robustness of technique for routine examination.

Table 6: Assay of tablets formulation

Conc. Present (µg/ml)			Replicate-1					
			Conc. Found (µg/ml)			% Conc. Found		
MET	TNG	PGL	MET	TNG	PGL	MET	TNG	PGL
10	0.15	0.2	9.95	0.14	0.2	99.50	93.3	100.00
10	0.15	0.2	9.98	0.15	0.19	99.80	100.0	95.00
10	0.15	0.2	9.96	0.15	0.18	99.60	100.0	90.00
10	0.15	0.2	9.95	0.14	0.19	99.50	93.3	95.00
10	0.15	0.2	9.96	0.14	0.2	99.60	93.3	100.00
					MEAN	99.60	96.00	96.00

Table 7: Outcomes of forced degradation studies of MET

Stress conditions	MET		TNG		PGL	
	Drug recovered (%)	Drug decomposed (%)	Drug recovered (%)	Drug decomposed (%)	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.90	0	99.85	0	99.45	0
Alkaline hydrolysis	92.28	7.62	89.98	9.87	96.65	2.8
Acidic hydrolysis	86.65	13.25	92.23	7.62	89.95	9.5
Oxidative degradation	94.45	5.45	94.47	5.38	94.88	4.57
Photolytic degradation	92.32	7.58	96.65	3.2	86.65	12.8

The method demonstrated high sensitivity for all three drugs. LOD of 0.50 µg/ml was recorded for MET, 0.03 µg/ml for TNG, and 0.15 µg/ml for PGL. LOD and LOQ values were 1.50 µg/ml for MET, 0.09 µg/ml for TNG, and 0.48 µg/ml for PGL, suggesting that the approach can detect and quantify extremely low amounts, especially for TNG. (Table 5).

The assay of tablet formulations showed that the method can accurately quantify the active ingredients in pharmaceutical products (Table 6). For MET, TNG, and PGL in tablet formulations, the mean recovery values were 99.60%,96.00%, and 96.00%,correspondingly. These results indicate that formulation contains the expected amount of active pharmaceutical ingredients, with minimal deviation. The individual results for each replicate showed slight variations around the mean, suggesting that technique is consistent and consistent for routine quality control of pharmaceutical tablets (Figure 3, Figure 4).

Forced degradation were showed to assess stability of MET, TNG, and PGL under various stress conditions (Figure 5). Outcomespresented that MET had least degradation, by99.90%recovery under standard conditions and 13.25% degradation under acidic hydrolysis (Table 7). TNG also showed good stability, with 99.85% recovery under standard conditions and 7.62%degradation under acidic hydrolysis (Table 7). PGL showed 99.45% recovery under standard conditions, but it was more sensitive to photolytic degradation, with 12.8% degradation (Table 7). For all three drugs, the degradation rates were relatively low under oxidative and alkaline conditions, suggesting that the method can be used to assess the stability of these drugs under various conditions[20].

CONCLUSION

In conclusion, developed technique is robust, accurate, and precise, creating it appropriate for the examination of MET, TNG, and PGL in both pure and formulated forms. The linearity, recovery, precision, and stability data support the method's effectiveness for repetitive quality control and stability testing. High sensitivity, as shown by the low LOD and LOQ values, ensures that even trace amounts of these drugs can be reliably quantified. Forced degradation studies offer valuable insight into stability profiles of drugs under various stress conditions, which is essential for their shelf-life prediction and regulatory compliance.

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