International Journal of Pharmaceutics and Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DAPAGLIFLOZIN AND SITAGLIPTIN IN MARKETED FORMULATION

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***Article History:**

Received: 14/08/2023
Revised: 29/08/2023
Accepted: 02/09/2023

ABSTRACT

This research aims to develop a stability-indicating analytical method for the accurate estimation of Dapagliflozin and Sitagliptin in marketed pharmaceutical formulations. Dapagliflozin and Sitagliptin are commonly used drugs for the management of Type 2 diabetes. The developed method involves high-performance liquid chromatography (HPLC) as the primary analytical technique, coupled with a suitable detector. Various chromatographic conditions, such as column selection, mobile phase composition, and detection wavelength, were optimized to separate and quantify Dapagliflozin and Sitagliptin efficiently. Validation parameters, including linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and robustness, were assessed according to International Conference on Harmonisation (ICH) guidelines. The method exhibited a wide linear range, good precision, and accuracy, meeting the requirements for routine pharmaceutical analysis. The developed stability-indicating method was successfully applied to the analysis of marketed formulations containing Dapagliflozin and Sitagliptin. The results demonstrated the method's reliability for quantifying the active ingredients in these formulations. In conclusion, the presented stability-indicating HPLC method offers a reliable and efficient means of analyzing Dapagliflozin and Sitagliptin in marketed formulations. This method can be employed for routine quality control analysis, ensuring the stability and potency of these essential drugs for diabetic patients.

Keywords: Dapagliflozin, Sitagliptin, Stability-indicating method, HPLC, Forced degradation, Marketed formulation, Pharmaceutical analysis.

INTRODUCTION

Dapagliflozin and Sitagliptin are two widely used pharmaceutical agents in the management of type 2 diabetes mellitus. Dapagliflozin is a sodium-glucose cotransporter 2 (SGLT2) inhibitor that works by reducing renal glucose reabsorption, thereby lowering blood glucose levels, while Sitagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor that enhances the action of incretin hormones, which regulate blood sugar (Bailey, 2010). Both of these drugs have been found to be effective in controlling hyperglycemia and improving glycemic control in diabetic patients (Kim *et al.,* 2013).

In the pharmaceutical industry, it is of utmost importance to develop reliable and robust analytical methods for the quantification of active pharmaceutical ingredients (APIs) in marketed formulations. The development of a stability-indicating method is essential because it enables the determination of the drug's stability and quality over time, as well as its compatibility with excipients and packaging materials. Stability-indicating methods ensure that the analytical method can effectively separate and quantify the drug substance from its degradation products (ICH; 2003).

Dapagliflozin is an oral anti-diabetic drug used in combination with diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus. It is an inhibitor of the sodium-glucose co-transporter 2 (SGLT2) which helps the kidneys to remove excess glucose from the body via the urine. Dapagliflozin is also approved for reducing the risk of hospitalization for heart failure and for reducing the risk of progression of kidney disease in adults with type 2 diabetes mellitus and moderately decreased kidney function (USP, 2018).

Sitagliptin is a prescription medication that is used to help lower blood sugar levels in people with type 2 diabetes. It belongs to a class of drugs called dipeptidyl peptidase-4 (DPP-4) inhibitors, which work to reduce the amount of sugar released from your liver, and help your body use insulin more effectively. Aim of the study is Stability indicating method development for the estimation of Dapagliflozin and Sitagliptin in marketed formulation.

MATERIALS & METHODS

Selection of mobile phase

Initially to estimate Dapagliflozin and Sitagliptin in fix dosage form number of mobile phase in different ratio were tried (Panchal *et al.,* 2014).

Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Acetonitrile: Methanol in the ratio of 50:50v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Preparation of stock solution

Accurately weighed 10 mg API of DPG and STG was transferred into 10 ml volumetric flask separately and added 5ml of methanol as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A).

Preparation of sub stock solution

5ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50 ml with diluent (methanol) to give concentration of 100µg/ml of DPG and STG respectively (Stock-B).

Preparation of different solution

0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (methanol). This gives the solutions of 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml

and 5µg/ml, for DPG. In same manner 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml of STG also prepared.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 1-5 µg/ml for DPG and 10-50µg/ml for STG were prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded at 254 nm and it was repeat for five times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

Validation of developed Method

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 1 to 5µg/ ml for DPG) and (10 to 50µg/ ml for (STG) and areas for each concentration were recorded three times and mean area was calculated ICH (1996).

The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure. The response ratio (response factor) was found by dividing the AUC with respective concentration.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components.

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed ICH (2006).

Precision

The stock solution was prepared. The precision are established in three differences:

Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 1, 2, 3, 4 and 5µg/ml for DPG and 5, 10, 15, 20 and 25µg/ml for STG indicates the precision under the same operating condition over short interval time.

Intermediate Precision

Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations.

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, Acetonitrile: Methanol (50:50 % v/v) to (45:55 % v/v).

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve ICH (2006).

Analysis of both the drug in tablet sample

Twenty tablets were accurately weighed and their mean weight was determined. The tablets were grinded to fine powder, an accurately weighed quantity of powder equivalent to 5mg of DPG and 50mg of STG was transferred to 10 ml volumetric flask containing methanol. The solution was sonicated for 25 min and the final volume was made with mobile phase. The mixture was then filtered through a 0.45 µm filter. The stock solution was further diluted sufficiently with methanol to get sample solution of drug concentration of 1µg/mL DPG and 5µg/mL STG respectively. The amounts of DPG and STG in tablets formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with formulation.

Forced degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were conducted on drug powder and the analysis was carried out by HPLC with a U.V. detector. 20µl of each of forced degradation samples were injected (Nagappan *et al.,* 2017).

Acid degradation:

50 mg of both the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 N HCl solutions was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

Alkaline hydrolysis:

50 mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

Oxidative degradation:

50 mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

Thermal degradation:

50 mg of the drug sample was taken in to a petri dish and kept in oven at 50ºC for 4 weeks. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

RESULTS AND DISCUSSION

Table 1: Results of linearity of Dapagliflozin and Sitagliptin

Parameter	DPG	STG
Beer's law limit (µg/ml)	1-5	10-50
Correlation Coefficient (r^2)*	0.999	0.999
Slope (m)*	141.2	28.55
Intercept (c)*	4.420	3.807

*Average of five determination

Table 2: Results of recovery studies on Marketed Formulations

Recovery Level %	% Recovery (Mean±SD)*	
	DPG	STG
80	98.94	98.75
100	98.09	99.64
120	99.18	99.87

Method: simultaneous equation method

Table 3: Results of validation (%R.S.D.)

Parameter		Method	
		DPG	STG
Precision (%R.S.D.)*	Repeatability	0.050	0.124
	Intra-day Precision	0.047	0.046
	Analyst to Analyst variations	0.032	0.047
	Robustness	0.051	1.353

*Average of five determination

Table 4: LOD and LOQ of DPG and STG

Name	LOD (µg/ml)	LOQ (µg/ml)
DPG	0.10	0.25
STG	0.50	1.55

Table 5: Result of assay of tablet formulation

	DPG*	STG*
Label Claim (mg)	**5mg**	**50mg**
% Found (mg)	4.95	49.85
% Assay	99.00	99.70
% RSD	0.125	0.221

*Average of three determination

CONCLUSION

The development of a stability-indicating method for the estimation of Dapagliflozin and Sitagliptin in marketed pharmaceutical formulations is a critical step in ensuring the quality, safety, and efficacy of these drugs for the management of Type 2 diabetes. This research successfully established a robust analytical method using high-performance liquid chromatography (HPLC) that can effectively separate and quantify Dapagliflozin and Sitagliptin in the presence of their degradation products.

The forced degradation studies provided strong evidence of the method's ability to distinguish between the active pharmaceutical ingredients and their degradation products under various stress conditions. This confirms the specificity of the method, a crucial characteristic for stability-indicating assays in pharmaceutical analysis. The validation parameters, in accordance with International Conference on Harmonisation (ICH) guidelines, demonstrate the method's reliability and suitability for routine pharmaceutical analysis. The method exhibited an extensive linear range, ensuring accurate quantification over a wide concentration range. Precision and accuracy were within acceptable limits, validating the method's reproducibility and reliability. The limits of detection (LOD) and quantification (LOQ) were established, allowing for the sensitive detection and quantification of trace amounts of Dapagliflozin and Sitagliptin.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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