Stability Indicating Rp-Hplc for Method Development and Validation for Simultaneous Estimation of Empagliflozin and Nateglinide in Bulk Drug

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ABSTRACT

A simple, accurate, and precise stability-indicating RP-HPLC method was developed and validated for the simultaneous estimation of Empagliflozin and Nateglinide in bulk drug form. Chromatographic separation was performed on a Shim-pack C-18 column (250 × 4.6 mm, 5 µm) using a mobile phase of Acetonitrile: Water (90:10, v/v) adjusted to pH 3. The UV detection wavelength was set at 216 nm, the flow rate was maintained at 1 mL/min, and the injection volume was 20 µL. The retention times for Empagliflozin and Nateglinide were found to be 2.770 and 3.682 minutes, respectively. This method was validated according to ICH Q2(R1) guidelines for linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness. A linear response was observed in the concentration range of 5–25 µg/mL for Empagliflozin and 10–50 µg/mL for Nateglinide. The LOD and LOQ for Empagliflozin were found to be 14.0502 µg/mL and 42.576 µg/mL, respectively, while for Nateglinide, they were 6.5398 µg/mL and 19.8178 µg/mL. Stress studies were performed in accordance with ICH Q1A(R2) guidelines. The drugs were subjected to stress conditions including photolytic, oxidative, thermal degradation, and acid/base hydrolysis to develop a stability-indicating method. The degraded products were effectively extracted and analyzed from the sample.

Keywords: Empagliflozin, RP-HPLC, Method validation, Stability indicating method, Nateglinide.

1. INTRODUCTION

Diabetes Mellitus (DM) is a chronic disease that affects protein, lipid and carbohydrate metabolism [1]. Diabetes Mellitus is characterized by a poor or inadequate insulin secretory response, causing hyperglycemia as well as inadequate utilization of carbohydrates (glucose). Diabetes Mellitus (DM), sometimes called "diabetes", is a common endocrine disease [2]. It is usually caused by a deficiency or deficiency of insulin or, in some cases, by a bad product of insulin (insulin resistance) [3]. Diabetes Mellitus (DM) is a disease that causes abnormal blood sugar levels, which is the main cause of prolonged hyperglycemic state in addition to recurrence of hypoglycaemia [4]. This disease is divided into groups 1 and 2, but there are other subtypes that can be caused by endocrine disorders, medications, diseases, immunity, genetics, or pancreatic disease [1,2]. These metabolic diseases lead to loss of life and can cause the decline of living beings by causing many problems affecting the heart, kidneys, blood vessels, eyes and nervous system [5].Approximately 20% of glucose in the body is controlled primarily by the hypothalamus through coordination with various hormones that influence food intake, energy, insulin utilization, hepatic glucose production, and glucose/fatty acid metabolism in adipose tissue and bone marrow2% of total body weight. Specifically for this purpose, the brain requires glucose to provide the energy needed by neurotransmitters to maintain the proper functioning of cells [6]. In addition to being involved in the pathogenesis of neurological diseases, glucose

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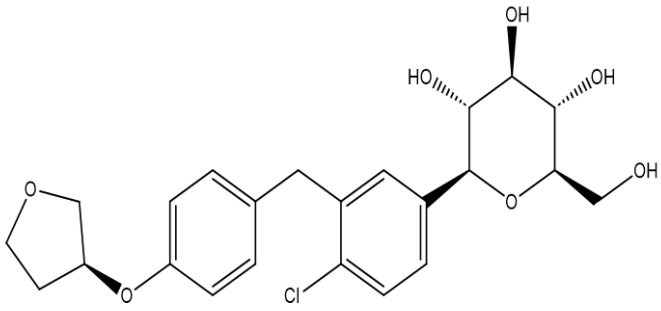
also plays an important role in regulatory pathways associated with oxidative stress, cell death, hypothalamic circuit disorders, and glucose and insulin sensitivity [7]. Therefore, blood sugar control is important. More than 400 million people worldwide suffer from diabetes (DM), a major public health problem. These metabolic diseases are increasingly associated with life-threatening micro vascular, europathic consequences [8]. These metabolic diseases are increasingly associated with life-threatening micro vascular, macro vascular and neuropathic consequences. Diabetes Mellitus is caused by insulin resistance resulting from inadequate insulin secretion, damage to pancreatic beta cells, or lack of insulin use [9]. A sedentary lifestyle may be a factor in the rise of diabetes worldwide. The elderly population (>65 years old) is expected to reach 366 million by 2030[10]. Many problems associated with diabetes include kidney disease, neuropathy, heart and kidney problems, retinopathy, and eating disorders and other problems [11]. There are two types of diabetes: type 1 and type 2. Type 1 diabetes is an autoimmune disease that affects cells in the pancreas, reducing or interfering with insulin [12]. Many hormones are responsible for maintaining the body's glucose homeostasis. On the other hand, glucagon and insulin are two hormones that control glucose homeostasis [13]. As blood sugar rises, beta cells secrete insulin. Insulin increases glucose production by the liver, muscle, and fatty tissue or inhibits the liver's ability to produce glucose through the processes of glycolysis and gluconeogenesis [14]. Diabetes risk is increased by a number of factors. The following is a list of the main factors:

If a child or adolescent has type 1 diabetes, they are more likely to develop the disease if a parent or sibling has the disease. Risk factors for developing type 2 diabetes include being overweight, following a special diet, being over age 45, having a family history of the disease, being physically inactive, having diabetes or pregnancy, having high cholesterol or triglycerides, and having diabetes story [15]. Your risk of developing gestational diabetes is increased if you are overweight and over the age of 25, have gestational diabetes, have given birth to a baby weighing more than 30 pounds, or have a family history of type 2 diabetes or polycystic ovary syndrome [16]. According to the World Health Organization (WHO) Diabetes Global Report, the disease affects 422 million people worldwide. This figure is almost four times higher than in 1980[17]. According to the International Diabetes Federation (IDF), 40.9 million people in India currently suffer from diabetes, and this number is expected to increase to 69.9 million by 2025[18]. The hormones glucagon and insulin are secreted by the pancreas. Beta and alpha cells are located in the pancreatic islets and secrete insulin and glucagon, respectively [19]. Insulin lowers blood sugar levels by transporting glucose to the muscles, liver, and fatty tissues and producing glycogen. Although red blood cells and arteries can use glucose without insulin, alpha cells play an important role in blood sugar control because they produce glucagon, which accelerates glycogen in the liver and raises blood sugar levels. Additionally, after birth, the foetus may be at increased risk for obesity, metabolic and cardiovascular disease and cancer 80-90% have type 2 diabetes [20].

1.1 EMPAGLIFLOZIN

Empagliflozin (Figure 1), a sodium glucose cotransporter 2 (SGLT2) inhibitor, is a new class of oral medications used to treat type 2 diabetes (T2DM) [21]. Empagliflozin has a unique non-insulin-dependent mechanism of action that increases glucose release and lowers blood sugar and has the advantage of not producing hypoglycaemic effects due to its non-insulin-dependent properties. Additionally, Empagliflozin is very slightly soluble in water (pH 1-7.4) [22]. Empagliflozin is also used in adults with heart failure to reduce the risk of hospitalization and death from heart disease and stroke. It is also used in adults with kidney disease to reduce the risk of kidney failure, the need for hospitalization, and the risk of death from heart disease [23]. Chemically it is (2S, 3R, 4R, 5S, 6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl] ox phenyl] methyl [Hydroxy]phenyl]-6 (hydroxy-methyl) oxane-3,4,5-triol [21].

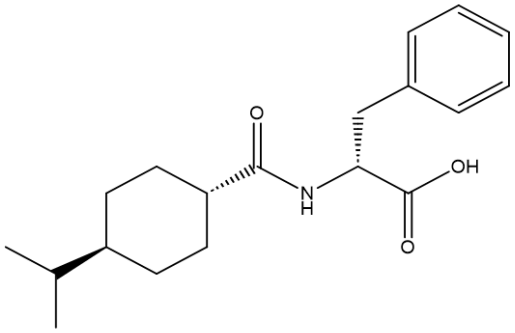


(2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol

Fig. 1. Chemical structure of Empagliflozin

1.2 NATEGINIDE

Nateglinide (NTG) [N(trans-4-isopropylcyclohexylcarbonyl)-d-phenylalanine] is a d-phenylalanine derivative without a sulfonylurea or amphetamine moiety and is a novel oral prandial glycaemic agent recently approved for the treatment of type 2 diabetes [24]. This meglitinide derivative works as follows: It stimulates insulin release from the beta cell membrane of the pancreas by closing ATP-dependent potassium channels, which leads to the opening of calcium channels [25]. The result of calcium influx causes insulin secretion. It is rapidly absorbed from the gastrointestinal tract, with blood concentration reaching peak in 0.5 to 1.0 hours. It is metabolized by the cytochrome P-450 system to inactive metabolites and eliminated with a half-life of 1.4 hours. Inactive metabolites and eliminated with a half-life of 1.4 hours [26].



(2R)-2-({[trans-4-(1-methylethyl)cyclohexyl]carbonyl}amino)-3-phenylpropanoic acid

Fig. 2. Chemical structure of Nateglinide

2. MATERIALS AND METHOD:

2.1 Chemicals and reagents:

Empagliflozin and Nateglinide is a gift sample from Yarrow Chem product, Mumbai, India. HPLC grade chemicals: Acetonitrile and Water were preferred for the development of the method, this was obtained from CDH (Central drug house) (P) Ltd.

2.2 Instruments:

Chromatography was performed on High performance Liquid Chromatography (Shimadzu), manual sampler,

software Win chrome and detector (UV- visible). The chromatographic separation was performed using Column C-18 (Shim-pack) 250 X 4.6 mm, particle size 5µm.

Selection of Wavelength: Wavelength was fixed at 216 nm by performing UV spectroscopy.

2.3 CHROMATOGRAPHIC CONDITION:

The method development for analysis of Empagliflozin and Nateglinide was performed using various solvents finally the separation was achieved using a mobile phase consisting of Acetonitrile: water [90:10 v/v] pH=3, pumped at a flow rate of 1 ml/min, and Rheodyne injector with 20µl loop was used for injecting sample. The eluent was monitored at using a UV- detector at a wavelength of 216 nm. Before being used, the mobile phase was filtered through a 0.22 µm nylon membrane filter and then degassed in an ultrasonic bath.

2.4 Preparation of Mobile phase: 450 ml of HPLC grade acetonitrile are added to 50 ml of water in a mobile phase reservoir then adjust the PH 3 by OPA and the mixture is kept for sonication for 10 minutes

2.5 Preparation of standard stock solution:

Standard stock solution was prepared by dissolving 10mg of Empagliflozin and Nateglinide in 100mL acetonitrile and water that gives the concentration of 100 µg/ml. this solution was diluted with mobile phase as needed to produce several standard solutions.

2.6 Method Validation:

The optimized analytical method was validated for system suitability, linearity, accuracy, precision, limit of detection and limit of quantification and robustness in accordance with ICH guidelines for analytical procedure Q2 [R1] [27].

2.6.1 Linearity and range:

The linearity was evaluated at five concentration levels in the range between 5- 25µg/ml for Empagliflozin and 10-50µg/ml for Nateglinide. A calibration curve was plotted by plotting concentration against corresponding peak area and linearity was using least square regression analysis. The analytical range formed by the highest and lowest conc. of analyte was acceptable linearity obtained.

2.6.2 Precision

Precision, as defined by ICH rules, includes both repeatability and intermediate precision. Six replicates of the sample injection were used to assess repeatability. To determine intraday precision, three different doses of Empagliflozin (5, 10, and 15 µg/mL) and Nateglinide (10, 20, and 30 µg/mL) were tested three times on the same day. The three concentrations indicated above were tested on three consecutive days for inter-day precision in order to assess day-to-day variability.

2.6.3 Accuracy:

Accuracy was determined by calculating recovery of the analyte of interest. A fixed amount of pre-analysed sample was taken, and the standard drug was added and 80%, 100% and 120% levels. The standard concentration was fixed as 15µg/ml of Empagliflozin and 20µg/ml for Nateglinide, and three concentration levels of 10µg/ml, 15µg/ml and 20µg/mL for Empagliflozin and 10µg/ml, 20µg/ml and 30µg/mL for Nateglinide were added to the standard concentration. Each level was repeated three times. The percentage recovery standard deviation [% RSD] were taken into consideration for testing accuracy.

2.6.4 Limit of detection:

The term "limit of detection" refers to the lowest concentration of an analyte in a sample that can be identified but is not usually defined as a precise value (LOD). The standard deviation of response and slope were used to calculate the LOD.

$$\text{LOD} = \frac{3.3\ X\ \sigma}{S}$$

The linearity curve was used to compute the slope and standard deviation for Empagliflozin and Nateglinide concentrations ranging from 5 to 25µg/mL and 10 to50µg/mL.

2.6.5 Limit of quantitation (LOQ):

LOQ is the smallest amount of analyte in a sample that can be quantitatively measured with sufficient precision

and accuracy. LOQ is determined using the standard deviation of the response and the slope. The data was derived from the linearity curve, and the LOQ was determined.

$$\text{LOD} = \frac{10X\,\sigma}{S}$$

The linearity curve was used to compute the slope and standard deviation for Empagliflozin and Nateglinide concentrations ranging from 5 to 25µg/mL and 10 to50µg/mL.

2.6.6 Robustness:

The robustness of the study was examined by purposefully changing a few factors slightly, in accordance with ICH recommendations. The capacity of the drug to stay unaffected by minute variations in parameters such as temperature, detecting wavelength, flow rate, and mobile phase composition is mostly associated with resilience. Little adjustments to the chromatographic settings, such as changes to the detection wavelength, and flow rate, can be used to assess how resilient the approach is. At a concentration of 10µg/mL, robustness was evaluated.

3. FORCE DEGRADATION STUDIES:

Forced degradation studies include subjecting the drug substance to various stress condition to observe the extent of degradation and rate of degradation which is likely to occur in the course of storage. The degradation pathways studied are acid hydrolysis, basic hydrolysis, and oxidative degradation, thermal and photolytic degradation [28-31].

3.1 Acid Hydrolysis: Acid hydrolysis was done by adding accurately weighed 10mg of Empagliflozin and Nateglinide into a clean and dry round bottom flask. 50ml of freshly prepared Acetonitrile and water (90:10) 0.1 N HCl solution transfer to it and it was refluxed in a water bath for 6 hours by maintaining the temperature at 60℃. After refluxing the solution 1ml sample withdraw at different intervals for 6 h which was neutralized and dilute to 10ml with acetonitrile and water (90:10). The 20 µL of the resulting solution (20 µg/ ml) was injected and analysed.

3.2 Basic Hydrolysis: To a clean and dry round bottom flask accurately weighed 10mg of Empagliflozin and Nateglinidewas transferred. 50ml of freshly preparedAcetonitrile and water (90:10) 0.1 NaOH was added to it and it was refluxed in a water bath for 6 hours by maintain the temperature at 60℃. After refluxing the solution 1ml sample withdraw at different intervals for 6 h which was neutralized and dilute to 10ml with solvent combination of Acetonitrile and water 90:10. The 20 µL of the resulting solution (20 µg/ ml) was injected and analysed.

3.3 Photolytic degradation: Photolytic degradation studies was carried out by taking 10mg of Empagliflozin and Nateglinidedrug into a clean and dry petri dish which is covered with a glass lid. The drugs are kept under UV light for 12h and then, 10 mg of Empagliflozin and Nateglinideadded in 50 mL of solvent combination (Acetonitrile: water 90:10). After extracting the solution (1 mL), solvent combination (Acetonitrile: water 90:10) was added to dilute it to 10 mL. After 20 µL of the resultant solution (20 µg/ml) was injected, it was examined.

3.4 Oxidation degradation: To a clean and dry round volumetric flask accurately weigh 10mg of Empagliflozin and Nateglinidedrug was taken to this 50 mL of solvent combination (Acetonitrile: water 90:10) (v/v) hydrogen peroxide solution was added & was kept in the dark for 6h. the solution (1ml) was diluted to 10mL with solvent. The 20 µL of resulting solutions (20 µg/ ml) was injected and analysed.

3.5 Thermal degradation: Photolytic degradation studies was carried out by taking 10mg of Empagliflozin and Nateglinide drug into a clean and dry petri dish which is covered with a glass lid. The drugs are kept under oven for 12h at 100^0 C and then, 10 mg of Empagliflozin and Nateglinideadded in 50 mL of solvent combination (Acetonitrile: water 90:10). After extracting the solution (1 mL), solvent combination (Acetonitrile: water 90:10) was added to dilute it to 10 mL. After 20 µL of the resultant solution (20 µg/ml) was injected, it was examined.

4. RESULTS AND DISCUSSION:

A stability-indicating and reverse-phase high-performance liquid chromatography (RP-HPLC) method were developed and validated for the accurate and precise estimation of Empagliflozin and Nateglinide in bulk. Various validation parameters, including stressed samples and different mobile phase compositions and flow rates, were explored to establish the method. Through multiple iterations and adjustments of chromatographic conditions, optimal parameters were identified and confirmed. Empagliflozin and Nateglinide exhibited well-defined peaks with excellent symmetry and a stable baseline using a mobile phase composed of Acetonitrile: Water (90:10 v/v) at a flow rate of 1.0 ml/min. The retention times for Empagliflozin and Nateglinide were determined to be 2.770 and 3.682, respectively, with distinct peaks observed at 216 nm.

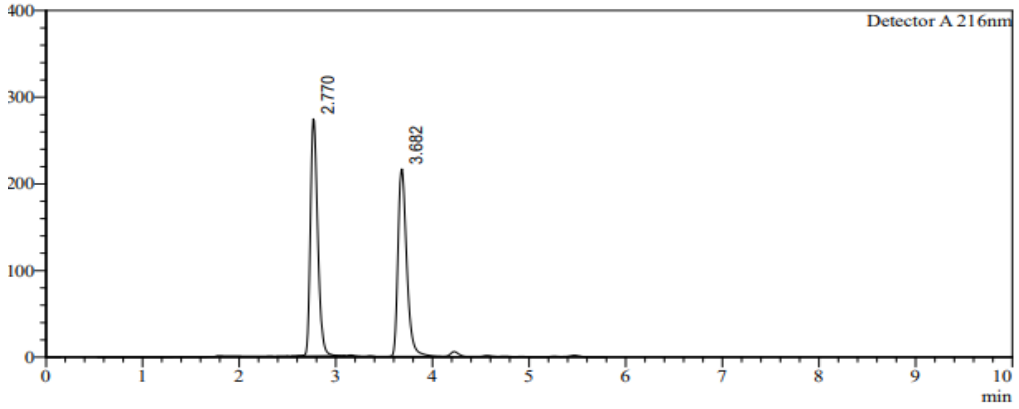


Fig.3. Chromatogram of Empagliflozin and Nateglinide

4.1 Linearity and range:

For linearity of Five-point concentration curve was obtained in concentration ranges 5 µg/mL- 25 µg/mL for Empagliflozin and 10 µg/mL- 50 µg/mL for Nateglinide. The response of the drugs was found to linear in the selected concentration range, the regression equation Y=172802x +110348, Y= 619704x - 352451 for Empagliflozin and Nateglinide and, the correlation coefficient (r^2) for Empagliflozin and Nateglinide were 0.9993 and 0.9992 respectively

Table 1. Linearity and Range data for Empagliflozin

Sr no.	Concentration µg/mL	Peak Area (mv)
1.	5 µg/mL	1012327
2.	10 µg/mL	1799519
3.	15 µg/mL	2699465
4.	20 µg/mL	3536841
5.	25 µg/mL	4463705
Average Area		2702371
Slope		172802
Y – intercept		110348
Correlation Coefficient		0.9993

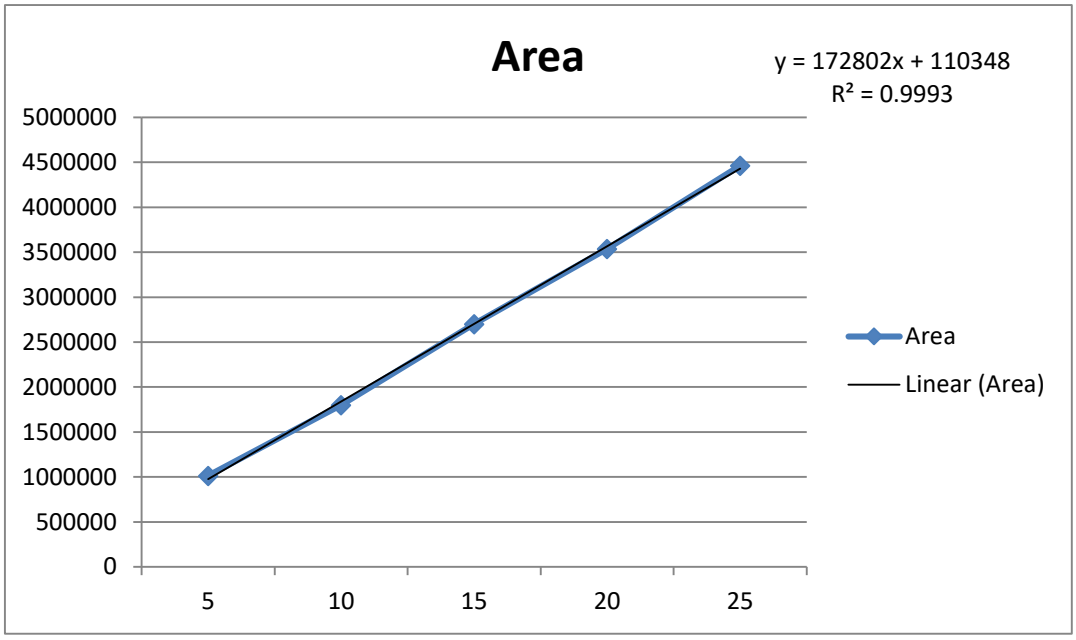


Fig.4. Calibration curve of Empagliflozin Y = 172802x + 110348Slope = 172802, Intercept = 110348, Correlation coefficient = 0.9993.

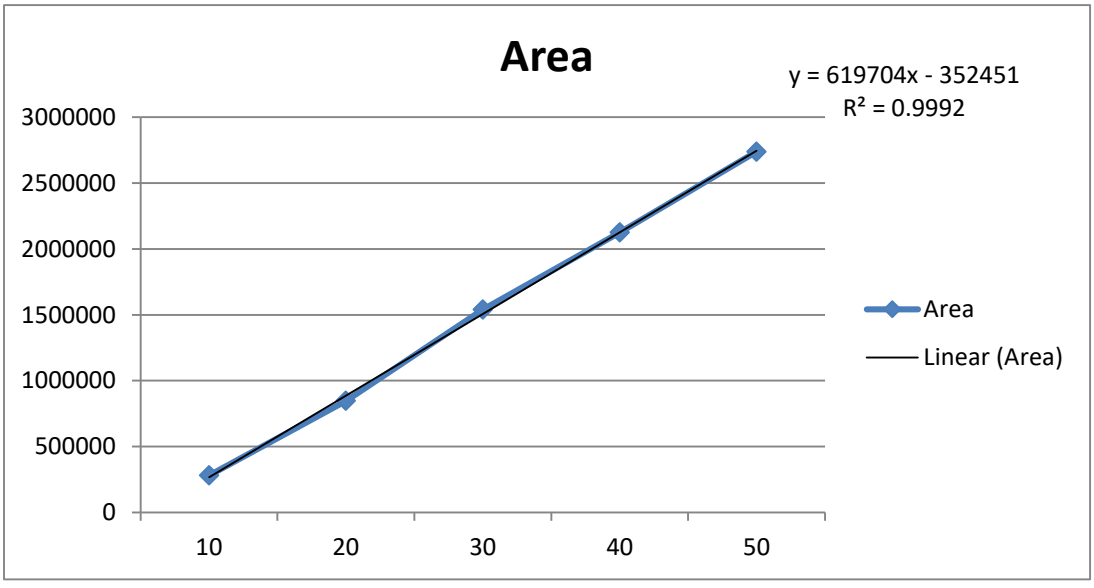


Fig.5. Calibration curve of Nateglinide. Y= 619704x - 352451Slope= 619704, Intercept = 352451, Correlation Coefficient = 0.9992

Table 2. Linearity and Range data for Nateglinide

Sr no.	Concentration µg/mL	Peak Area (mv)
1.	10 µg/mL	280427
2.	20 µg/mL	847141
3.	30 µg/mL	1540315
4.	40 µg/mL	2125807
5.	50 µg/mL	2739613
Average Area		1506661
Slope		619704
Y – intercept		352451
Correlation Coefficient		0.9992

4.2 Precision:

The analytical method's precision is characterized by the concordance level among individual test outcomes derived from applying the method across various samples. Precision assessments of the proposed method were conducted, encompassing evaluations for repeatability and intermediate precision (both within and between days). The performance of the HPLC instrument was assessed under chromatographic conditions by repetitively injecting 10 µg/mL of Empagliflozin and Nateglinide. The Relative Standard Deviation (RSD) was ascertained to be within acceptable limits, signifying the enhanced accuracy of the proposed methodology. Precision results are shown in Tables 3, 4, and 5.

Table.3. Interday precision of the developed method for Empagliflozin and Nateglinide

Interday precision										
Empagliflozin						**Nateglinide**				
Sr.no.	**Conc. (µg/ml)**	**Peak Area**	**Mean**	**S. D**	**%RSD**	**Conc. (µg/ml)**	**Peak Area**	**Mean**	**S. D**	**%RSD**
1.	**5**	1012327 1012445 1012352	1012375	62.179895	0.006142	**10**	280427 281154 280957	280846	375.996	0.13388
2.	**10**	1799519 1785491 1776497	1787169	11602.37	0.649204	**20**	847141 836549 859642	847777.3	11559.64	1.363524
3.	**15**	2699465 2697257 2683581	2693434	8604.356	0.319457	**30**	1540315 1542698 1541263	1541425	1199.765	0.077835

Table.4. Intraday precision of the developed method for Empagliflozin and Nateglinude

Intraday precision										
Empagliflozin						**Nateglinide**				
Sr.no.	**Conc. (µg/ml)**	**Peak Area**	**Mean**	**S. D**	**%RSD**	**Conc. (µg/ml)**	**Peak Area**	**Mean**	**S. D**	**%RSD**
1.	**5**	1012698 1025612 1010256	1016189	8251.682	0.812023	**10**	296554 295345 263216	285038.3	18908.36	6.63362
2.	**10**	1885649 1895478 1883654	1888260	6329.774	0.335217	**20**	877638 854412 884569	872206.3	15795.2	1.810948
3.	**15**	2597212 2696882 2696892	2663662	57547.39	2.160461	**30**	1890264 1865493 1890246	1882001	14296.35	0.759636

Table.5. Repeatability of the developed method for Empagliflozin and Nateglinide

Empagliflozin			**Nateglinide**	
Sr.no	**(Conc.(ug/ml)**	**Peak Area**	**(Conc.(ug/ml)**	**Peak Area**
1	5	1012327	10	280427
2.	5	1012341	10	280425
3.	5	1012356	10	280431
4.	5	1012356	10	280451
5.	5	1012356	10	280451
6.	5	1012351	10	280429
7.	**Mean**	1012348	**Mean**	280435.7
8.	**S. D**	11.75443	**S. D**	12.04436
9.	**%RSD**	0.001161	**%RSD**	0.004295

4.3 Accuracy:

The percentage recovery of the spiked sample was with 100±2% which ensures the accuracy of the developed method. Result of accuracy shown in Table.6,

Table.6. Accuracy of the developed method for Empagliflozin

Empagliflozin							
Sr. no.	**Unfortified sample**			**Fortified sample**			**%Recovery**
	Conc. (ug/ml)	**Area**	**Mean (Area)**	**Conc. (ug/ml)**	Area	**Mean (Area.)**	
1.	**10**	2065996	2066353	**10+15**	4935826	4935713	**100.02%**
		2066521			4934659		
		2066541			4936654		
2.	**15**	3085466	3088801	**15+15**	5546712	5551106	**100.07%**
		3085475			5547125		
		3095462			5559482		
3.	**20**	3954841	3886503	**20+15**	5016984	5019012	**99.88%**
		3859432			5024571		
		3845236			5015482		

Table.7. Accuracy of the developed method for Nateglinide

Nateglinide							
Sr. no.	**Unfortified sample**			**Fortified sample**			**%Recovery**
	Conc. (ug/ml)	**Area**	**Mean (Area)**	**Conc. (ug/ml)**	**Area**	**Mean (Area.)**	
1.	**10**	311549	316328	**10+20**	1825841	1803014	**102.64%**
		312694			1756621		
		324741			1826581		
2.	**20**	874695	879354.7	**20+20**	2468741	2469894	**99.40%**
		889821			2484561		
		873548			2456379		
3.	**30**	1736951	1730263	**30+20**	3015941	3020099	**100.04%**
					3018743		
					3025613		
		1725694					
		1728143					

4.4 Limit of detection and Limit of Quantification

Limit of detection (LOD) and limit of quantification (LOQ) was estimated from the standard deviation of the y-intercepts and slope of the calibration curve of Empagliflozin and Nateglinide. The LOD were found to be for Empagliflozin14.0502, for Nateglinide 6.539873 µg/ml and LOQ were found to be forEmpagliflozin42.57638, for Nateglinide 19.8178 µg/ml. This showed that developed method can detect and quantify at lower concentration was highly sensitive and other less sensitive.

4.5 Robustness:

Robustness of Empagliflozin and Nateglinide was determined by studying the small changes in chromatographic condition as change in flow rate (± 2mL/ min), wavelength detection (±2 nm) respectively. Robustness was assessed at concentration 5 µg/ ml and 10 µg/ ml. The standard deviation of response was calculated for each parameter and % RSD was found to less than 2% indicating that the method is robust as shown in Table.8,9

Table.8. Robustness results of the proposed RP- HPLC method for Empagliflozin

Sr.no	Parameters			Peak Area	RT	USP	
	Optimized		Used			Plate Count	Tailing factor
1.	Flow Rate (±2)	1ml/min	0.8ml/min 1.2ml/min	957654 112365	2.423 3.894	5239 5843	1.085 1.272
2.	Wavelength detection (±2)	216nm	214 nm 218 nm	1254789 1156984	2.751 2.512	5088 5120	1.380 1.375

Table.9. Robustness results of the proposed RP- HPLC method for Nateglinide

Sr.no	Parameters			Peak Area	RT	USP	
	Optimized		Used			Plate Count	Tailing factor
1.	Flow Rate (±2)	1ml/min	0.8ml/min 1.2ml/min	260569 309161	3.657 2.397	3654 2569	1.005 0.954
2.	Wavelength detection (±2)	216nm	214 nm 218 nm	275632 293741	3.156 3.178	1209 1141	0.678 0.459

4.6 Force Degradation:

The chromatograms obtained from samples exposed to be acidic, basic, oxidative, thermal and photo degradation depicted well- separated peaks of Empagliflozin and Nateglinide, its having retention time 2.770, 3.682 and some additional peaks at different values. Acid degradation for empagliflozin show 1 extra peak and for Nateglinide show additional peaks. In base degradation for empagliflozin show two additional peaks and for Nateglinide show additional peaks. In case of oxidative, photodegradation and thermal degradation, they show 2 additional peaks for Empagliflozin, and for Nateglinide, it shows additional peaks. The percentage of degradation product is listed in Table 10. Results of force degradation are shown in Fig.6,7,8,9,10,11,12,13,14,15.

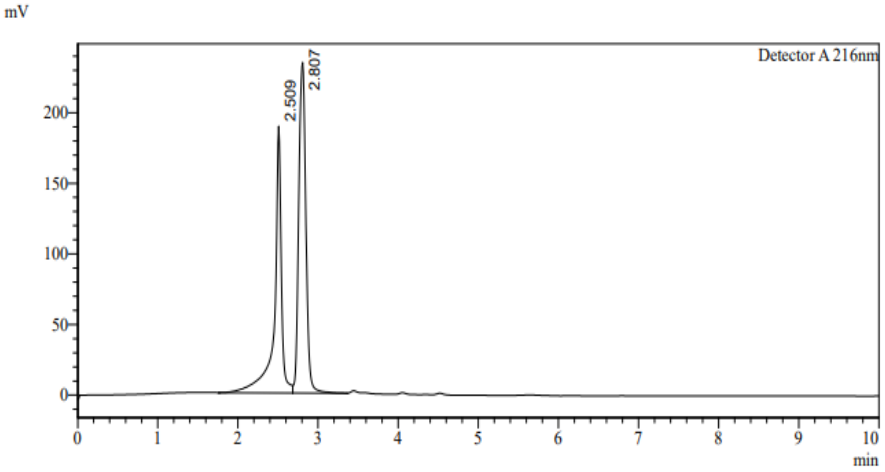


Fig. 6 Acid degradation of Empagliflozin

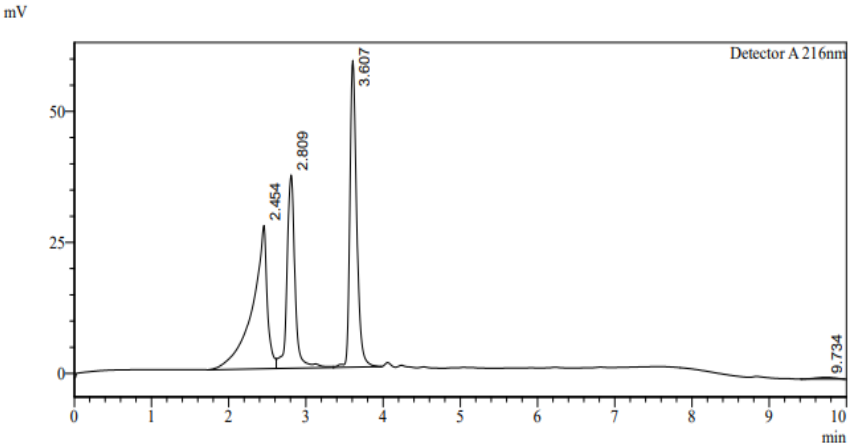


Fig. 7 Base degradation of Empagliflozin

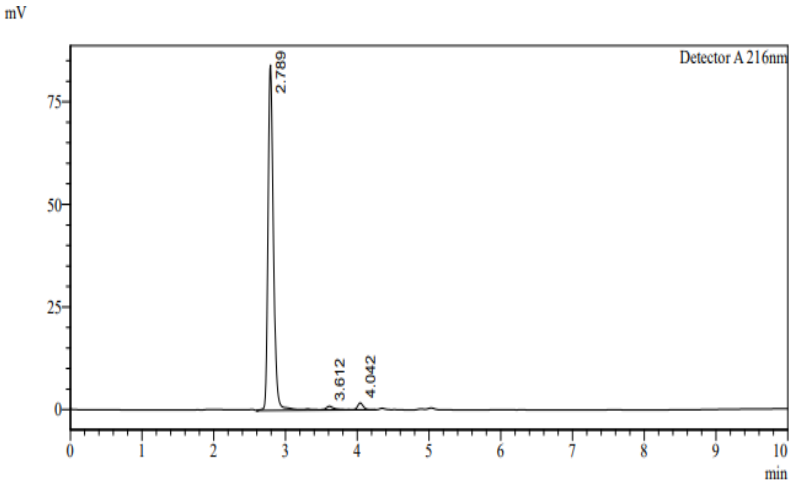


Fig. 8 Photo degradation of Empagliflozin

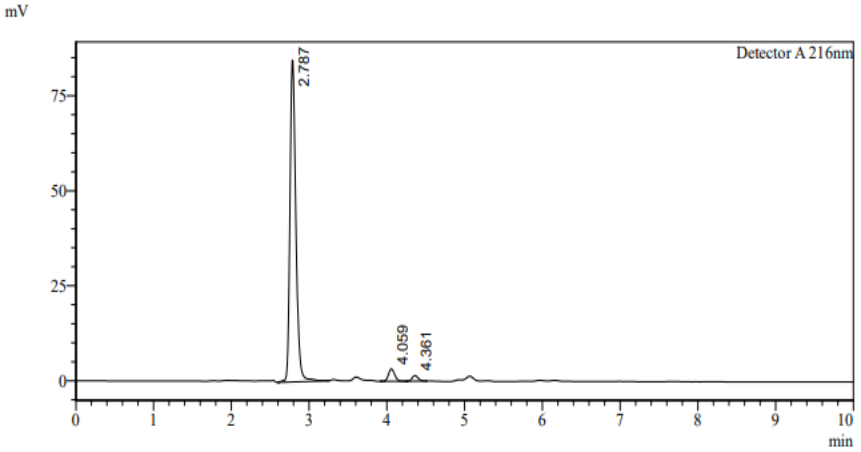


Fig. 9 Thermal degradation of Empagliflozin

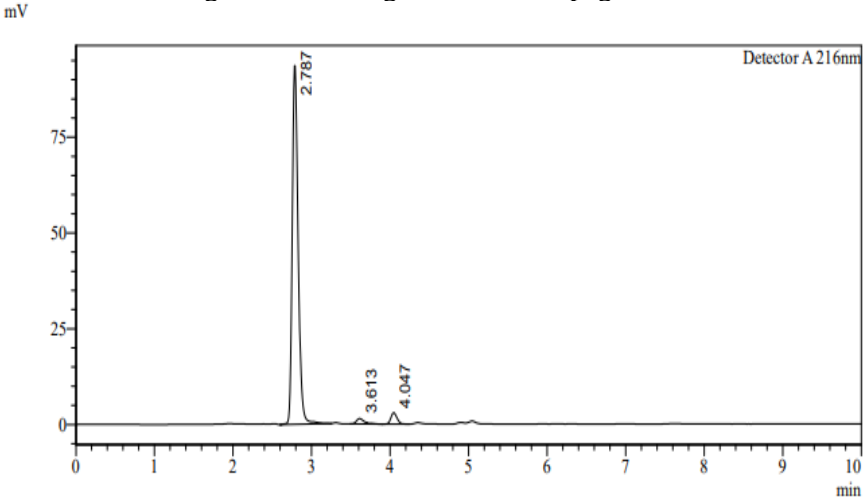


Fig. 10 Oxidative degradation of Empagliflozin

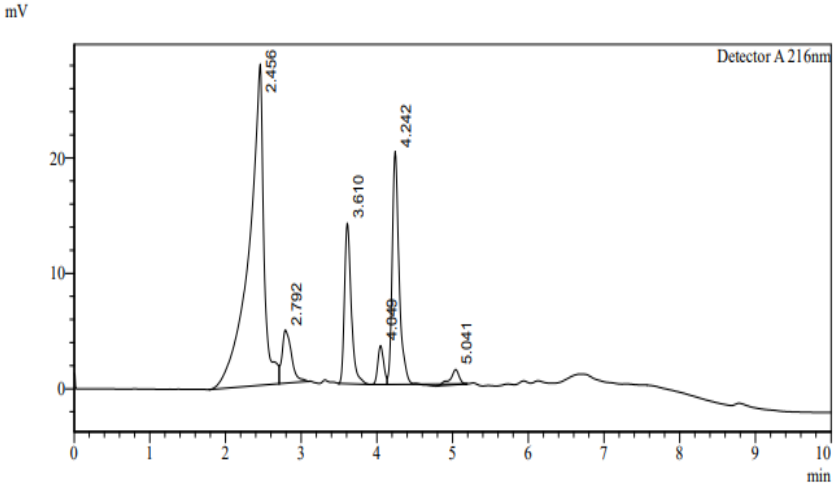


Fig. 11 Acid degradation of Nateglinide

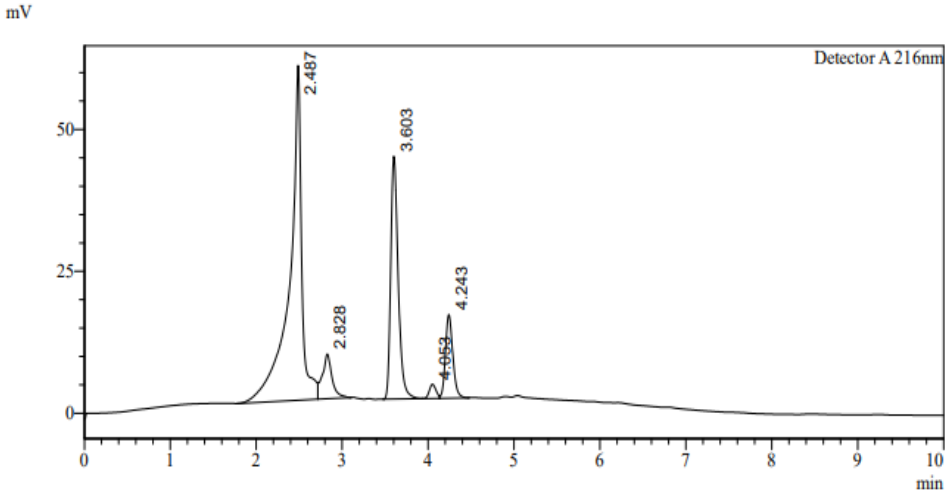


Fig. 12 Base degradation of Nateglinide

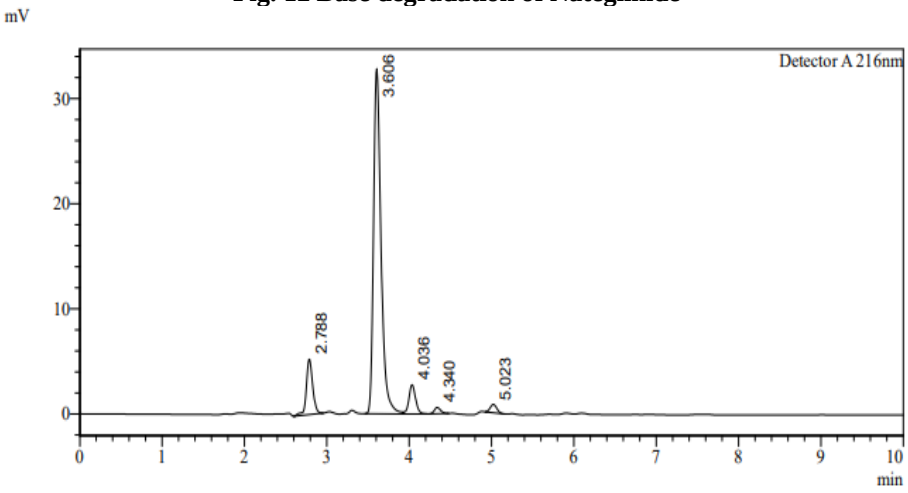


Fig. 13 Photo degradation of Nateglinide

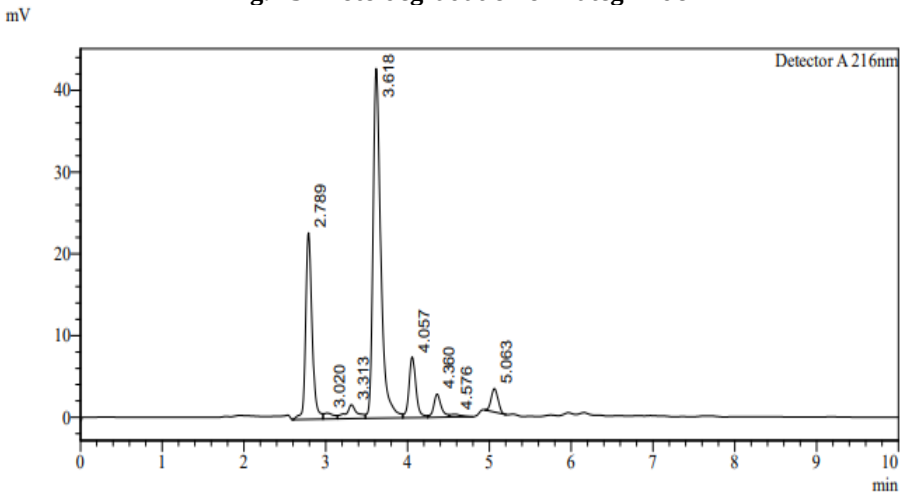


Fig. 14 Thermal degradation of Nateglinide

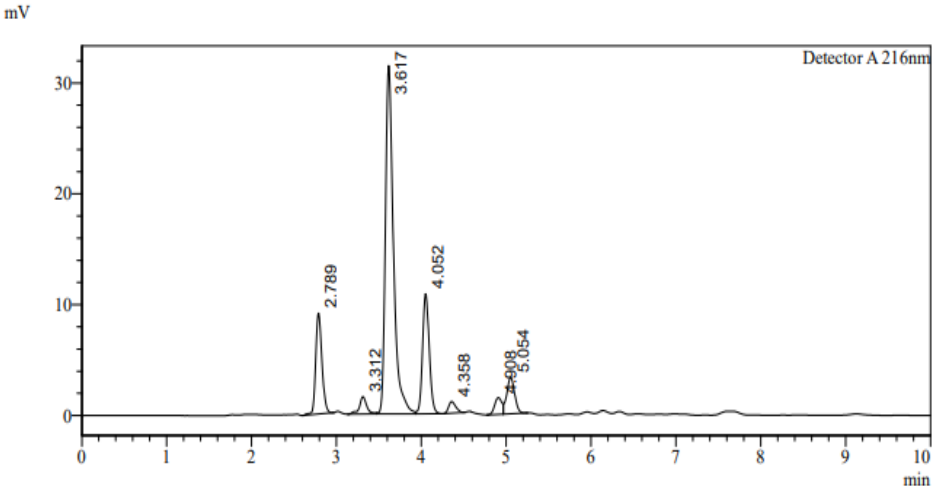


Fig. 15 Oxidative degradation of Nateglinide

Table.10. Force degradation study data of Empagliflozin and Nateglinide

	Empagliflozin			Nateglinide		
Type of degradation	**Area**	**% Recovered**	**%Degradation**	**Area**	**% Recovered**	**%Degradation**
Acid	1449208	44	**56%**	86794	87	**13%**
Base	265973	74	**26%**	259043	74	**26%**
Oxidation	490651	6	**94%**	20741	43	**57%**
Photo degradation	445996	4	**96%**	207411	20	**80%**
Thermal degradation	449292	6	**94%**	282913	45	**55%**

5. CONCLUSION

The study shows that the developed RP-HPLC method is fast precise, accurate, specific and stability. The proposed method applied for the simultaneous estimation of both the drugs in bulk form. These are within short analysis time and the low value of % RSD indicate that the proposed method is highly precise. The stability indicating method was developed and validated according to the ICH guidelines for simultaneous estimation of Empagliflozin and Nateglinide in bulk drug by RP-HPLC. Finally, concluded that the method is suitable for user in routine quality control analysis of Empagliflozin and Nateglinide in active pharmaceutical ingredient.

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التطوير والتحقق من طريقة RP-HPLC المؤشرة على الثبات للتقدير المتزامن للإمباغليفلوزين والناتيغلينيد في المادة الدوائية الخام

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ملخص

تم تطوير وتحقق طريقة RP-HPLC بسيطة ودقيقة ومؤشرة على الثبات للتقدير المتزامن للإمباغليفلوزين والناتيغلينيد في المادة الدوائية الخام. تم إجراء الفصل الكروماتوغرافي على عمود) Shim-pack C-18بأبعاد 250 × 4.6 مم، 5 ميكرومتر)، باستخدام الطور المتحرك أسيتونتريل: ماء (90:10 (v/vبدرجة حموضة 3 لتطوير هذه الطريقة. تم ضبط طول موجة الكشف بالأشعة فوق البنفسجية عند 216 نانومتر، ومعدل التدفق عند 1 مل/دقيقة، وحجم الحقن عند 20 ميكرولتر. تم العثور على زمن الاحتجاز للإمباغليفلوزين والناتيغلينيد ليكون 2.770 و 3.682 دقيقة على التوالي. تم التحقق من هذه الطريقة وفقًا لإرشادات ICH Q2 R1 من حيث الخطية، الدقة، التكرارية، حد الكشف ((LOD، حد التقدير ((LOQ، والمتانة. تم ملاحظة استجابة خطية في نطاق تركيز 5–25 ميكروغرام/مل للإمباغليفلوزين و10–50 ميكروغرام/مل للناتيغلينيد. كانت حدود الكشف والتقدير للإمباغليفلوزين 14.0502 و 42.576 ميكروغرام/مل، وللناتيغلينيد 6.5398 و 19.8178 ميكروغرام/مل على التوالي. تم إجراء دراسات الإجهاد وفقًا لإرشادات ICH Q1A R2، حيث تعرضت الأدوية لظروف إجهاد تشمل التحلل الضوئي، الأكسدة، التحلل الحراري، التحلل الحمضي والقلوي، وذلك لتطوير نهج مؤشِّر على الثبات. تم استخراج المنتجات المتدهورة بفعالية من العينة.

الكلمات الدالة: إمباغليفلوزين، RP-HPLC، التحقق من الطريقة، طريقة مؤشرة على الثبات، ناتيغلينيد.

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