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METHOD DEVELOPMENT AND VALIDATION OF VILDAGLIPTIN AND METFORMIN HCl IN PHARMACEUTICAL DOSAGE FORM BY REVERSE PHASE–HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC)

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ABSTRACT: Determination of the anti-diabetic drugs vildagliptin and metformin is wildly used to validate drug efficiency in diabetic patients. Here we describe a new and simple chromatographic method to analyze both drugs simultaneously in their commercial pharmaceutical dosage forms. A reverse Phase-high performance liquid chromatography (RP-HPLC) separation method was applied using an Xterra C18 column (250 mmL×4.6 mm I.D × 5µ) with acetonitrile: phosphate buffer (pH 6.0): water (65: 20:15v/v/v) as a mobile phase at a flow rate of 1.0 ml/min. Quantification of these drugs by this method was achieved using an ultra violet detector at λ = 239 nm. The limit of detection (LOD) for vildagliptin was 0.0040 µg/ml and 0.025 µg/ml for metformin using this RP-HPLC method. A linear calibration curves were reached at a concentration range of 4-34 µg/ml and 8-54 µg/ml for vildagliptin and metformin, respectively. The developed technique was validated for concentration linearity, robustness, accuracy and precision, and results were statistically analyzed according to the International Conference on Harmonisation (ICH) guidelines. The results presented in this report revealed the development of simple, rapid, precise and accurate RP-HPLC method for immediate determination and validation of vildagliptin and metformin in their pharmaceutical dosage forms.

INTRODUCTION: Diabetes mellitus type two (T2DM) is a chronic disease that wants a mixture of anti-diabetic drugs to have different mechanisms of action to succeed glycaemic goals [1]. The widely used of metformin and a sulphonylurea (SU) as dual therapy fails to improve glycaemic control and the adding of a third anti-hyperglycaemic drug is necessary [2].

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Vildagliptin (VGT) [(S)-1-[N-(3-hydroxy-1-ada-mantyl) glycyl] pyrrolidine-2-carbonitrile], **Fig. 1**, is a new oral anti-diabetic drug belonging to the class of dipeptidyl peptidase-4 inhibitor (reduces glucose-induced glucagon-like peptide 1 and gastric inhibitory polypeptide secretion) [3] and is used as mono therapy in adults with type 2 diabetes mellitus treatment especially in patients inadequately controlled by diet and exercise alone [4, 5].

Vildagliptin can be used as dual oral therapy in combination with; metformin, in patients with insufficient glycemic control despite maximal tolerated dose of monotherapy with metformin [6, 7]. It has similar efficacy as it is used with metformin when compared to sulphonylurea and reduced

hypoglycaemia risk with no weight gain [8]. Vildagliptin has a complement pharmacological effect as metformin, where it improves the glucose-dependent insulin secretion and inhibits glucagon release, thus increasing the glycaemic control and weight control and reduced hypoglycaemia [9].

In addition, vildagliptin used with a sulphonylurea, in patients with insufficient glycaemic control despite maximal tolerated dose of a sulphonylurea and for whom metformin is inappropriate due to contraindications or intolerance. Also, used with a thiazolidinedione, in patients with insufficient glycaemic control and for whom the use of a thiazolidinedione is appropriate. Furthermore, it was used with sulphonylurea and metforminas triple oral therapy when diet and exercise plus dual therapy with these medicinal products do not provide adequate glycaemic control [10]. Vildagliptin is also indicated for use in combination with insulin (with or without Metformin) when diet and exercise plus a stable dose of insulin do not provide adequate glycaemic control. The general formula for VGT is $C_{17}H_{25}N_3O_2$ and the molar mass 303.40 g/mol.

Metformin (MTF) is chemically known as [1-carbamimidamido-N, N-dimethylmethanimidamide] (**Fig. 2**) is an oral anti-diabetic drug in the class of biguanides. It is used as the first-line drug for noninsulin-dependent diabetes mellitus treatment [11]. It works as improving glycemic control factor through decreasing hepatic glucose production, decreasing glucose absorption, and increasing the insulin-mediated uptake of glucose. Therapeutic indications of metformin competent is indicated as second line treatment of type 2 diabetes mellitus adult patients, particularly overweight patients, who are unable to achieve sufficient glycaemic control at their maximally tolerated dose of oral metformin alone [12]. The mechanism through which metformin HCl decreases blood glucose and lipid concentrations is by activation of the enzyme AMP-activated protein kinase (AMK) and the Peutz-Jeghers protein, LKB1, to regulate AMPK [13].

Therefore, a complementary mechanism of action in treatment of patients with type 2 diabetes was achieved by using a decreasing hepatic glucose production. The possible relation between metformin and sugar was studied in rats' plasma [14]. The general formula for MTF is $C_4H_{11}N_5$ and the molar mass 129.16 g/mol. Many studies used HPLC to prepare an easy, rapid, precise and accurate method to evaluate compounds in different pharmaceutical dosage forms [15 - 17].

Several methods were developed for the analysis of both vildagliptin and metformin in combination such as UV-Vis spectroscopies, HPLC and LCMS/MS methods. Instantaneous estimation of these compounds by RP-HPLC methods were showing more time of analysis and complicated procedures; hence the present study was focused on chromatographic analysis of vildagliptin and metformin in a less time consuming simultaneous analysis of these compounds inactive ingredient (API) and Pharmaceutical dosage form which found in the pharmaceutical market [18 - 21].

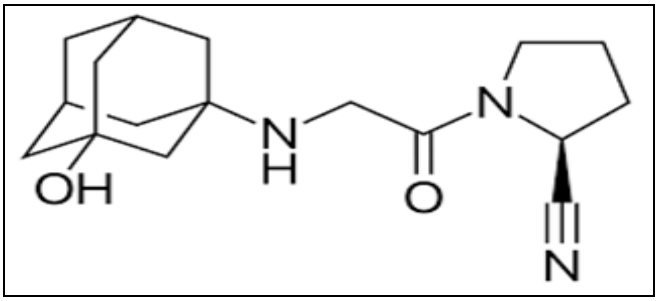


FIG. 1: STRUCTURE OF VILDAGLIPTIN

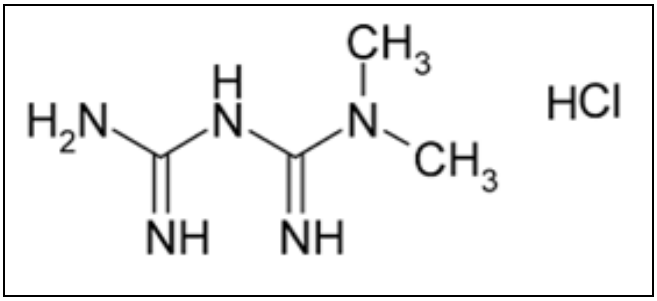


FIG. 2: STRUCTURE OF METFORMIN HCl

MATERIALS AND METHODS:

Chemicals Reagents and Instrumentation: Chemicals and reagents used in this experiment, Active Pharmaceutical Ingredients (API) of vildagliptin and metformin HCl (TQ Pharma Pharmaceutical Technology), HPLC grade acetonitrile (Merck), potassium dehydrogenate orthophosphate (sigma chemicals) Thomas Baker, Ortho phosphoric acid (sigma chemicals), HPLC grad water (Hikma - Pharma) and marketed formulation obtained from local Jordanian Market.

HPLC (WATERS EQ, Aliance 2796 Model, and Detector 2678 with Em.power 2.3 software), UV spectrophotometer (Make: Labindia, Model: UV-3000 with UV win 5 software). Weighing balance (Make: Ascoset, Model: ER.200A), Sonicator (Make: Enertech, Model: SE60.US), pH Meter (Make: ADWA, Model: AD102U), nylon filter Paper 0.45 microns (Make: Milli Pore), and column used was XTerra c18 (150×4.6mm, 5µ).

Chromatographic Conditions: HPLC (WATERS EQ.: Aliance 2796 Model, Detector 2678 with Em. power 2.3 software). The column used was symmetry C18 (4.6 x 250mm, 5 µm, Make: X. Terra 1). The mobile phase consisting of phosphate buffer (6.0pH), acetonitrile and water (65:20:15v/v/v), with flow rate 1.0 ml/min, the run time (min) was6.6 min, columns temperature was maintained at room temperature in normal laboratory condition, injection volume 20 µl and detection wavelength 239 nm.

Buffer Solution Preparation: 6.75gms of KH_2PO_4 was dissolved with water in 1000 ml beaker, then the volume was adjusted with HPLC grade water. After sonication and filtration using nylon G filter the pH was adjusted to 6.0 ± 0.01 with 1M sodium hydroxide (NaOH). The mobile phase was prepared by mixing 15% of above salt KH_2PO_4 prepared buffer (150 ml), 20 % of water (200 ml) and 65 % of acetonitrile (650ml).

Standard Stock Solution Preparation: Stock solution of 500µg/ml and 1000 µg/ml of vildagliptin and metformin, respectively was prepared by taking accurately weighing 5 mg of Vildagliptin (VGT) and 10 mg of Metformin (MTF) working standard in to a clean 10ml volumetric flask individually. 2, 5 -3.0 ml of mobile phase was used for dissolving completely and then the volume was made up to the mark with mobile phase. More dilutions were prepared with mobile phase. 0.3 ml of both the standard stock was diluted with mobile phase up to 10ml to get mixed standards of 15µg/ml vildagliptin and 30µg/ml metformin. Analysis of marketed formulation weigh an equivalent to 10 mg of marketed formulation was transferred to a clean 10ml volumetric flask and added with 3 ml of mobile phase for dissolving the content and the volume was made up to 10 ml with mobile phase. Further 0.3 ml of the solution was taken into a clean conical flask and volume was made to 10ml with mobile phase. The resulting solution was sonicated and filtered through nylon filter 0.45µ membrane filter. This solution was injected into the HPLC; the chromatograms are shown in **Fig. 3**.

Validation Method: The validation method was developed as per the ICH guidelines and accordingly the parameters evaluated were specificity, precision, accuracy, linearity, ruggedness, robustness and system suitability studies. For all the parameters %RSD were calculated [22 - 25].

System Suitability Parameters: System suitability test is commonly applied to validate resolution, column efficiency, and repeatability of a chromatographic system to ensure its capability for a specific analysis. The stock solution was injected into the chromatographic system and system suitability parameters were determined **Table 1**.

Specificity: Specificity of the pharmaceutical analysis is the ability to measure accurately and specifically the concentration of API, without interference from other active ingredients, diluents, mobile phase. Solutions of mobile phase, sample solution, standard solution were injected into liquid chromatography. Retention times of samples and standard were compared.

Linearity: Linearity of an analytical procedure is its ability to elicit test results that are directly proportional to the concentration of analyte in samples within given range.

TABLE 1: PARAMETERS OF SYSTEM SUITABILITY

S. no.	Parameter	Vildagliptin	Metformin
1	Retention Time (t) ,min.	2.28	4.27
2	Theoretical Plates (N)	2405	3562
3	Tailing Factor (Tf)	1.59	1.41
4	Area (AUC)	3161561	935842
5	Resolution (Rs)	6.87	

Linearity of the method was studied by analyzing five analyte concentrations of drug ranging from 5-25ppm for VGT and 9.99-49.99 ppm MTF are tabulated in **Table 2** and linearity plot is given in the **Fig. 3A** and **3B**.

Accuracy: Accuracy is the closeness of a measured value to a standard value. Accuracy was studied by means of recovery experiments for 50%, 100% and 150 %. Each level was injected three times. The accuracy was calculated in the form of percentage the test of the analyte recovered by the assay and the dated are shown in **Table 3**.

Precision: The precision expresses the closeness of agreement between a series of measurement obtained from multiple sampling of same homogenous sample under prescribed conditions. This experiment was conducted to prove the repeatability of the assay results obtained by quantification methodology. System precision, method precision and intermediate precision was performed for the homogeneous sample, according tom ICH guideline.

20 µl of standard solution was injected for six times and measured the peak area for all six injections in HPLC. The % RSD for the area of six replicate injections was calculated for system precision and shown in **Table 4**. 20 µl of sample solution was injected for six times and the peak area of the resulting chromatogram was used for the calculation of standard deviation and relative standard deviation for method precision.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The detection and quantification limits for the VGT and MTF were performed and calculated using S/N ratio method.

Robustness: Robustness measures the lack of internal influences on the test results. As part of the robustness, deliberate change in the flow rate and mobile phase composition was made to evaluate the impact on the method.

Change in flow rate the flow rate was varied at 0.80 ml/min to 1.00ml/min. Standard solution 15µg/ml of VGT and 30µg/ml of MTF were prepared and analyzed using the varied flow rates along with method flow rate and tailing around 1.60 **Table 5** and **6**.

Change in organic composition: the organic composition in the mobile phase was varied to ± 5 %. Standard solution 15 µg/ml of VGT and 30 µg/ml of MTF were prepared and analyzed using the varied mobile phase composition along with the actual mobile phase composition in the method **Table 7**.

RESULTS AND DISCUSSION: Vildagliptin and metformin can be effectively analyzed by the RP-HPLC method with phosphate Buffer, pH around 6.0 and composition of acetonitrile: water (20: 65: 15v/v/v) at a flow rate of 1.0 ml/minute and detection wavelength of 239 nm.

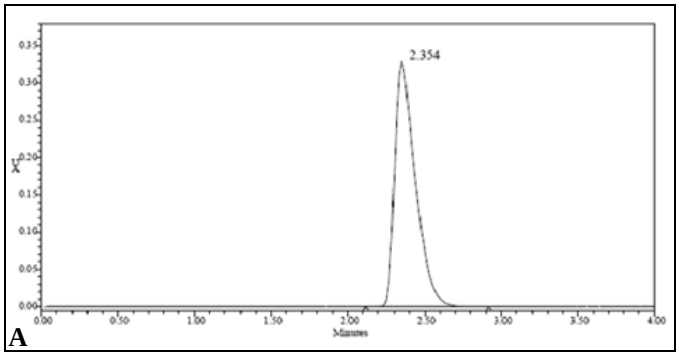
The retention time of the drugs was 2.32 and 4.29 minute for vildagliptin and metformin respectively. The assay limits for vildagliptin and metformin was 92-109% and the results were obtained for vildagliptin and metformin was found to be 99.66%, 101.44% hence the results were within the limits.

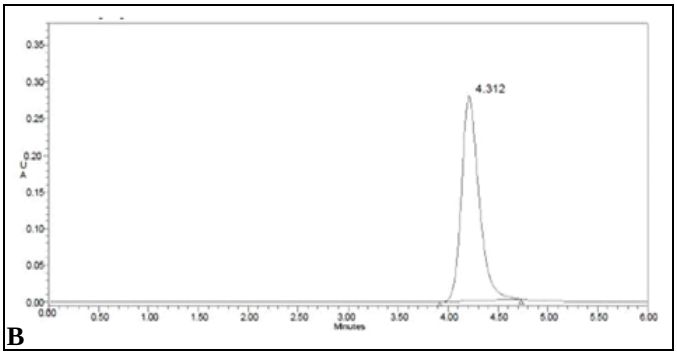
Specificity: The method was found to be specific since there was no interference of mobile phase or placebo in the retention time of the analyte peak **Fig. 3**.

Linearity: The linearity range was found to be 5-25 µg/ml for vildagliptin and 10-50 µg/ml for metformin. Calibration curves were plotted between the peak area and the concentrations and the linear regression coefficients for both drugs VGT and MTF were found to be 0.999 and 0.998 respectively (**Table 2**, **Fig. 4** and **5**). Hence the results obtained within the limits.

TABLE 2: LINEARITY MEASUREMENTS OF VILDAGLIPTIN AND METFORMIN

S. no.	Vildagliptin (VGT)		Metformin (MTF)	
	Concentration (ppm)	Area (AUC)	Concentration (ppm)	Area (AUC)
1	5	1361972	10	378569
2	10	2353521	20	696421
3	15	3637763	30	1085234
4	20	4663749	40	1511310
5	25	5762657	50	1917886
	Correlation Coefficient (R^2)	0.9999	Correlation Coefficient (R^2)	0.9998





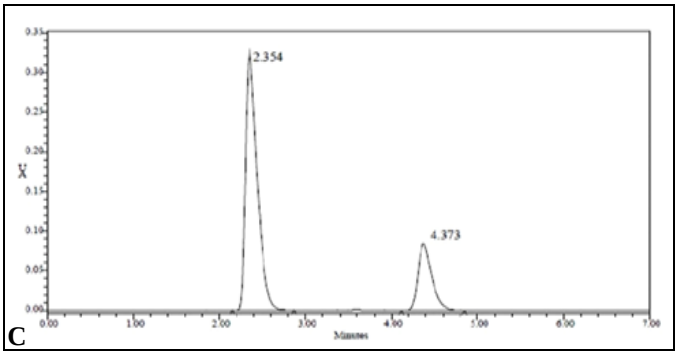


FIG. 3: CHROMATOGRAMS OF (3-A) VILDAGLIPTIN, (3-B) METFORMIN AND (3-C) MIXED STANDARD VILDAGLIPTIN AND METFORMIN

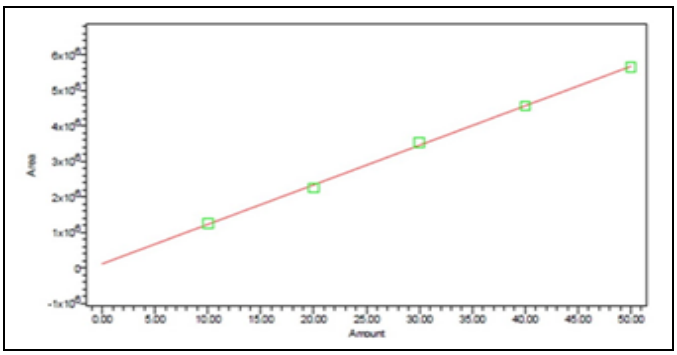


FIG. 4: CALIBRATION CURVE OF PEAK AREA VERSUS CONCENTRATION (PPM) FOR VILDAGLIPTIN

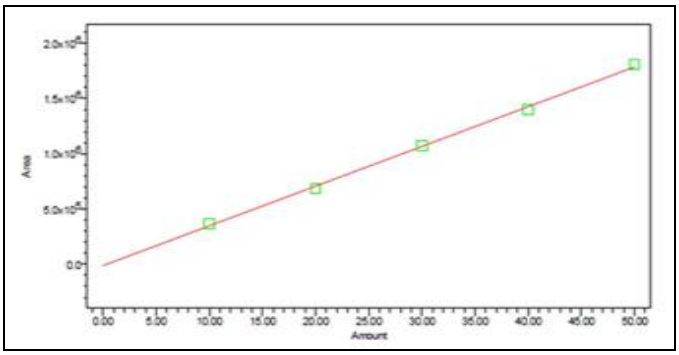


FIG. 5: CALIBRATION CURVE OF PEAK AREA VERSUS CONCENTRATION (PPM) FOR METFORMIN

Accuracy: The accuracy studies were shown as % recovery for Vildagliptin (VGT) and Metformin (MTF) at three levels; 50 %, 100 % and 150 % (**Table 3**). The mean % recovery of the vildagliptin was 99.6% and of the metformin was 99.8 %.

The limits of % recovery of drugs were 98-102 % and the above results which indicates that the method was accurate the limits of % recovered should be in range of 98-102 %.

TABLE (3) ACCURACY VALUES OF VILDAGLIPTIN AND METFORMIN

Analyte	Accuracy %	Standard addition	Formulation	Percent recovery %	Mean recovery %
Vildagliptin	50 %	7.5	15	99.5 %	
	Low	7.5	15		
		7.5	15		
	100 %	15	15	99.6 %	
	Mid	15	15		99.6 %
		15	15		
	150 %	22.5	15	99.7 %	
	High	22.5	15		
		22.5	15		
Metformin	50 %	15	30	99.9 %	
	Low	15	30		

	15	30		
100 %	30	30	99.8 %	
Mid	30	30		99.8 %
	30	30		
150 %	45	30	99.7 %	
High	45	30		
	45	30		

Precision: In the System precision study, %RSD was found to be less than 2%. For VGT 1.03% and MTF 0.32% which system precision indicates has good reproducibility.

TABLE 4: PRECISION VALUES OF VILDAGLIPTIN AND METFORMIN

Injection no.	System Precision		Intermediate Precision (System)		Intermediate Precision (Method)	
	VGT Area	MTF Area	VGT Area	MTF Area	VGT Area	MTF Area
Injection no. 1	3403811	951802	3272548	956856	362187	959695
Injection no. 2	3450890	958267	3266849	953165	3617752	954256
Injection no. 3	3464625	954481	3276987	956887	3647049	958687
Injection no. 4	3495173	952151	3266847	956224	3659050	958342
Injection no. 5	3501597	952151	3355848	956745	3643518	958845
Average	3463219	953664	3285816	956969	3645987	956571
Standard Deviation (SD)	35474.7	95481.8	35184.53	1584.07	19492.57	9570.67
Relative Standard Deviation (% RSD)	1.03	0.32	1.19	0.171	0.67	0.92

In the method precision study % RSD was found to be less than 2 %. For VGT 1.19 % and MTF 0.171 % which indicates that the method has good repeatability. In the Intermediate System precision study, % RSD was found to be less than 2 %. For VGT 0.67 % and MTF 0.92 % which indicates that the system has good reproducibility.

The results obtained for precision (RSDs) for both vildagliptin and metformin are shown in the **Table 4**.

Limit of Detection and Limit of Quantification: The limit of detections (LOD) was 0.0040 µg/ml for vildagliptin and 0.025 µg/ml for metformin.

TABLE 5: ROBUSTNESS VALUES OF VILDAGLIPTIN (CHANGE IN FLOW RATE)

S. no.	Flow rate (ml/min)	AUC	Relative standard deviation (% RSD)	System suitability results	
				Plate count	Tailing factor
1	Less flow 0.80	4032572	0.017	2300	1.65
1		4033772			
1		4032625			
2	Actual flow 0.90	3303901	0.826	2331	1.57
2		3350004			
2		3354625			
3	More flow 1.00	3050006	0.317	2253	1.55
3		3050125			
3		3059621			

TABLE 6: ROBUSTNESS VALUES OF METFORMIN (CHANGE IN FLOW RATE)

S. no.	Flow rate (ml/min)	AUC	Relative standard deviation (% RSD)	System suitability results	
				Plate count	Tailing factor
1	Less flow 0.80	1230000	0.473	3975	1.37
1		1239890			
1		1239001			
2	Actual flow 0.90	952100	0.351	3684	1.35
2		958467			
2		955691			
3	More flow 1.00	920012	0.098	3338	1.46
3		928502			
3		929654			

TABLE 7: ROBUSTNESS VALUES OF VILDAGLIPTIN (CHANGE IN ORGANIC PHASE)

S. no.	Mobile phase	Area	Relative standard deviation (% RSD)	System suitability results	
				Plate count	Tailing factor
1	Less Organic	3509807 3539807 3548795	0.15	3215	1.57
2	Normal	3202811 3208976 3215314	0.87	3310	1.63
3	More Organic	3541605 3550100 3523230	0.31	2370	1.58

TABLE 8: ROBUSTNESS VALUES OF METFORMIN (CHANGE IN ORGANIC PHASE)

S. no.	Mobile phase	Area	Relative standard deviation (% RSD)	System suitability results	
				Plate count	Tailing factor
1	Less Organic	1038103 1034231 10375414	0.099	2515	1.34
2	Normal	3303799 3348987 3355331	0.798	2232	1.31
3	More Organic	1123036 1130252 1122187	0.545	4165	1.32

Robustness: The effect of changes in flow rate and mobile phase composition were studied for both drugs and are shown in the **Table 5** and **7** for vildagliptin and **Table 6** and **8** for metformin.

CONCLUSION: The developed and validated proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous determination of vildagliptin and metformin in pharmaceutical dosage form in Jordanian Market.

Moreover, this method introduces a simple extraction procedure with a little chromatographic run time, that make this method appropriate for the analysis of large number of samples of the pharmacokinetic, bioavailability or bioequivalent studies of vildagliptin and metformin hydrochloride. This developed method was validated as per ICH guidelines. All sample recoveries in all formulations were in good agreement with their respective label claims.

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CONFLICT OF INTEREST: The authors declare that no conflict of interest for this research.

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