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A Validated LC-MS/MS Method for Simultaneous Estimation of Dapagliflozin and Metformin in Pharmaceutical Dosage Form

Sahil Kalyan*[1] and Amrita Parle[2]

1. *Department of Quality Assurance, Delhi Institute of Pharmaceutical Sciences and Research, New Delhi - 110017, India.*
2. *Department of Quality Assurance, Delhi Institute of Pharmaceutical Sciences and Research, New Delhi - 110017, India.*

Corresponding Author: Sahil Kalyan
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ABSTRACT
A precise and highly sensitive LC-MS/MS method was developed and validated for the simultaneous estimation of Dapagliflozin and Metformin in pharmaceutical dosage form. Chromatographic separation was performed on Agilent InfinityLab Poroshell 120 EC-C18 (2.1×100 mm, 2.7 µm) column. Isocratic elution was achieved using 5mM ammonium acetate: acetonitrile (20:80, v/v) as mobile phase with column temperature at 35°C and flow rate at 0.2 mL min^{-1}.The mass spectrometer was operated under multiple reaction monitoring (MRM) mode using electrospray ionization by monitoring the transition pair (precursor to product ion) of m/z 426.20-107.20 and 130.10-60.10 for dapagliflozin and metformin respectively in the positive mode. The method showed linearity in the concentration range of 25-500 ng/mL for dapagliflozin and 100-2000 ng/mL for metformin. The limit of detection (LOD) and limit of quantitation (LOQ) obtained were6.83 ng/mL and 20.70 ng/mL respectively for dapagliflozin and 29.45 ng/mL and 89.24 ng/mL respectively for metformin. The developed method was validated in accordance with the International Conference on Harmonization (ICH) guidelines. The developed method was successfully applicable for the simultaneous estimation of dapagliflozin and metformin in tablet dosage form.
KEYWORDS: Dapagliflozin, Metformin, LC-MS/MS, Validation, Spectroscopy.

I. INTRODUCTION

Type 2 diabetes is a challenging disease as it requires continuous glycaemic control. Metformin (Fig. 1b) is the first-line drug for the treatment of type 2 diabetes along with dietary and lifestyle changes. When the satisfactory glycaemic control is not achieved by metformin alone, patients require additional hyperglycaemic therapy. SGLT2 (Sodium-glucose co-transporter 2) inhibitors like dapagliflozin (Fig.1a) are added to acquire the required glycaemic control. They are relatively new entrants in anti-diabetic therapy which work by inhibiting the SGLT2 proteins responsible for reabsorption of glucose in the kidney. Combination of dapagliflozin and metformin lower blood glucose levels effectively because of their different mechanisms of action in the kidney and liver respectively [1, 2].

Analytical methods available for the simultaneous estimation of dapagliflozin and metformin include HPLC methodfor the API [3], ultraviolet spectrophotometric method for the API utilizing first order derivatizationtechnique [4] and LC-MS/MS method for simultaneous estimation of dapagliflozin and metformin in human plasma [5]. LC-MS/MS is a sophisticated technique having benefits of better sensitivity and selectivity when compared with other techniques of analysis. No LC-MS/MS method is available for simultaneous estimation of Dapagliflozin and metformin in tablet dosage form. Thus, the present study was designed for the simultaneous estimation of dapagliflozin and metformin using LC-MS/MS technique for tablet formulation.

(a) (b)

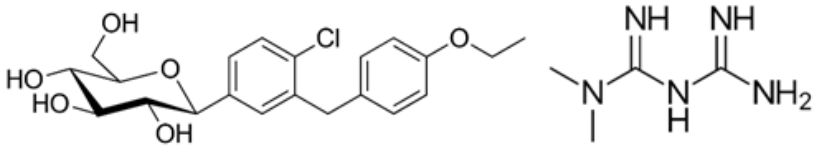

Fig. 1.(a) Chemical structure of dapagliflozin. (b) Chemical structure of metformin

II. MATERIAL AND METHODS

2.1 Chemicals and reagents

Dapagliflozin (99% purity) and Metformin (100.3% purity) were provided asgift samples by Sun Pharmaceuticals limited (Gurgaon, India) with Certificate of Analysis for both drugs. HPLC grade acetonitrile was purchased from Merck (Mumbai, India) and analytical reagent grade ammonium acetate was procured from Fluka Analytical (St. Louis, MO, USA). Water used in the entire analysis was prepared usingMillipore Direct-Q 3UV water purification system by Millipore (Bangalore, India).Xigduo XR tablets containing 10 mg of Dapagliflozin and 500 mg of Metformin, manufactured by AstraZeneca were purchased from market.

2.2 Liquid chromatographic and mass spectrometric conditions [6]

The analysis was performed with the help of Agilent 1200 HPLC system equipped with Agilent 6410 triple quadrupole LC-MS and an electrospray ionization (ESI) source. Chromatographic separation was achieved using an Agilent InfinityLab Poroshell 120 EC-C18 (2.1×100 mm, 2.7 µm) column. The isocratic mobile phase utilized consisted of 5mM ammonium acetate: acetonitrile (20:80, v/v) and was delivered at a constant flow rate of 0.2 mL/min. The column temperature and autosampler temperature were maintained at 35°C and 5°C respectively.The injection volume was kept at 10 µL.

Detection was carried out using the multiple reaction monitoring (MRM) mode to measure the transition pair (precursor to product ion) of m/z 426.20-107.20 for dapagliflozin-ammonium adduct ions $[M+NH_4]^+$and 130.10-60.10 for metformin ions $[M+H]^+$ in the positive mode. The optimized fragmentor value and collision energy were 140 and 40 respectively for the dapagliflozin and 80 and 10 respectively for the metformin and the dwell time was set at 200 ms for both. Quantitation was carried out using Agilent MassHunter Workstation software version B.06.00.

2.3 Preparation of standard solutions

The standard stock solutions were prepared by dissolving 10 mg of each drug in 10 mL diluent i.e. acetonitrile: water (50:50 v/v) to acquire a concentration of 1000 µg/mL of each drug. Working standard solutions containing 25, 50, 100, 250, 500 ng/mL of dapagliflozin and 100, 200,400, 1000,2000 ng/mL of metformin were prepared by serial dilutions from the respective standard stock solutions.

2.4 Sample preparation

Ten tablets were firstly weighed and then finely powdered, an amount of powder equivalent to 10 mg of dapagliflozin and 500 mg of metformin was weighed precisely and transferred to a 10 mL volumetric flask. Required amount of diluent was added to make up the volume, the solution was sonicated for 15 min and filtered using 0.22 µm nylon syringe filter. From the filtrate, measured volume was taken and diluted with the diluent to achieve the final concentrations of 25 ng/mL of dapagliflozin and 1250 ng/mL for metformin.

2.5 Validation of the analytical method

The proposed LC-MS/MS method was validated in accordance with the International Conference on Harmonization (ICH) guidelines [7]. The parameters evaluated were linearity, precision, accuracy, limit of detection, limit of quantitation, specificity and robustness.

III. RESULTS AND DISCUSSION

The analytical method was developed and optimised for the simultaneous estimation of Dapagliflozin and Metformin. Based on signal intensity and reproducibility, transition pair (precursor to product ion) of m/z 426.20-107.20 for dapagliflozin and 130.10-60.10 formetformin are selected for the analysis as shown in Fig. 2, Fig. 3 and Fig. 4.

The representative MRM chromatograms of dapagliflozin and metformin are depicted in Fig. 5, the retention time was found to be 1.44 and 1.32 min for Dapagliflozin (DAPA) and Metformin(MET) respectively.

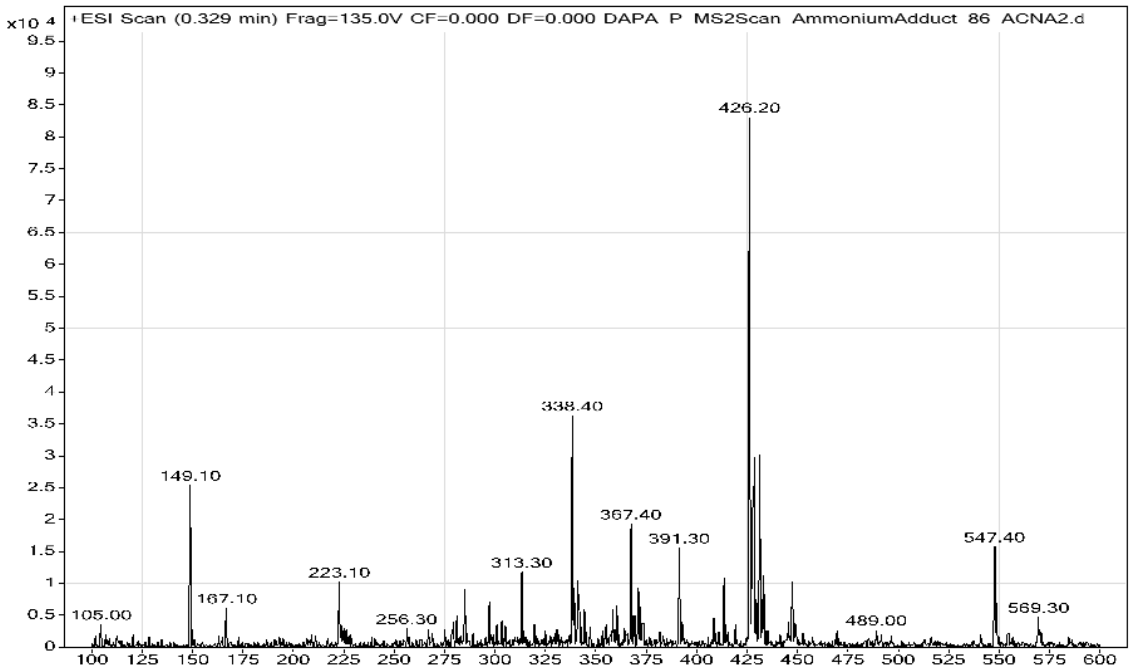

Fig. 2.Full scan mass spectra of dapagliflozin.

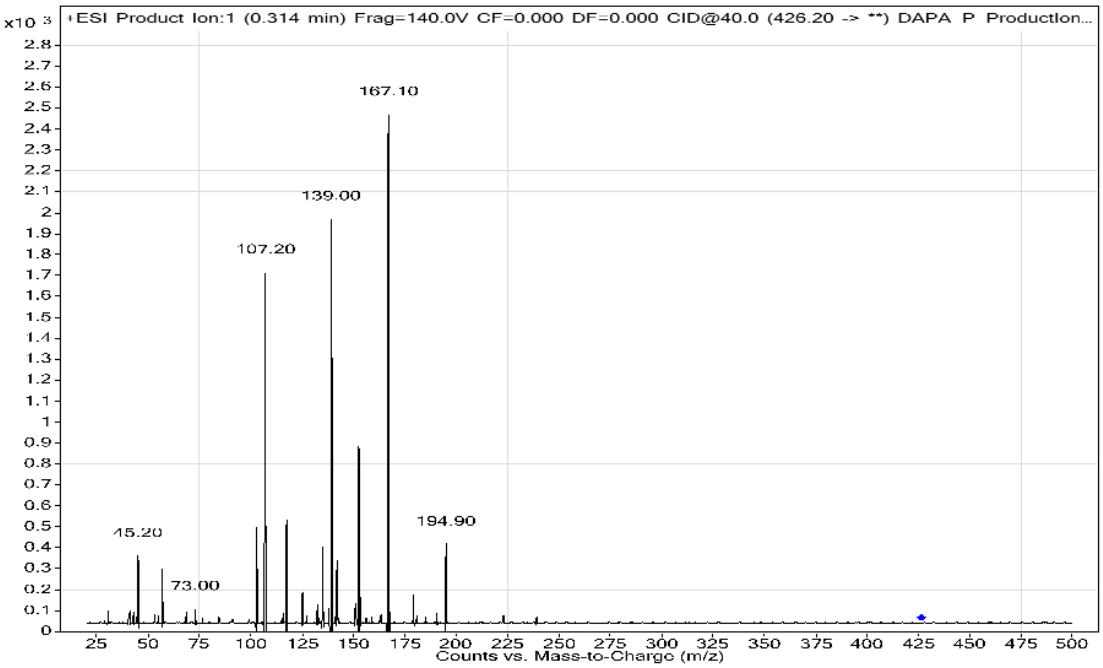

Fig. 3.Product ion mass spectra of dapagliflozin.

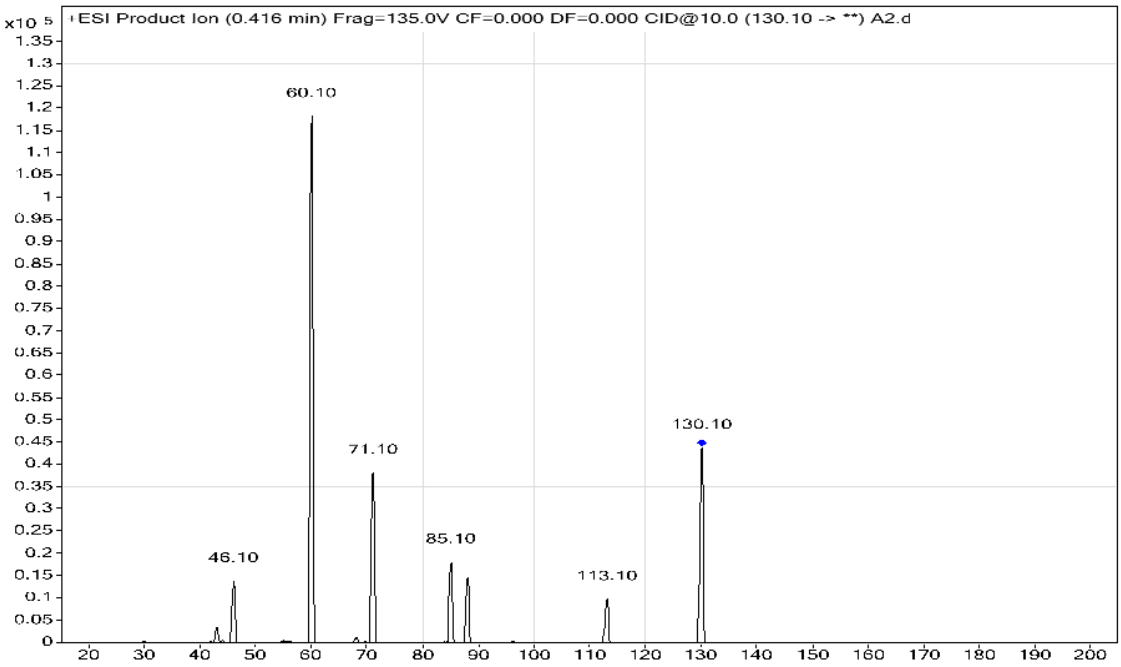

Fig. 4.Product and precursor ion mass spectra of metformin.

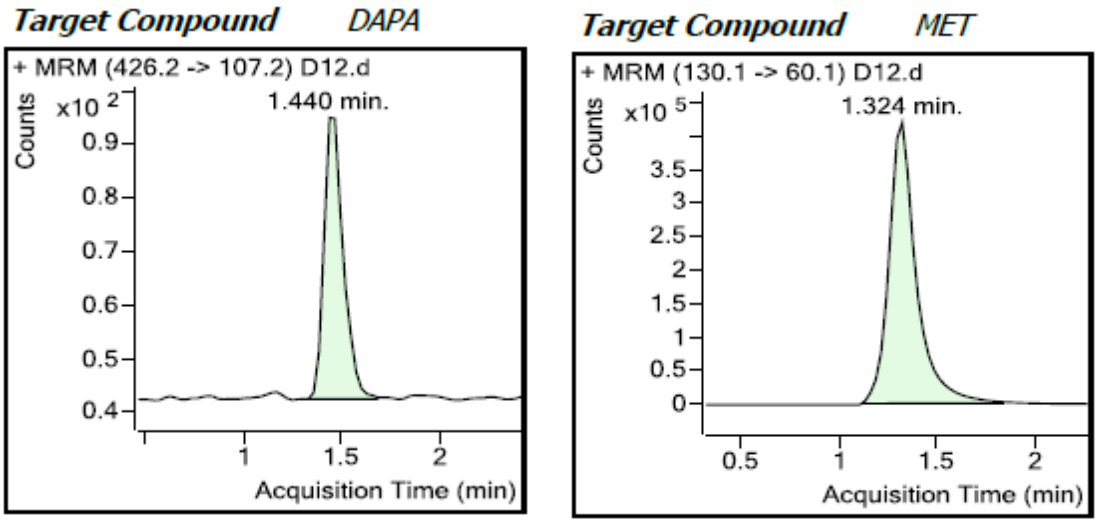

Fig. 5. MRM chromatogram of dapagliflozin and metformin.

3.1 Method validation

The method was validated as per ICH guidelines and the results of validating parameters are discussed below.

3.1.1 Linearity and range

Calibration curve was obtained by plotting the area under the peak (AUP) against the concentrations. Linearity was found to be acceptable over the concentration range of 25-500 ng/mL for dapagliflozin and 100-2000 ng/mL for metformin. Calibration curvesare shown in Fig. 6 and Fig. 7.

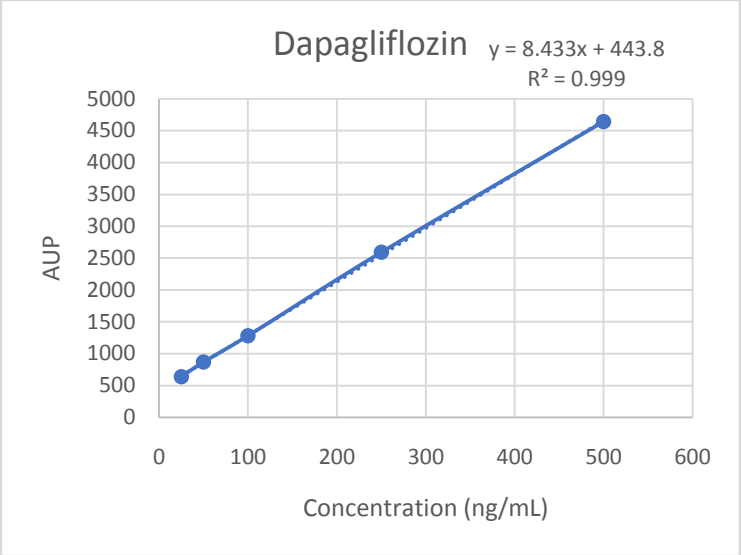

Fig. 6.Calibration curve of dapagliflozin.

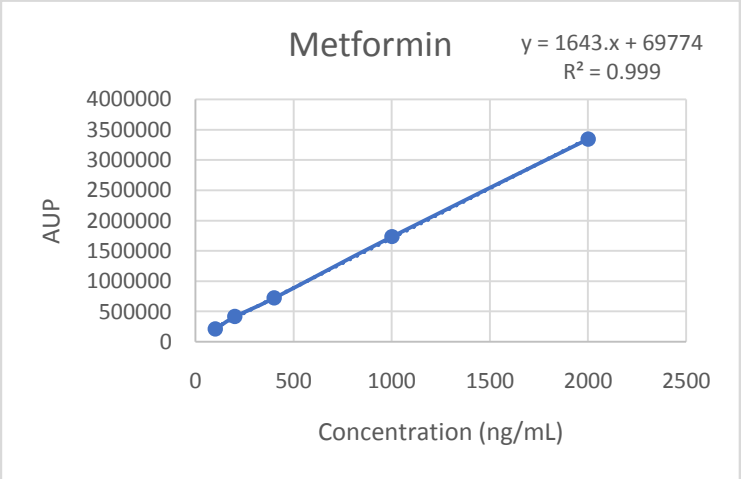

Fig. 7.Calibration curve of metformin.

3.1.2 Limit of Detection (LOD) and limit of Quantitation (LOQ)

The minimum concentrationlevel at which the analyte can be reliably detected (LOD) and quantified (LOQ) were found to be 6.83 ng/mL and 20.70 ng/mL respectively for dapagliflozin and 29.45 ng/mL and 89.24 ng/mL respectively for metformin.The calculation was based on the standard deviation of the response and the slope.

3.1.3 Precision

Precision of the method was established by determining repeatability, intra-day and inter-day precision. Repeatability of the method was confirmed by injecting six replicates of 100 ng/mL of Dapagliflozin and 400 ng/mL of Metformin.

Intra-day and Inter-day precision study was carried out by selecting three concentrationsfrom the linearity range and analysing them in triplicate, on same day and for three successive days.The acceptable%RSD for precision is ≤2. The method was found to be precise with %RSD within acceptable limits. Results for repeatability, intra-day and inter-day precision are presented in Table 1 and Table 2respectively.

Table1

Results for repeatability.

S. No.	Dapagliflozin area	Metformin area
1	1258	722988
2	1269	718640
3	1278	718416

4	1243	724260
5	1285	722624
6	1265	720384
Mean	1266.33	721218.7
Standard deviation	14.88	1431.31
%RSD	1.18	0.34

Table2

Intra-day & inter-day precision results for dapagliflozin and metformin.

Drug	**Conc. (ng/mL)**	**Intra-day (n=3)**		**Inter-day (n=3)**	
		Avg. area	**%RSD**	**Avg. area**	**%RSD**
Dapagliflozin	50	859	0.81	863.33	0.88
	100	1257	0.84	1260.67	0.79
	250	2564	0.82	2575.33	0.82
Metformin	200	407284	0.59	416932	1.03
	400	725066	0.52	728312	0.86
	1000	1751025	0.55	1755260	0.83

3.1.4 Accuracy

Accuracy of the method was studied by spiking the pre-analysed conc. at 50%, 100% and 150%, and calculating the % recovery.Acceptable % recovery is 100 ±2% and the accuracy of the methodwas within the specified range. Data for accuracy is shown in Table 3.

Table3

Results for accuracy

Drug	**% level**	**Amount spiked (ng/mL) n=3**	**Amount recovered (± SD) ng/mL**	**% recovery**
Dapagliflozin	50	50	50.37 (±0.72)	100.75
	100	100	100.10 (±0.48)	100.1
	150	150	149.65 (±0.33)	99.77
Metformin	50	200	201.04 (±0.0.46)	100.52
	100	400	399.16 (±0.28)	99.79
	150	600	599.49 (±0.52)	99.91

3.1.5 Robustness

Deviations in chromatographic conditions like flow rate (± 1%), mobile phase ratio (± 2%) and column temperature (± 5°C) were studied to determine the robustness of the method, %RSD ≤2% is acceptable for a method to be robust.Developed method was robust with a %RSD<1%.Results are shown in Table 4.

Table4

Results for robustness

Drug	**Parameter**	**Variation (n=3)**	**%RSD**
Dapagliflozin	Flow	0.202	0.63
	Flow	0.198	0.61
	Mobile phase	81.6	0.4
	Mobile phase	78.4	0.38
	Temperature	40	0.62

	Temperature	30	0.64
Metformin	Flow	0.202	0.65
	Flow	0.198	0.64
	Mobile phase	81.6	0.45
	Mobile phase	78.4	0.42
	Temperature	40	0.51
	Temperature	30	0.53

3.1.6 Application on the pharmaceutical dosage form

Application of the method on pharmaceutical formulation was tested by performing the assay on the Xigduo (10 mg Dapagliflozin and 500 mg Metformin) tablets.The acceptable % assay should be 100±2% and the % assay was found to be 99% for dapagliflozin and 100% for metformin. It can be successfully applied for the simultaneous estimation of dapagliflozin and metformin in tablets. Results for the assay are shown in Table 5.

Table5

Results for assay of dapagliflozin and metformin combination tablets

Pharmaceutical formulation	**Drug**	**Claimed conc. (ng/mL)**	**Mean (± SD) amount found (n=6)**	**%Assay**
Xigduo XR 10/500 mg tablets	Dapagliflozin	25	24.83 (±0.43)	99.32
	Metformin	1250	1251.37 (±0.35)	100.11

IV. CONCLUSION

The established LC-MS/MS method for pharmaceutical dosage form analysis utilizes positive ion electrospray ionization technique for the simultaneous estimation of dapagliflozin and metformin in tablet dosage form while the previous method available for the estimation of dapagliflozin and metformin was a bioanalytical method developed using negative ion electrospray ionization technique. The proposed method was validated for linearity, precision, accuracy and robustness as per ICH guidelines. The developed method is proved to be highly sensitive, accurate and reproducible.This LC-MS/MS method adds on a sophisticated analytical tool in the repertoire of analyst for simultaneous estimation of dapagliflozin and metformin for tablet dosage form.

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Conflicts of interest

Authors declare that there are no conflicts of interest.

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