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Development and Validation of RP-HPLC Method for Simultaneous Estimation of Teneligliptin, Pioglitazone Hydrochloride and Metformin Hydrochloride in Bulk and Their Pharmaceutical Dosage Form

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ABSTRACT

The pharmaceutical combined dosage forms of teneligliptin, pioglitazone hydrochloride and metformin hydrochloride are used for the treatment of type II diabetes mellitus. A reverse-phase high-performance liquid chromatography method has been developed for simultaneous estimation of teneligliptin, pioglitazone hydrochloride and metformin hydrochloride available in combined tablet dosage form. The method was developed using Kromasil 100 C18 column (150 mm x 4.5 mm x 5 µm), and mobile phase composition of acetonitrile:10 mM phosphate buffer (pH 3.5): methanol in the ratio of (30:50:20 v/v/v). The flow rate was adjusted at 0.8 ml/min for isocratic elution and detection was performed at 238 nm with UV detector. The retention time for metformin hydrochloride, teneligliptin and pioglitazone hydrochloride was found to be 1.74 min, 2.77 min and 5.98 min, respectively. The method was validated as per ICH guidelines. The linearity range was found to be 5-25 µg/ml for teneligliptin and pioglitazone hydrochloride and 150-750 µg/ml for metformin hydrochloride. The percent recovery was found to 101.9% for teneligliptin, 102.6% for pioglitazone hydrochloride and 99.7% for metformin hydrochloride, which indicate that method is accurate. The %RSD was found to be less than 2%, which indicates the developed method is precise. The proposed methods were successfully applied for the quantification of teneligliptin, pioglitazone hydrochloride and metformin hydrochloride in pharmaceutical formulations without any interference from excipients.

Keywords: Teneligliptin, Pioglitazone hydrochloride, Metformin hydrochloride, RP-HPLC

Abbreviations: TEN- Teneligliptin; PIO- Pioglitazone hydrochloride, MET- Metformin hydrochloride

INTRODUCTION

Currently, multicomponent formulation is used in various combinations of medications for improved therapeutic index, immediate relief, and lesser adverse effects. In diabetic mellitus treatment, combination rationales for the most commonly used fixed drug combinations (FDC) medications are to provide rationale drug regulatory mechanism and enhance drug therapeutic effectiveness.[1]

Subsequent the widespread approval of two drugs fixed drug combinations (FDC) for oral antidiabetic drug; Indian pharmaceutical companies have introduced triple FDC of teneligliptin, pioglitazone hydrochloride and metformin hydrochloride.[2]

Fixed drug combinations have been associated with better compliance and enhanced glycaemic control.[3] Reducing the number of drugs, which reduces the difficulty of the treatment, so that greater patient adherence is expected with combined dosage forms. In general, these kinds of multicomponent dosage forms are useful for effective therapy and enhance patient compliance. Method developed can be conveniently used for quality control and routine determination of drug in pharmaceutical preparation in pharmaceutical industry.

Teneligliptin, pioglitazone hydrochloride and metformin hydrochloride are combination of three antidiabetic medications.

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Teneligliptin is a third-generation dipeptidyl peptidase-4 (DPP-4) inhibitor, chemically, it is [(2S,4S)-4-[4-(5-methyl-2-phenylpyrazol-3-yl) piperazin-1-yl] pyrrolidin-2-yl] -(1,3-thiazolidin-3- yl) methanone (Figure 1a). Teneligliptin increases the release of insulin from the pancreas and decreases the hormone (glucagon) that raises blood sugar levels. This reduces the fasting and post meal sugar levels. [4,5]

Pioglitazone, chemically is 5-[[4-[2-(5-ethylpyridin-2-yl) ethoxy] phenyl] methyl]-1,3- thiazolidine-2,4-dione; hydrochloride, belonging to thiazolidinediones category of antihyperglycemic agents (Figure 1b). Pioglitazone primarily acts on muscle, fat, and liver cells to enhance their response to insulin, making it easier for these cells to take up glucose from the bloodstream. It also reduces the production of glucose by the liver, which is a common problem in people with type 2 diabetes. [6,7]

Metformin HCl is N,N-Dimethylimidodicarbonimidic diamide belonging to biguanides category of antihyperglycemic agents. It is used to reduce blood sugar released into the bloodstream from the liver and increasing the body's use of glucose (Figure 1c). Metformin decreases the amount of glucose absorbed from your food and the amount of glucose made by the liver. It also increases the body's response to insulin, a natural substance that controls the amount of glucose in the blood. [8]

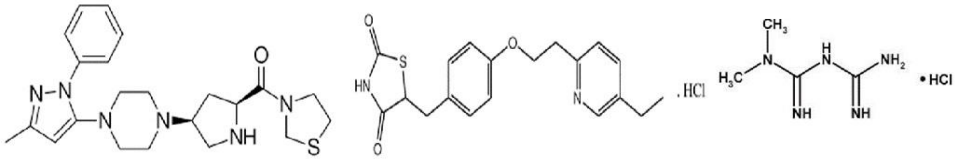

Fig.1. Chemical structure of a) Teneligliptin b) Pioglitazone HCL c) Metformin HCL

Formulations containing various components put challenges in analysis and therefore simultaneous analysis has become interest for analytical chemists for the last few years in the field of analytical chemistry. The analytical methods for simultaneous determination are proposed to ensure that the formulation must have an equal quantity of active ingredients as claimed in the label. Out of various analytical methods, chromatography is ideal in the quantification of multi-component dosage forms due to their benefits of cost-effectiveness, robustness, accuracy, and high specificity. [9]

Several analytical methods have been stated for analysis of the studied drugs separately, and in combination with others drugs. However, to the best of our knowledge, no RP-HPLC method has been reported in literature for the simultaneous estimation of these three commonly used important antidiabetics: teneligliptin, pioglitazone hydrochloride and metformin hydrochloride in bulk and pharmaceutical formulation. A review of the literature revealed that several analytical methods, such as UV-visible spectroscopy[10-12], RP-HPLC[13-21], RP-UHPLC[22], stability-indicating HPLC[23], and RP-UFLC[24] have been stated thus far for the measurement of TEN, PIO and MET alone or in combination with other medications. Comparing to the individual analysis of these drugs as per their respective official methods in pharmacopoeias, developing a single isocratic RP-HPLC method for simultaneous analysis of these three antidiabetics has substantial advantages like it avoids time consuming extraction and separation, minimize the use of costly regents and method further being accurate and precise. There is requirement of developing an accurate, precise and robust reversed phase HPLC method for simultaneous quantification of teneligliptin, pioglitazone hydrochloride and metformin hydrochloride in bulk and their combined dosage form.

MATERIAL AND METHODS

Materials

The Standard drug substance of teneligliptin and metformin hydrochloride were obtained as gift samples from Zydus life sciences, Ahmedabad, India and pioglitazone hydrochloride was obtained as a gift sample from Torrent Pharmaceuticals, Ahmedabad, India. The marketed tablet formulation of teneligliptin, pioglitazone HCl and metformin HCl (Zita-Pio Met_{500}) by Glenmark Pharmaceuticals were purchased from local market. AR grade of potassium di-hydrogen ortho-phosphate, ortho- phosphoric acid, and HPLC grade methanol, water, and acetonitrile were used in research.

Instrumentation

HPLC system, LC-2030 plus (Shimadzu Co.) with Lab solutions software was used for method development and validation. Shimadzu 1800 series UV double beam spectrophotometer with UV-Probe, version 2.33 software for obtaining the UV spectra of all three drugs. The pH of solution was measured with Electro quip pH metre (PHCAL).

Chromatographic Condition

The separation was performed on kromasil 100 C_{18} column (250mm x 4.5mm x 5 µm) using isocratic mobile phase comprising of acetonitrile:10 mM phosphate buffer (pH3.5): methanol (30:50:20, v/v/v), pH 3.5 adjusted with ortho-phosphoric acid. The detection was carried out at 238 nm. The flow rate was maintained at 0.8 ml/min and temperature of column kept at 30 ± 2 ºC. The sample were injected in volume of 10 µl.

Preparation of mobile phase and diluent

A 10 mM Potassium phosphate buffer (pH 3.5) was prepared in 1000 mL of HPLC water by dissolving potassium di-hydrogen ortho-phosphate. The pH of the solution was then adjusted to 3.5 ± 0.2 with ortho-phosphoric acid. The mobile phase was prepared by mixing acetonitrile: potassium phosphate buffer pH, and methanol in ratio of (30:50:20 v/v/v) and filtered with cellulose acetate filter paper of 0.45 microns using vacuum pump and same mobile phase was used as diluent.

Preparation of standard stock solution of teneligliptin, pioglitazone hydrochloride and metformin hydrocholoride (1000 µg/ml)

Accurately weighed 10 mg of teneligliptin, pioglitazone hydrochloride and metformin HCl were transferred separately into a 10 ml volumetric flask, and the volume was made up to the mark with methanol to get a concentration of 1000 µg/ml.

Preparation of a working stock solution of teneligliptin, pioglitazone hydrochloride and metformin hydrocholoride

From the above standard stock solution, 0.1 ml of teneligliptin, 0.1 ml of pioglitazone hydrochloride and 3 ml of metformin HCl were transferred into a 10 ml volumetric flask and diluted with diluent to get a concentration of 10 µg/ml for teneligliptin, 10 µg/ml for pioglitazone HCL and 300 µg/ml for metformin HCL.

Preparation of test sample

Average weight of 20 tablets (Zita-Pio Met_{500}) were noted and tablets were powdered using mortar pestle. Tablet powder equivalent to 20 mg of teneligliptin, 15 mg of pioglitazone HCl and 500 mg of metformin HCl was accurately weighed and transferred into 50 mL volumetric flask and dissolved in diluent. The solution was sonicated for 10 minutes using ultra-sonicator and volume was made up to mark with diluent. The solution was filtered through 0.45 µm whatman filter paper. A pipette out 0.5 mL of the above solution into 10 mL volumetric flask and volume was made up to mark with diluent, and injected into HPLC.

METHOD VALIDATION

Method was validated according to International Council on Harmonization guideline Q2 (R1) for validation of analytical method.

Linearity

The linear response was determined by analysing six independent levels of the calibration curve in the range of 5–25 µg/ml for TEN, PIO and 150-750 µg/ml MET. Each solution was analysed in triplicate. Peak area was recorded, and the calibration curve was plotted by plotting peak area vs. concentration. The result should be expressed in terms of the correlation coefficient.

Precision

Intraday and Interday precision were performed by injecting three replicates of 10 µg/ml, 15 µg/ml and 20 µg/ml of teneligliptin and pioglitazone HCl, and 300 µg/ml, 450 µg/ml and 600 µg/ml of metformin HCl. The results of precision are expressed as the relative standard deviation.

Accuracy

The accuracy of the method was performed by spiking a previously analysed test sample with three different concentrations of standard at 80%, 100%, and 120%, respectively. Accuracy can be expressed as % recovery by the assay of a known, added amount of a standard of drugs.

Limit of detection and Limit of quantification

The calibration curve was repeated five times, and the standard deviation (SD) of the intercepts (response) and slope were calculated. Then LOD and LOQ were measured by using mathematical expressions: LOD = 3.3 δ/S, LOQ = 10 δ/S, where S is the slope of the calibration curve and δ is the standard deviation of the y-intercept of the regression line.

Robustness

Robustness measures the method's reliability under a variety of conditions. Robustness was typically carried out by deliberately varying critical method parameters such as the flow rate, pH, mobile phase composition, and detection wavelength. The method's robustness is evaluated based on its ability to produce results within acceptable ranges of accuracy, precision, and other performance characteristics, despite the variations.

Specificity

Specificity focuses on demonstrating that an analytical method can accurately identify and quantify the analyte in the presence of other substances. It helps in distinguishing the analyte from other components and ensures that the measurement is not confounded by external factors. Specificity was determined by injecting sample, standard and blank solution and resultant chromatograms were compared to check the interference.

System Suitability

System suitability was performed to check that the analytical method is suitable for the intended analytical procedure and to check that the method is performing as expected before analysing actual samples. The parameters used in these were asymmetry of the chromatographic peak, theoretical plates, retention time, and resolution.

RESULT AND DISCUSSION

HPLC method optimization

To establish and validate an efficient RP-HPLC method for quantification of teneligliptin, pioglitazone HCl and metformin HCl, different chromatographic conditions were applied. Various ratio of water: acetonitrile, water: methanol, methanol-acetonitrile to water were tried, but proper separation was not achieved. In order to get a successful separation, phosphate buffer pH 3.5 with methanol and acetonitrile was utilised. Finally, separation was performed using a Kromasil 100 C18 column (250 mm x 4.5 mm x 5 µm), with a pH 3.5 mobile phase (acetonitrile: 10 mM phosphate buffer pH 3.5: methanol in ration of 30:50:20 V/V/V), a flow rate of 0.8 ml/min, and a detection wavelength of 238 nm. Figure 2 displays the chromatogram of the TEN, PIO and MET standard solutions. TEN, PIO and MET were shown to have retention time of 1.74, 2.77 and 5.98 minutes, respectively. The method was validated including validation parameters like specificity, linearity, precision, accuracy, limit of detection, limit of quantification and robustness as per ICH guidelines. The results are shown in the following tables.

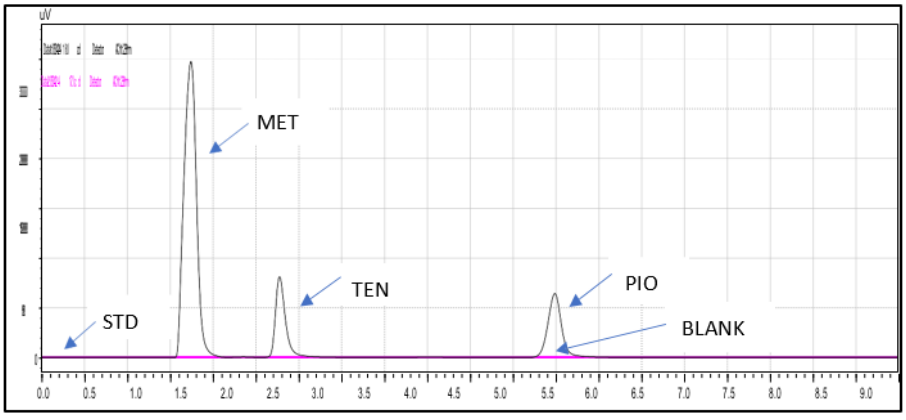

Fig. 2. Overlaid HPLC Chromatogram of blank and standard of TEN, PIO and MET

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Method Validation results

Linear responses were observed in linearity range of 5–25 µg/ml for TEN, PIO and 150 -750 µg/ml for MET. It was discovered that the calibration curves of TEN, PIO and MET had correlation values of 0.9975, 0.9977, 0.9946, respectively. In **Figures 3-5**, the calibration curve is displayed. **Table 1** includes the linearity data for TEN, PIO and MET. The precision study, which is typically represented as a percentage of RSD. TEN, PIO and MET were found to have mean percentage RSD values for intra-day precision of 0.184, 0.316 and 0.163, respectively, and mean percentage RSD values for inter-day precision of 0.184, 0.310 and 0.267, respectively. The low RSD values show that the proposed method is precise. **Tables 2** show the precision study's results. TEN, PIO and MET were discovered to have LOD values of 0.180 µg/ml, 0.187 µg/ml and 3.108 µg/ml, respectively, and LOQ values of 0.548 µg/ml, 0.569 µg/ml and 9.420 µg/ml, respectively. The developed method is sensitive enough to identify and quantify smaller concentrations of drugs, as evidenced by the lower values of LOD and LOQ. The accuracy study results are represented as a percentage of recovery and are obtained by adding various concentrations of a standard solution to a test solution. It was discovered that TEN, PIO and MET had recovery rates of 98-101.9%, 98.87-102.6% and 99.07-101.5%, respectively. The accuracy of the suggested procedure is demonstrated by the recovery data. **Table 3** presents the accuracy study's results. This method was specific because no interfering peaks were observed, and the retention time of the test sample was the same as that of standard teneligliptin, pioglitazone hydrochloride and metformin hydrochloride. The results of the robustness study can be expressed as % RSD for peak area for TEN, PIO and MET. The results indicate an insignificant difference in results, indicating that the proposed method is robust. The results of the robustness study are shown in **Table 4. Table 5** presents the findings of the system suitability analysis and makes it abundantly evident that the suggested method is appropriate for the effective separation of TEN, PIO and MET with good peak symmetry, number of HETP, and resolution.

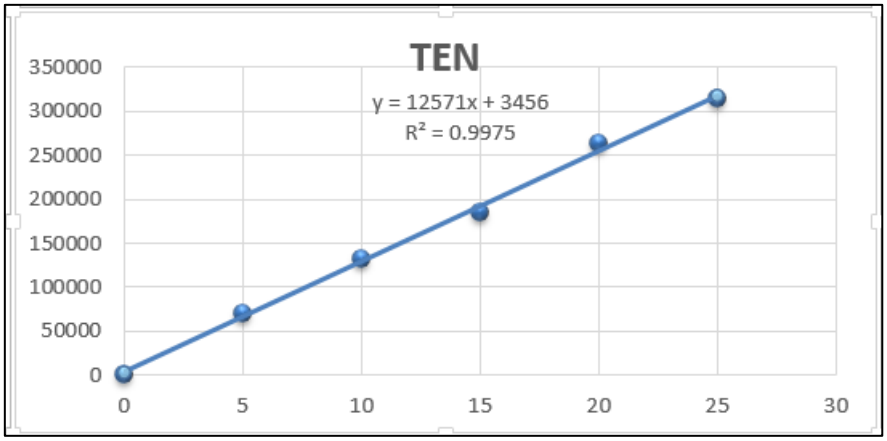

Fig. 3: Calibration curve of TEN (5 -25 µg/ml)

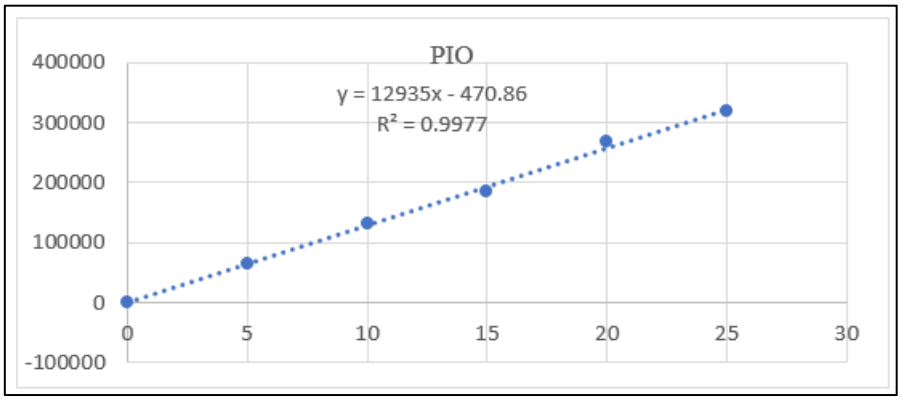

Fig. 4: Calibration curve of PIO (5- 25 µg/ml)

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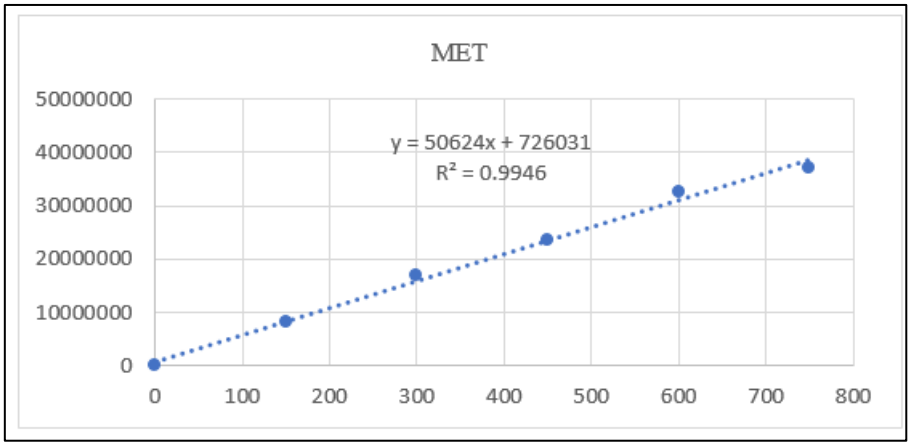

Fig. 5: Calibration curve of MET (150- 750 µg/ml)

Table 1: Result of Linearity

Conc (µg/m)	Peak area (mean ± S.D, n=3)	%RSD	Conc (µg/ml)	Peak area (mean ± S.D, n=3)	%RSD	Conc. (µg/ml)	Peak area (mean ± S.D, n=3)	%RSD
TEN			**PIO**			**MET**		
5	69862.33±520.2	0.744	5	63464±348.7	0.549	150	8145173.33±31175.5	0.382
10	132570.3±293.1	0.221	10	131674.7±592.2	0.449	300	16826164±20054.1	0.123
15	184431.7±272.9	0.147	15	185220±550.2	0.297	450	23617958±34326.2	0.145
20	262929.7±900.9	0.384	20	267113.3±890.2	0.333	600	32468820±63086.7	0.194
25	313777±1347.7	0.429	25	319825±1302.1	0.407	750	37203163±89823.5	0.241

Table 2: Result of Precision

Parameter	Peak area (mean±S.D, n=3)	% RSD	Peak area (mean ± S.D, n=3)	% RSD	Peak area (mean ± S.D, n=3)	% RSD
Intraday precision	196675.2±243.1	0.357	210722.6±450.6	0.310	24781714.4±14326.5	0.267
Interday precision	197566.8±356.7	0.184	196932.4±630.2	0.316	24696403.4±23343.4	0.163

Table 3: Result of Accuracy study

Drug	% Level	Test Conc. (µg/ml)	Amount of standard drug added (µg/ml)	Total Conc. (µg/ml)	Found Conc. (µg/ml)	% recovery ± S.D.
TEN	80	10	8	18	17.64	98.00 ± 0.55
	100	10	10	20	20.38	101.9 ± 0.12
	120	10	12	22	21.65	98.40 ± 0.35
PIO	80	7.5	6	13.5	13.45	99.62 ± 0.43
	100	7.5	7.5	15	15.40	102.6 ± 0.23
	120	7.5	9	16.5	16.15	98.87 ± 0.55
MET	80	250	200	450	457.09	101.5 ± 0.32
	100	250	250	500	495.39	99.07 ± 0.44
	120	250	300	550	558.20	101.4 ± 0.53

Table 4: Result of System Suitability study

Parameter	MET	TEN	PIO
Retention time (min)	1.74	2.77	5.98
Theoretical plates	4289 ± 56	4952 ± 27	5647 ± 65
Asymmetry	1.03 ± 0.11	1.44 ± 0.08	1.04 ± 0.18
Resolution	-	3.57 ± 0.9	6.87 ± 0.4

Table 5: Result of robustness study

Parameter	TEN (% RSD)	PIO (% RSD)	MET (%RSD)
Flow rate	0.103	0.210	0.130
Mobile phase composition	0.268	0.808	0.179
Wavelength	0.280	0.345	0.145
pH	0.160	0.175	0.071

Analysis of the marketed formulation by the proposed method

Teneligliptin (20 mg), pioglitazone HCl (15 mg) and metformin hydrochloride (500mg) in marketed tablets were analysed using assays, and the results showed good agreement with the contents as stated. The marketed formulation contained 99.35% teneligliptin, 98.60% pioglitazone HCl and 99.07% metformin hydrochloride. Since there was no evidence of excipient interaction with the peaks of interest, and the suggested method might be effectively used to estimate teneligliptin, pioglitazone HCL and metformin HCL simultaneously in a combination tablet dose form. **Table 6** presents the assay findings.

Table 6: Estimation of TEN, PIO and MET in marketed formulation

Drug	Label claim	Amount Found	% Assay ± S.D.
PIO	20 mg	19.67	99.35 ± 0.30
TEN	15 mg	14.79	98.60 ± 0.27
MET	500 mg	495.38	99.07 ± 0.32

CONCLUSIONS

The developed RP-HPLC method can be utilised for routine analysis of TEN, PIO and MET in bulk as well as their pharmaceutical formulations. It was proven to be accurate, precise, sensitive, and specific. The method was successfully applied to the available marketed formulation without any interference due to the excipients which indicates method is specific and can have an application in the industry.

CONFLICT OF INTEREST: Authors declare no conflicts of interest.

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