International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.3, No.3, pp 1459-1466, July-Sept 2011

Development of UV Spectrophotometric method for the simultaneous estimation of Simvastatine and Ezetimibe in tablet dosage form by simultaneous Equation and Absorbance ratio method.

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Abstract: versatile, accurate, precise and economic method for simultaneous determination of simvastatin and ezetimibe in fixed dose combination products was developed. The absorbance values at 238.2 nm and 247.6 nm and 243.3nm (isoabsorptive point) were used for the estimation of simvastatin and ezetimibe, respectively without mutual interference. This method obeyed Beer's law in the concentration range of 3–18 µg /ml for simvastatin and 5-30 µg /ml for ezetimibe. The results of analyses have been validated statistically for linearity, accuracy and precision, LOD and LOQ of the proposed method.
Keywords: Simvastatin (SMV), Ezetimibe (EZE), methanol, distilled water, Simultaneous equation method, Absorption ratio method.

INTRODUCTION

Simvastatin (SMV) is chemically is 2,2-Dimethyl butanoic acid (1S,3R,7S,8S,8aR)-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-[(2R,4R)- tetrahydro-4-hydroxy-6 oxo2H pyran-2yl]ethyl]1-napthalenyl ester used as a HMG-CoA reductase inhibitors.[1-2] Ezetimibe (EZT) is chemically (3R,4S)-1-(4-Fluoro phenyl)-3- [(3S)-3-(4-floro phenyl]-3-hydroxyl propyl]- 4-(4-hydroxy phenyl)-2-azetidinone used as a Cholesterol absorption Inhibitors3-4. SIM is official in Indian Pharmacopoeia and EZM is official in USP. By the literature survey HPLC, Stability Indicating HPLC, LC-MS methods have been reported for the estimation of SIM while UV, HPLC and LC-MS methods have been reported for EZM. Moreover the literature survey revealed that so far, no method has been reported for estimation of SMV and EZT in combined dosage form by Q- absorbance equation and simultaneous equation methods using UV spectroscopy. Hence we attempt to develop simultaneous spectrophotometric estimation of Simvastatin and ezetimibe in tablet dosage form.[3-4]

MATERIALS AND METHODS:

A Shimadzu UV/Visible double beam spectrophotometer (UV model– 1700) and 1cm UV

matched quartz cells were used. Gift samples of SIM and EZM were obtained from Lupine pharmaceuticals Ltd, Pune,Methanol AR Grade, Distilled Water.

INSTRUMENTATION

A Shimadzu UV/Visible spectrophotometer, model 1700 (Japan) was employed with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm, with automatic wavelength correction employing a pair of quartz cells. A Shimadzu electronic analytical balance (AX-200) was used for weighing the sample.

PREPARATION OF STANDARED STOCK SOLUTION:

A. Standard Simvastatine stock solution (100 µg/mL)

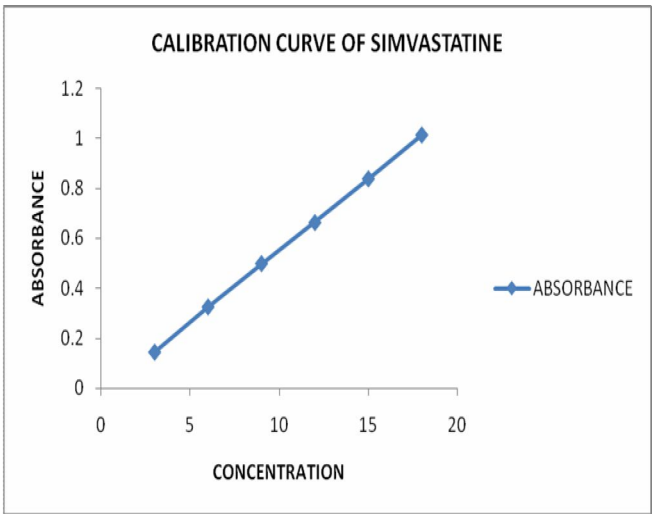
Simvastatine standard stock solution was prepared by weighing 10 mg of Simvastatine and transferred to a 100 ml volumetric flask and volume was made up to 100 ml with Methanol & Water in the ratio of 40: 60(Methanol: Water) to get a concentration of 100µg/ml, The prepared solution is sonicated for 5 minutes and filtered through the whatman filter patae no. 41. [5-7]

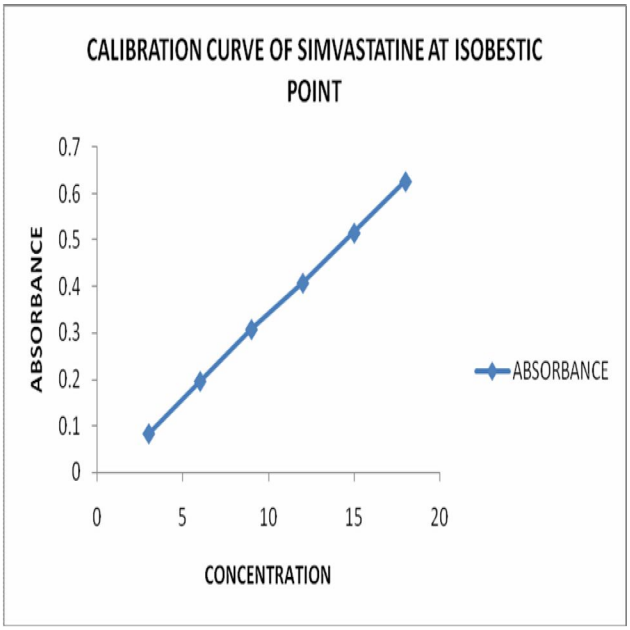
B. Standard Ezetimibe stock solution (100 µg/mL)

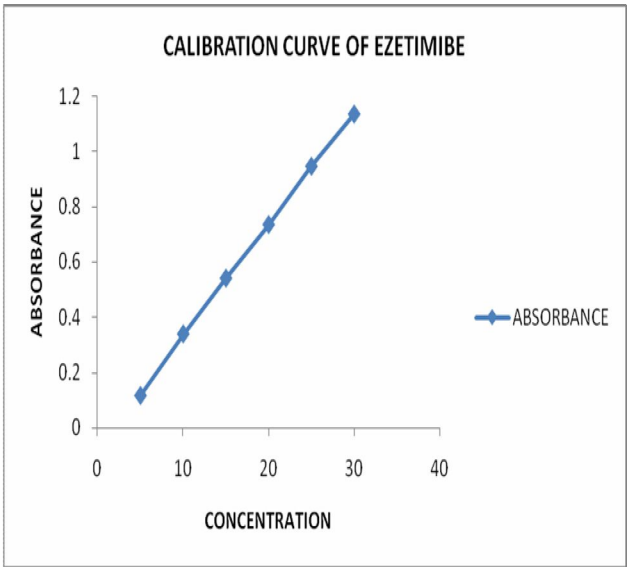
Ezetimibe standard solution was prepared by weighing 10 mg of EZT to a 10 ml volumetric flask and volume was made up to 100 ml with with Methanol & Water in the ratio of 40: 60(Methanol: Water) to get a concentration of 100 µg/ml. The prepared solution is sonicated for 5 minutes and filtered through the whatman filter paper no. 41.[7-9]

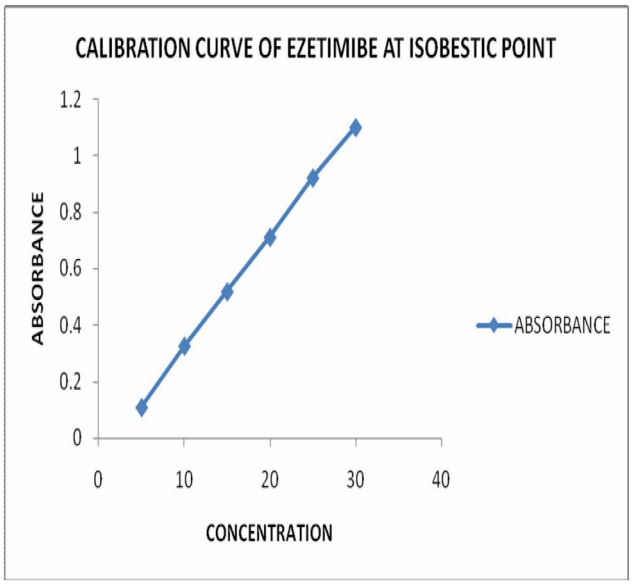
CALIBRATION CURVE

A calibration curve was plotted over a concentration range of 3-18 µg/mL Simvastatine 5-30µg/ml Ezetimibe. Accurately measured standard stock solution of Simvastatine (0.3, 0.6, 0.9, 1.2, 1.5 & 1.8mL) and standard stock solution of Ezetimibe (0.5, 1, 1.5, 2, 2.5 & 3mL) were transferred to a separate series of 10 mL of volumetric flasks and diluted to the mark with Methanol and Water in the proportion of 40:60. The absorbance of each solution was measured at the wavelengths 238.2 nm 243.3nm and 247.6.nm.Calibration curves were constructed for Simvastatine and Ezetimibe by plotting absorbance versus concentrations at both wavelengths. Each reading was average of five determinations.[10-12]









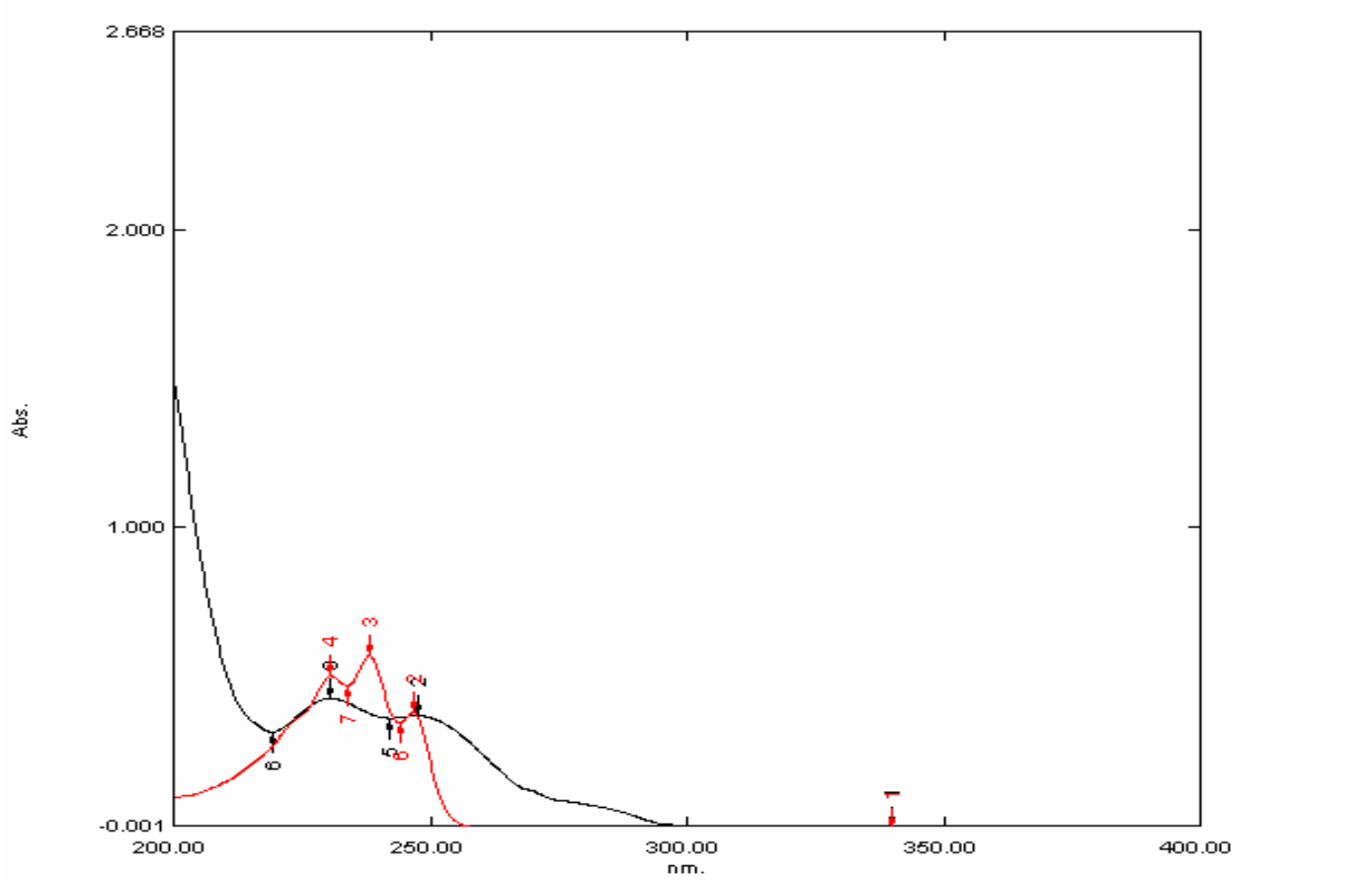
SELECTION OF ANALYTICAL WAVELENTH

For selection of analytical wavelength for the Q-absorbance method (Method-1)

The stock solutions of SMV and EZT were separately diluted in Methanol and water in the ratio of 40:60 to get a concentration of 10 µg/ml of SIM and 10 µg/ml of EZB respectively and scanned in the wavelength range of 200 -400 nm. From the overlay spectra of both drugs, wavelengths 243.3 nm (isoabsorptive point) , 247.6 nm (λ max of EZB) and 238.2 nm(λ max of SIM) were selected for the formation of Q-absorbance equation. The absorbance of various dilutions of Simvastatine measured at 238.2 nm and calibration curves were plotted. Similarly the absorbance of various dilutions of Ezetimibecmeasured at 247.6 nm , calibration curves were plotted. The absorptivities (A1%, 1cm) of each drug at both the wavelengths were also determined.

The absorbance and absorptivity values at the particular wavelengths were calculated and substituted in the following equation, to obtain the concentration.[12-15]

OVERLAY SPECTRUM OF SIMVASTATINE AND EZETIMIBE



METHODS

A) ABSORPTION RATIO/Q METHOD

ANALYSIS [15-17]

From the over line spectrum of Simvastatine and Ezetimibe, one wavelength was selected for the estimation of both drugs, which is known as isoabsorptive point (at 243.3nm). The dilutions of standard and sample solutions were prepared.The absorptivity values were determined at 243.3nm.The method employs Q values and the concentrations of drugs in sample solution were determined by using the following formula,

C_{SIM} = (QM – QY) X A1 / (QX – QY) X ax1,
C_{EZB} = (QM - QX) X A1/ (QY – QX) X ax2,
Where,
C_{SIM} = concentration of Simvastatine ,
C_{EZB} = concentration of Ezetimibe respectively,
A1 = absorbance of sample at 243.3 nm,

For Simvastatine-
ax1 = the absorptivity of Simvastatine 243.3nm

QX, QY & QM was obtained using the following equation
QX = (absorptivity of Simvastaine at 238.2 nm) / (absorptivity of simvastatine at 243.3 nm)
QY = (absorptivity of Ezetimibe at 238.2 nm) / (absorptivity of Ezetimibe at 243.3 nm) and
QM = (absorbance of sample at 238.2 nm) / (absorbance of sample at 243.3 nm).

For Ezetimibe -
ax2 = the absorptivity of Ezetimibe 243.3 nm
QX,QY & QM was obtained using the following equation
QX = (absorptivity of Ezetimibe at 247.6 nm) / (absorptivity of Ezetimibe at 243.3 nm),
QY = (absorptivity of Simvastatine at 247.6 nm) / (absorptivity of Simvastatine at 243.3 nm)
and
QM = (absorbanc eof sample at 247.6 nm) / (absorbance of sample at 243.3 nm).

B) SIMULTANEOUS ESTIMATION METHOD [17-19]

The spectra of Simvastatine and Ezetimibe of method 1 was used and wavelength 247.6 and 238.2 nm (λ max of EZT and λ max of SMV) were selected for the formation of the simultaneous equations. For calibration curves, stock solutions of Simvastatine and Ezetimibe in the concentration of range of 3 – 18 µg/ml and 5 – 30 µg/ml respectively. The absorbance of Simvastatine and Ezetimibe were measured at 238.2 and 247.6 nm, calibration curves were plotted. The absoptivities of both the drugs at both the wavelengths were determined.

The absorbance and the absorptivity values at the particular wavelength were calculated and substituted in the following equation, to obtain the concentration.

C_{SIM} = (A1ax2 – A2ax1) / (ax2ay1 – ax1ay2).
C_{EZB} = (A2ay1 – A1ay2) / (ax2ay1 – ax1ay2).
Where,
C_{SIM} = Concentration of Simvastatine
C_{ezb} = Concentration of Ezetimibe respectively,

A 1 = absorbance of sample at 238.2 nm,
A 2 = absorbance of sample at 247.6 nm,
ax1 = absorptivity of Simvastatine at 238.2 nm and
ax2 = absorptivityof Simvastatine at 247.6 nm,
ay1 = absorptivity of Ezetimibe at 238.2 nm and
ay2 = absorptivity of Omeprazole at 247.6 nm.

OPTICAL CHARACTERISTICS DATA

Parameter	Method I Q-Absorbance Ratio Ratio		Method II Simultaneous Equation Method	
	Simvastatine	Ezetimibe	Simvastatine	Ezetimibe
Working λmax	238.2 & 243.3	247.6 & 243.3	238.2	247.6
Beer's Low Limit	3-18µg/ml	5-30 µg/ml	3-18 µg/ml	5-30 µg/ml
Correlation coefficient*	0.9997	0.9994	0.9999	0.9995
Intercept*	-0.0217	-0.0814	-0.0227	-0.0767
Slope*	0.0360	0.0397	0.0575	0.0406
Molar Absorptivity(lit/mol/cm)	15068.52	16253.18	24067.76	16621.64
Regression Equation	Y=0.0360x-0.0217	Y=0.0397x-0.0814	y=0.0575x-0.0227	Y=0.0406x-0.0217

*Average of six determination., SIM=Simvastatine., ZB=Ezetimibe.

ANALYSIS OF FORMULATION

Twenty Tablets of brand Adilip (Intas Pharma) containing 10 mg of Simvastatine and 10 mg of Ezetimibe were weighed, average weight determined and finely powdered with the help of mortor and pestle. Appropriate quantity of powder from each tablet equivalent to 10 mg of Simvastatine was accurately weighed transferred to a 100 ml volumetric flask and volume was made up to 100 ml with methanol and water in the proportion of 40:60 shaken vigorously for 15 minutes then sonicated for 5 minutes and filtered through the Whatman filter paper no.41. Necessary dilutions of filtrate were made with methanol and water to get final concentration 8 µg/ml of Simvastatine and 8 µg/ml of Ezetimibe. Absorbance of this solution was measured at 238.2 nm (λ max of Simvastatine 247.6nm((λ max of Ezetimibe), and 243.3 nm (Isobestic Point), values were substituted in the respective formulae of (Method 1 & 2) to obtain concentration .Results are shown in the following table.[20-21]

VALIDATION [20-24]

Validation of the developed method was done accordingto the USP 2006, Asian edition.

LINEARITY

The linearity of the method is its ability to elicit test results that are directly proportional to the concentration of the analyte in samples. The calibration curve was taken in the range of 3-18µg/ml for Simvastatine and 5-30 µg/mL for Ezetimibe at the respective λmax. The correlation coefficient of the linearity was found to be 0.999 at each wavelength for both drugs as shown in table 1.

RECOVERY STUDIES

In order to ensure the reliability and suitability of the proposed method, recovery studies were carried out. It was done by mixing known quantity of standard drug with formulation sample and the content were reanalysed by the proposed method. To a quantity of formulation equivalent to 10 mg of Simvastatine, standard drugs of Simvastatine and Ezetimibe were added at 80%, 100% and 120% levels. This was extracted diluted and reanalysed as per the formulation procedure. Absorbance were noted at respective wavelength. Recovery studies were repeated for six times and the results are shown in following table.[21]

RESULT OF ANALYSIS OF TABLET FORMULATION

Method	Drug Name	Lable Claim in mg	% Lable Claim Found*	Amount Found in mg
I	Simvastatine	10 mg	96.25 %	9.6 mg
	Ezetimibe	10 mg	83.75%	8.3 mg
II	Simvastatine	10 mg	97.5%	9.7 mg
	Ezetimibe	10 mg	92.5%	9.2 mg

SIM- Simvastatine

EZB-Ezetimibe

*Average of six estimation of tablet formulation.

RECOVERY RESULT OF SIMVASTATINE AND EZETIMIBE

Method	Recovery Level	% Recovery	S.D	% RSD OR %COV	% Recovery	S.D	% RSD OR %COV
		Simvastatine			Ezetimibe		
I	80 %	94.20	0.0158	0.1872	95.66	0.0529	0.6145
	100%	95.50	0.06670	0.7059	96.60	0.01732	0.1793
	120%	94.36	0.03240	0.3120	96.36	0.06123	0.5776
II	80%	94.77	0.0254	0.2988	98.11	0.05147	0.5829
	100%	97.50	0.05522	0.5664	96.20	0.07516	0.7813
	120%	95.81	0.0158	0.1872	97.27	0.0500	0.4673

Day	Method I		Method II	
Interday	% of lable claim estimated (Mean± % RSD)			
	Simvastatine	Ezetimibe	Simvastatine	Ezetimibe
	102.87±1.15	99.92±0.4568	100.55±0.304	101±0.4648

PRECISION

The precision of an analytical method is determined by assaying a sufficient number of aliquots of a homogeneous sample to be able to calculate statistically valid estimate of % Relative Standard Deviation (%RSD). Intermediate precision was done to express within laboratory variation, on different days. Five replicates of 8 µg/mL concentration of the working standard mixture and sample solution were analysed %RSD was found to be less than 2%.[22]

SPECIFICITY

Results of tablet solution showed that there is no interference of the excipients when compared with the working standard solution. Thus, the method was said to be specific.

LIMIT OF DETECTION

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. Limit of detection can be calculated using following equation as per ICH guidelines.[23]

$LOD = 3.3 \times N/S$

Where,

N = Standard deviation of the responce and
S = Slope of the corresponding calibration curve.

LIMIT OF QUANTIFICATION

It is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guidelines.[24]

$LOQ = 10 \times N/S$

Where,

N = Standard deviation of the response and
S = Slope of the corresponding calibration curve.

The overlain spectra of both the drugs showed that the peaks are well resolved, thus satisfying the criteria for obtaining maximum precision, based on absorbance ratio. The criteria being the ratios (A2 / A1) / (ax2 / ax1) and (ay2 / ay1) / (A2 / A1) should lie outside the range 0.1 – 2.0 for precise determination of (Y) and (X) respectively. Where A 1/A2 represents the absorbance of mixture at λ1 and λ2, ax1 and ax2 denote absorptivities of (X) at λ1 and λ2 and ay1 and ay2 denote absorptivities of (Y) at λ1 and λ2 respectively. In this context, the above criterion was found to be satisfied for SMV (X) and EZT(Y). Where λ1 (243.3 nm) and λ2 (247.6 nm) for Q-absorbance method, λ1 (238.2 nm) and λ2 (247.6 nm) for simultaneous equation method.

Validation Parameter	Method II (Simultaneous estimation method)		Method I (Q- Absorbance Ratio Method)	
	Simvastatine	Ezetimibe	Simvastatine	Ezetimibe
LOD(µg/ml)	0.33068	1.3360	1.4980	0.13584
LOQ(µg/ml)	1.0073	4.0485	4.539	4.1160

RESULT AND DISCUSSION

The proposed methods for simultaneous estimation of Simvastatin and Ezetimibe in combined tablet dosage form were found to be simple accurate economical and rapid. The % RSD was found to be less than 2% in the developed method. Hence proposed method may be used for routine analysis of these drugs in combined dosage forms.

ACKNOWLEDGEMENT

The authors are thankful to Lupine Pharmaceuticals Pvt Ltd. for providing Simvastatin and Ezetimibe as gift samples for this work. And also Thanks to Prof., K.B.Burade,(Incharge Principal) GOVERNMENT COLLEGE OF PHARMACY, KARAD, for providing the required facilities for research work. I am also greetfull of Mrs.A.S.Kulkarni (Assistant Professor) for guiding and supporting me.

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