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Newer Rp-Hplc Method Development and Validation for the Simultaneous Estimation of Lafutidine and Rabeprazole in Dosage Form

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validation of Lafutidine and Rabeprazole, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Phenomenex Gemini C18 (4.6×250mm) 5µ column using a mixture of Methanol: TEA Buffer (65:35 v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 230nm. The retention time of the Lafutidine and Rabeprazole was 2.121, 3.643 ±0.02min respectively. The method produce linear responses in the concentration range of 10-50mg/ml of Lafutidine and 20-100mg/ml of Rabeprazole. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Lafutidine, Rabeprazole, RP-HPLC, validation.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the

identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. [1, 2]

The primary objective of proposed work is

- ✓ To develop new simple, sensitive, accurate and economical analytical method for the simultaneous estimation of Lafutidine and Rabeprazole.
- ✓ To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Lafutidine and Rabeprazole in dosage form.

MATERIALS AND METHODS

Table 1: Instruments used

S.No	Instruments And Glasswares	Model
1	HPLC	WATERS, software: Empower 2, Alliance 2695 separation module. 996 PDA detector.
2	pH meter	LabIndia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Table 2: chemicals used

S.No	Chemical	Brand names
1	Lafutidine	Sura labs
2	Rabeprazole	Sura labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC METHOD DEVELOPMENT

TRAILS

Preparation of standard solution: Accurately weigh and transfer 10 mg of Lafutidine and Rabeprazole working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.3 ml of Lafutidine and 0.6ml of Rabeprazole from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was methanol: Water, Methanol: Phosphate buffer and ACN: Water with varying proportions. Finally, the mobile phase was optimized to TEA buffer (pH 4.0), Methanol in proportion 65:35 v/v respectively.

Optimization of Column: The method was performed with various C18columns like Symmetry, X terra and ODS column. Phenomenex Gemini C18 (4.6×250mm) 5µ was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used : Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature : 40ºC
Column : Phenomenex Gemini C18 (4.6×250mm) 5µ
Mobile phase : Methanol: TEA Buffer (65:35 v/v)
Flow rate : 1ml/min

Wavelength : 230nm
Injection volume : 10µl
Run time : 6minutes

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of Triethylamine buffer (pH-4.0): Take 6.0ml of Triethylamine in to 750ml of HPLC water in a 1000ml volumetric flask and mix well. Make up the volume up to mark with water and adjust the pH to 4.0 by using Orthophosphoric acid, filter and sonicate.

Preparation of mobile phase: Accurately measured 350 ml (35%) of TEA buffer and 650 ml of HPLC Methanol (65%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram

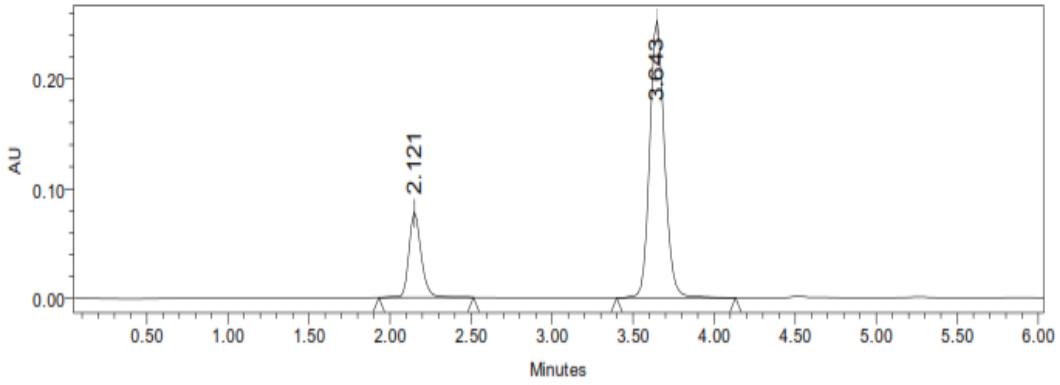

Fig 1: Optimized Chromatogram (Standard)

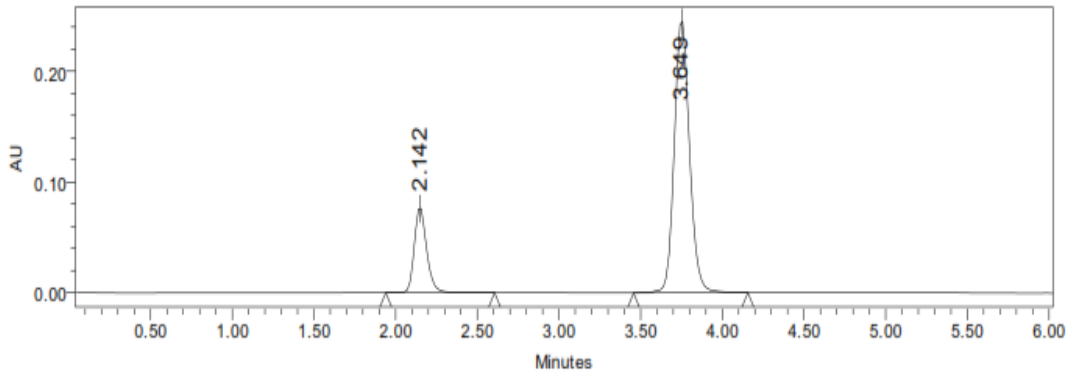

Fig 2: Optimized Chromatogram (Sample)

System suitability

Table 3: Results of system suitability for Lafutidine

S.No			Area	Height		
1	Lafutidine	2.152	382726	70725	5271	1.2
2	Lafutidine	2.157	382621	70625	5928	1.2
3	Lafutidine	2.141	389172	70617	5283	1.2
4	Lafutidine	2.133	384152	70718	5763	1.2
5	Lafutidine	2.166	389721	70172	6222	1.2
Mean			385678.4			
Std. Dev.			3497.932			
% RSD			0.906956			

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table 4: Results of system suitability for Rabeprazole

S.No			Area	Height			Resolution
1	Rabeprazole	3.674	1562821	227365	5827	1.1	10.1
2	Rabeprazole	3.631	1562726	226748	6183	1.1	10.1
3	Rabeprazole	3.625	1567361	227163	5029	1.1	10.1
4	Rabeprazole	3.692	1562811	226948	4920	1.1	10.1
5	Rabeprazole	3.629	1563816	226452	5183	1.1	10.1
Mean			1563907				
Std. Dev.			1982.03				
% RSD			0.126736				

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

SPECIFICITY

Table 5: Peak results for assay standard of Lafutidine

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Lafutidine	2.152	406538	77074	1.2	4009	1
2	Lafutidine	2.198	409975	76001	1.2	4136	2
3	Lafutidine	2.179	402283	76048	1.2	5263	3

Table 6: Peak results for assay standard of Rabeprazole

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Rabeprazole	3.646	1609924	251956	1.1	7849	1
2	Rabeprazole	3.604	1601840	246020	1.1	7819	2
3	Rabeprazole	3.610	1602832	248287	1.1	7826	3

Table 7: Peak results for Assay sample of Lafutidine

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Lafutidine	2.152	406538	77074	1.2	4009	1
2	Lafutidine	2.150	409975	76001	1.2	4136	2
3	Lafutidine	2.187	402911	77823	1.2	5173	3

Table 8: Peak results for Assay sample of Rabeprazole

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Rabeprazole	3.646	1609924	251956	1.1	7849	1
2	Rabeprazole	3.651	1601840	246020	1.1	7819	2
3	Rabeprazole	3.601	1603821	240291	1.1	6812	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

=1605195 /1604865 ×10/60×60/0.0254×99.5/100×0.0382/15×100
= 99.7%
The % purity of Lafutidine and Rabeprazole in pharmaceutical dosage form was found to be 99.7%

LINEARITY

Table 9: CHROMATOGRAPHIC DATA FOR LINEARITY STUDY OF LAFUTIDINE

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	10	135005
66	20	277120

100	30	405128
133	40	534643
166	50	672357

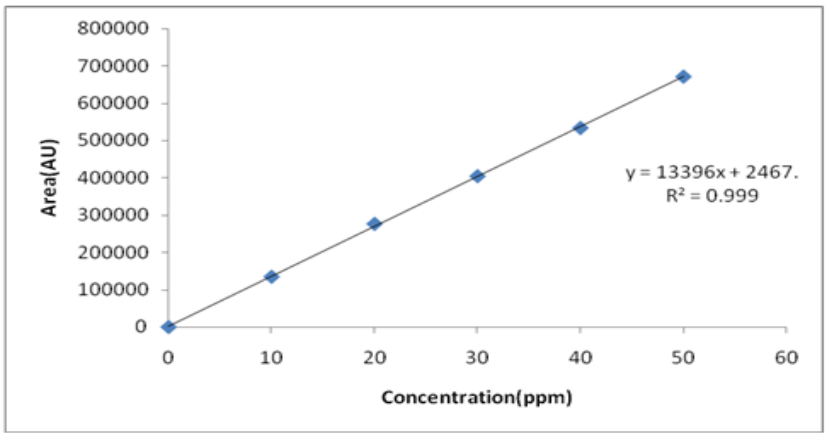

Fig-3: Calibration Curve of Lafutidine

Correlation Coefficient (r) is 0.99, and the intercept is 2467. These values meet the validation criteria.

Table 10: CHROMATOGRAPHIC DATA FOR LINEARITY STUDY OF RABEPRAZOLE

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	20	469094
66	40	1149397
100	60	1657592
133	80	2150412
166	100	2748444

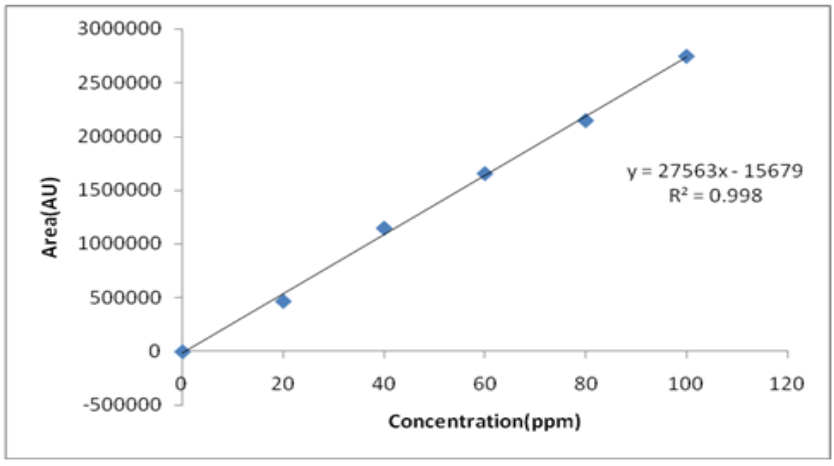

Fig 4: Calibration Curve of Rabeprazole

Correlation Coefficient (r) is 0.99, and the intercept is 15679. These values meet the validation criteria.

Precision

REPEATABILITY

Table 11: Results of repeatability for Lafutidine

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing	%Assay
1	Lafutidine	2.157	400459	70717	1.2	4987	99%
2	Lafutidine	2.159	402118	71819	1.2	5019	99.4%
3	Lafutidine	2.186	405412	73930	1.2	5126	100%

4	Lafutidine	2.160	406506	73333	1.3	4999	100%
5	Lafutidine	2.170	407673	72623	1.2	5214	100%
Mean			404433.6				
Std.dev			2716.809				
%RSD			0.671757				

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 12: Results of repeatability for Rabeprazole

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing	%Assay
1	Rabeprazole	3.603	1617864	226985	1.1	7045	98.7%
2	Rabeprazole	3.608	1618493	234764	1.1	7399	98.8%
3	Rabeprazole	3.600	1628262	227712	1.2	7159	99.4%
4	Rabeprazole	3.696	1615796	235459	1.1	7896	98.6%
5	Rabeprazole	3.629	1619626	242158	1.1	7965	98.8%
Mean			1620008				
Std.dev			4310.623				
%RSD			0.266086				

Intermediate precision

Table 13: Results of Intermediate precision Day 1 for Lafutidine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	%Assay
1	Lafutidine	2.198	405262	70572	5672	1.2	100%
2	Lafutidine	2.196	405637	70516	5639	1.2	100%
3	Lafutidine	2.160	405628	70572	6183	1.2	100%
4	Lafutidine	2.160	405647	70372	5923	1.2	100%
5	Lafutidine	2.160	405948	70592	6739	1.2	100%
6	Lafutidine	2.186	408732	70526	5837	1.2	100%
Mean			406142.3				
Std. Dev.			1287.197				
% RSD			0.316933				

- %RSD of five different sample solutions should not more than 2

Table 14: Results of Intermediate precision Day 1 for Rabeprazole

S.No	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution	%Assay
1	Rabeprazole	3.623	1608292	235473	5372	1.1	10.1	98%
2	Rabeprazole	3.611	1609283	235938	5927	1.1	10.1	98.2%
3	Rabeprazole	3.696	1617836	235738	6129	1.1	10.1	98.7%
4	Rabeprazole	3.696	1619743	235963	5284	1.1	10.1	99.7%
5	Rabeprazole	3.696	1614262	231938	5284	1.1	10.1	98.5%
6	Rabeprazole	3.642	1608471	235948	6347	1.1	10.1	98.2%
Mean			1611315					
Std. Dev.			6077.093					
% RSD			0.377151					

Table 15: Results of Intermediate precision Day 2 for Lafutidine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	%Assay
1	Lafutidine	2.198	405423	70572	5672	1.2	100%
2	Lafutidine	2.196	405927	70516	5639	1.2	100%
3	Lafutidine	2.178	405029	70572	6183	1.2	100%
4	Lafutidine	2.142	405432	70372	5923	1.2	100%
5	Lafutidine	2.177	405062	70592	6739	1.2	100%
6	Lafutidine	2.177	408417	70526	5837	1.2	101%
Mean			405881.7				
Std. Dev.			1283.857				
% RSD			0.316313				

- %RSD of five different sample solutions should not more than 2

Table 16: Results of Intermediate precision Day 2 for Rabeprazole

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution	%Assay
1	Rabeprazole	3.611	1638732	244384	5363	1.1	10.1	100%
2	Rabeprazole	3.623	1637438	235827	6282	1.1	10.1	100%
3	Rabeprazole	3.684	1638474	236382	5938	1.1	10.1	100%
4	Rabeprazole	3.697	1634273	239183	6194	1.1	10.1	99.7%
5	Rabeprazole	3.684	1636372	231931	5402	1.1	10.1	99.8%
6	Rabeprazole	3.684	1639283	234356	5837	1.1	10.1	100%
Mean			1637429					
Std. Dev.			1860.366					
% RSD			0.113615					

ACCURACY

Table 17: The accuracy results for Lafutidine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	201472.3	15	14.8	98.6	99.7%
100%	406193	30	30.1	100.3	
150%	607144	45	45.1	100.2	

- The percentage recovery was found to be within the limit (98-102%).

Table 18: The accuracy results for Rabeprazole

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	826527.7	30	30.5	101.6	99.6%
100%	1622241	60	59.4	99	
150%	2422702	90	88.4	98.2	

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

LAFUTIDINE

Result: = 3.3×4269.822/13396

= 1.05µg/ml

RABEPRAZOLE

Result: =3.3×57796.93/27563

= 6.9µg/ml

QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S
Where
σ = Standard deviation of the response
S = Slope of the calibration curve
LAFUTIDINE
Result: =10×4269.822/13396
=3.1µg/ml
RABEPRAZOLE
Result: =10×57796.93/27563
=20.9µg/ml

Robustness

Table 19: Results for Robustness Lafutidine

Parameter used for sample	Peak Area	Retention Time	Theoretical	Tailing factor
Actual Flow rate of 1.0 mL/min	406433	2.121	4009	1.2
Less Flow rate of 0.9 mL/min	398841	2.210	3800.8	0.9
More Flow rate of 1.1 mL/min	389947	2.184	4800.8	
Less organic phase	413898	2.200	4890.8	0.9
More Organic phase	389578	2.172	4190.8	0.7

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Table 20: Results for Robustness Rabeprazole

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical	Tailing factor
Actual Flow rate of 1.0 mL/min	1592811	3.643	7849	1.1
Less Flow rate of 0.9 mL/min	1613422	4.498	3312.2	0.9
More Flow rate of 1.1 mL/min	1619138	3.505	4312.2	0.8
Less organic phase	1616104	4.504	4392.2	0.9
More organic phase	1623185	3.512	4292.2	0.9

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Lafutidine and Rabeprazole in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Lafutidine and Rabeprazole are freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: Triethylamine Buffer was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Lafutidine and Rabeprazole in bulk drug and in Pharmaceutical dosage forms.

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