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CHEMOMETRIC ASSISTED RP-HPLC METHOD FOR ACECLOFENAC AND PANTOPRAZOLE ESTIMATION

Shine Sudev*

Research Scholar, Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan.

***Corresponding Author: Shine Sudev**
Research Scholar, Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan.

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ABSTRACT

Aceclofenac is (2-2[-[2[2,6 –dichlorophenyl)amino]phenyl] acetyl] oxyacetic acid with a Molar mass: 353.0216g/mol. Aceclofenac is a pain reliever. It treats rheumatoid arthritis, ankylosing spondylitis, and osteoarthritis by reducing pain and inflammation. It is a kind of nonsteroidal anti-inflammatory medication (NSAID). It works by inhibiting the release of particular chemical messengers responsible for pain and inflammation .The medication works by blocking the function of cyclooxygenase, which is involved in the creation of PG, which is responsible for pain, swelling, inflammation, and fever.Pantoprazole is Sodium;5-(difluoromethoxy)-2-[(3,4-dimethoxypyridin-2yl)methylsulfinyl] benzimidazol-1-ide. Pantoprazole is a proton pump inhibitor that reduces acid production in the stomach. It is used to treat erosive esophagitis (esophageal damage caused by gastroesophageal reflux disease, or GERD) in adults and children over the age of 5. The LC18 analytical Supelcosil (Supelco) column was used to achieve the chromatographic conditions (5 micron -LC18, 250x4.6mm) The mobile phase was composed of phosphate buffer (PH 4.3), acetonitrile, and methanol in the following proportions: 50:40:10. The sample was injected at a volume of 20 microliters, and the mobile phase was degassed using an infrared digital ultra sonicator before being pumped into the HPLC system. The flow rate was set at 0.8 ml per minute, and the wave length 284nm was chosen for detection. The column temperature was kept at 30^{o}C.

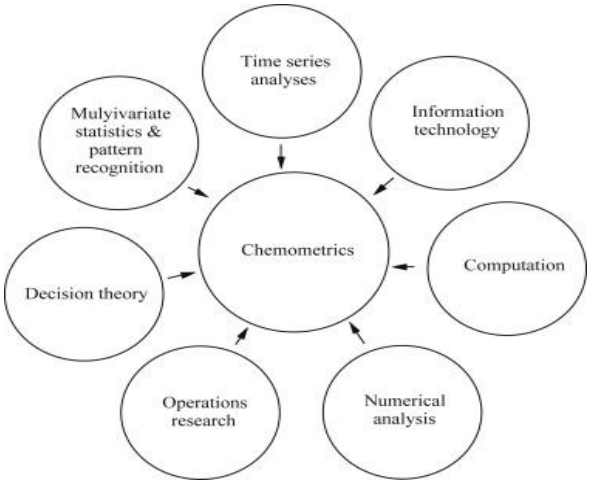
KEYWORDS: Aceclofenac, Pantoprazole, Chromatography.

INTRODUCTION

Chemometrics can be utilised in the chromatography laboratory to achieve method development to speed up and multivariate calibration is becoming more successful, impurity detection and monitoring. Chemometric techniques can be used on chromatographic data to create models that allow clinicians to differentiate between disease states based on patterns in body fluids or cellular material. All living systems are made up of chemical components, and the relative distribution of these constituents can be used to classify samples using a biological fingerprint. Matching techniques on chromatographic patterns are extensively used to classify bacteria, yeast, and moulds. One example is the use of HPLC to identify the bacterium causing tuberculosis and kindred mycobacterial species. Chemometric approaches allow to derive useful information from environmental measurements. These pattern recognition and modelling techniques have resulted in more accurate DNA fingerprinting, forensics investigation. The challenge in forensic analysis is not determining the concentration of various chemical elements, but rather determining whether a chromatographic trace is associated to a known sample.

Chromatographers today have tasks that go beyond simply collecting, analysing, and reporting on individual samples. The true result of the analytical effort is the integration of these individual analyses into an assessment of the chemical system as a whole. They compare a fresh sample to a collection of our existing knowledge and use nonspecific analytical procedures to infer qualities of interest, and so on. Chemometrics can be used to condense big assembly projects into more manageable time frames; modelling allows you to accelerate method development and understanding of complex chromatographic patterns. Multivariate models can be used in an expert system setting to Chemometrics seeks to extract as much information as possible from chemical problems by applying statistics and mathematics. Chemometrics has evolved into an important chemical discipline with a significant impact on analytical chemistry, including significant improvements in the design and selection of optimal experimental procedures, calibration of analytical

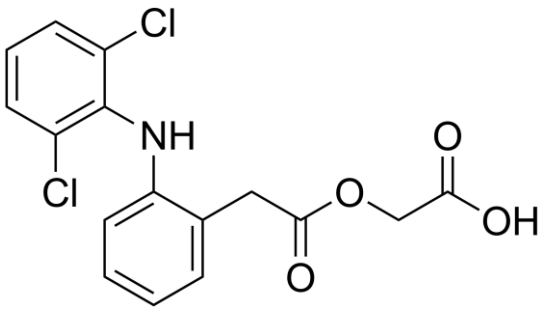
instrumentation, and advanced methods for chemical data analysis. The application of chemometric approaches to separation science, specifically chromatography and capillary electrophoresis, has followed the same upward trend as any other branch of analytical chemistry.



Chemometrics can be used for Adulteration Detection Tool. Poor-quality medications can be found on the market for two reasons: inadequate production standards (which mostly result in defective medicines) and fraud attempts. Counterfeited medications may contain a variety of frauds/adulterations, such as no active pharmaceutical ingredient (API), a different API from the one reported, or a different (lower)API strength. Multiple procedures have been developed in order to detect substandard/counterfeit medications; among these, those based on the application of spectroscopic techniques in combination with various chemometric methods play a significant role. The importance of these methodologies stems from the fact that spectroscopy (particularly NIR) combined with exploratory data analysis, classification, and regression methods can result in effective, high-performing, fast, non destructive, and in some cases, online methods for checking pharmaceutical quality and compliance with production and/or Pharmacopoeia standards. Nonetheless, the available chemometric methods for dealing with spectroscopic (but not simply those) data are numerous, and there is plenty of space for them to be misused.

A new chemometric technique was devised and applied to the simultaneous determination of utilising high performance liquid chromatography (HPLC) with photodiode array (PDA) detection. The peak area at multiwavelength PDA detector responses were subjected to chemometric calibration approaches, classical least squares (CLS), principle component regression (PCR), and partial least squares (PLS). HPLCCLS, HPLCPCR, and HPLCPLS are acronyms for the combining of HPLC with chemometric calibration procedures. For purposes of comparison, the HPLC method known as classical HPLC method was utilised to corroborate the results produced from combined HPLC Chemometric calibration procedures. Various formulations of medicines used to treat coughs and colds include combinations of more than two or three substances (multicomponent system). Conventional analytical techniques, such as UV spectrophotometry, make it challenging to analyse such multi-component mixtures. However, chemometric calibration techniques such as inverse least squares (ILS), classical least squares (CLS), principal component regression (PCR), and partial least squares (PLS) have been widely applied to the spectrophotometric resolution of such multi component formulations without preliminary separation in recent years. PLS and PCR are particularly well suited for multi component analysis, especially for mixes with strongly overlapped spectra. Although the HPLC method provides an appropriate approach for analysis, it necessitates numerous trials, expensive and high purity solvents, and proves to be time consuming. A few HPLC Systems have also looked into anti-inflammatory medicine like aceclofenac and created a completely automated narrow bore high-performance liquid chromatography from human plasma samples. Aceclofenac is the chemical name for [[2-[(2,6-Dichlorophenyl)amino]phenyl]acetyl]oxy]acetic acid.

DRUG PROFILE
ACECLOFENAC

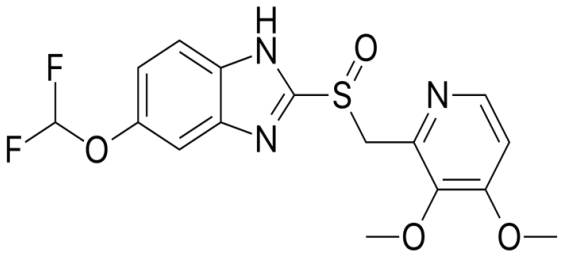


Chemical name: (2-2[-[2[2,6 – dichlorophenyl)amino]phenyl] acetyl]oxyacetic acid
Molar mass: 353.0216g/mol
Pharmacokinetics:

- MP: 149-153%
- Insoluble in water

Aceclofenac is a pain reliever. It treats rheumatoid arthritis, ankylosing spondylitis, and osteoarthritis by reducing pain and inflammation. Aceclofenac is a kind of nonsteroidal anti-inflammatory medication (NSAID). It works by inhibiting the release of particular chemical messengers responsible for pain and inflammation (redness and swelling). It is used to treat rheumatoid arthritis and osteoarthritis pain and inflammation. The medication works by blocking the function of cyclooxygenase, which is involved in the creation of PG, which is responsible for pain, swelling, inflammation, and fever.

PANTOPRAZOLE



Sodium;5-(difluoromethoxy)-2-[(3,4-dimethoxypyridin -2yl)methylsulfinyl]benzimidazol-1-ide Pantoprazole is a proton pump inhibitor that reduces acid production in the stomach. Pantoprazole is used to treat erosive esophagitis (esophageal damage caused by gastroesophageal reflux disease, or GERD) in adults and children over the age of 5. Pantoprazole is typically used for up to 8 weeks at a time to help your oesophagus repair. It is also used to treat Zollinger-Ellison syndrome and other stomach acid-related diseases.

MATERIALS AND METHOD

Chemicals and the Reagents

Greensmed lab supplied pantaprazole and aceclofenac working standards. In Chennai, the tablet dosage forms aceclofenac 100 mg from Madhav Biotech Pvt. Ltd. and pantaprazole tablets 40 mg from Cadila Pharmaceuticals Ltd. were obtained from the local market. The weight equivalent of powder to be calculated from the labelled claim and the average weight obtained by randomly picking 20 tablets. Merck provided HPLC quality water, methanol, and acetonitrile, whereas NICE provided sodium dihydrogen orthophosphates and orthophosphoric acid of analytical reagent (AR) grade.

Buffer preparation

1.75 gm of sodium dihydrogen ortho phosphate was accurately weighted into a 1L volumetric flask, then 700 ml of HPLC grade water was added and sonicated for a few minutes to degas before making up the volume with HPLC water and the PH was corrected to 4.3 using dilute orthophosphoric acid.

Standard preparation

Weigh 100mg precisely. Pantaprazole and Aceclofenac were transferred separately into a 100 ml clean dry separate standard flask and diluted with 50 ml of buffer and sonicated for 30 minutes, and both the standards flasks were made up to the final volume with buffer, and 1 ml of each of the above stock solutions was pipetted out into a 100 ml standard flask and then makeup to the final volume with buffer (to get 10mcg/ml solution).

Sample preparation

The weight equivalent of powder (100mg) to be taken from the formulation obtained from the local market is calculated from the labelled claim and average weight, the weight equivalent of powder is weighed, and the powder is transferred into clean dry separate standard flasks of 100 ml capacity each. The powder was dissolved in 50 ml of buffer solution, which was then increased to 100 ml. The contents of both flasks are filtered, and 1 ml of each filtrate is put into a clean dry 100 ml standard flask and properly diluted to obtain a combination of 10 mcg/ml solution of each medication.

Chromatographic conditions

The LC18 analytical Supelcosil (Supelco) column was used to achieve the chromatographic conditions (5 micron -LC18, 250x4.6mm) The mobile phase was composed of phosphate buffer (PH 4.3), acetonitrile, and methanol in the following proportions: 50:40:10. The sample was injected at a volume of 20 microliters, and the mobile phase was degassed using an infrared digital ultra sonicator before being pumped into the HPLC system. The flow rate was set at 0.8 ml per minute, and the wave length 284nm was chosen for detection. The column temperature was kept at 30^{o}C.

Method development

Several trials were conducted to refine the approach, and the best peak with the least fronting factor was discovered in the fifth trial, with a response time of 5.568 for pantaprazole and 7.356 for Aceclofenac.

The best optimum separation conditions are presented in Table Number 1, and the related chromatography is displayed in Figures 1–3.

Table no 1

SL.No	Chromatographic Conditions	
1	Mode of separation	Isocratic Elution
2	Mobile phase	Buffer (PH 4.3) Acetonitrile and Methanol (50:40:10)
3	Column	LC18 analytical Supelcosil (Supelco) column (5 micron -LC18, 250x4.6mm)
4	Flow rate	0.8ml/min
5	Detection wave length	284
6	Injection volume	20 micro liter
7	Column over temperature	30^{o}C
8	Run time	10 min

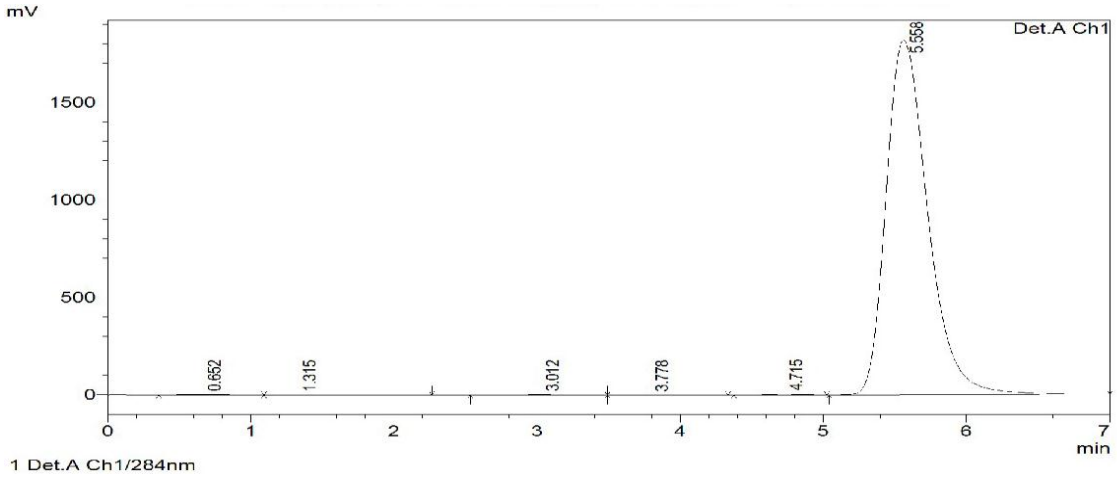


Fig 1: chromatogram showing the retention time for Pantoprazole standard.

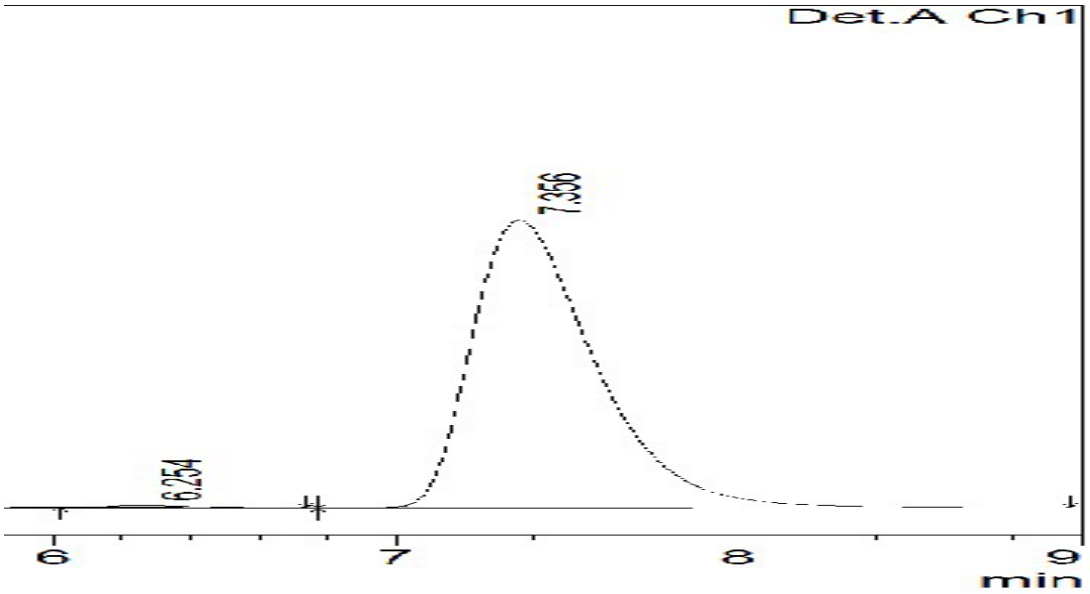


Fig 2: chromatogram showing the retention time for Aceclofenac standard.

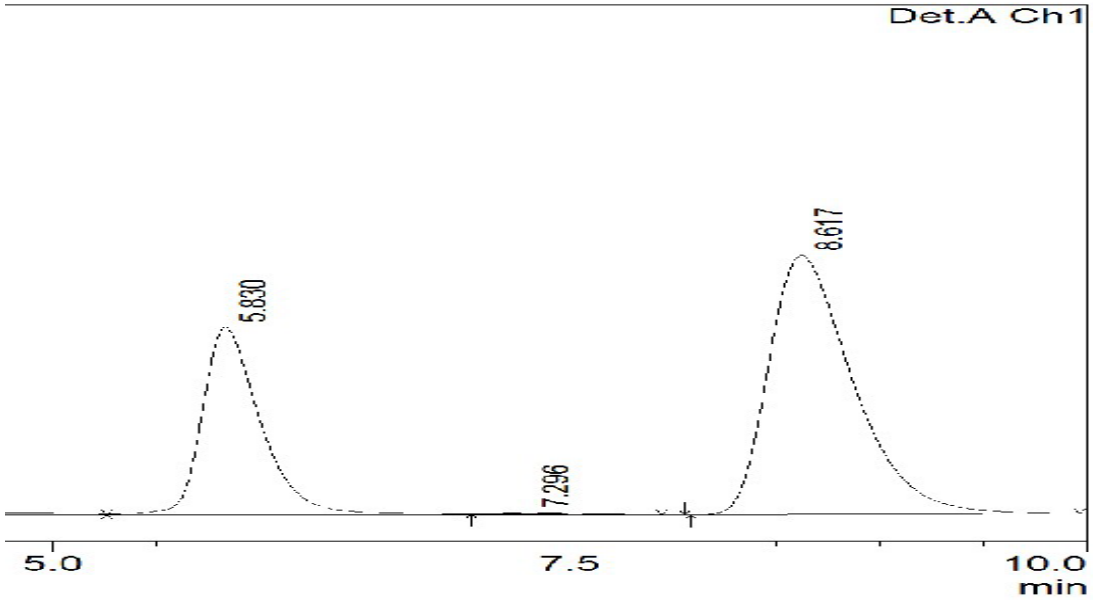


Fig 3: chromatogram showing the retention time for combined dosage form.

RESULTS AND DISCUSSION

Method validation

The final test settings were validated using the validation parameters given in the ICH recommendations. Analytic parameters such as specificity, accuracy, precision, linearity, detection, and quantitation limits were assessed in accordance with the ICH Protocol.

System suitability

As part of the HPLC procedures, system suitability tests were done. These tests are used to ensure that the chromatographic system is adequate for the purpose intended. The system suitability test was carried out for theoretical plates (more than 2000) with a tailing factor less than 2. Table 2 summarises the results that were within acceptable limits.

Table no 2.

Sl. No	Parameters	Pantaprazole	Aceclofenac	Acceptable Criteria
1	Tailing Factor	1.496	1.579	Less than 2
2	Theoretical Plates	2230.674	2095.630	Not less than 2000
3	Retention time	5.568	7.356	Less than 10
4	Area	5087421	4958763	
5	% RSD	0.09	0.58	Less than 2%
6	HETP	67.244	71.578	
7	Resolution	2.038	1.940	

Linearity

Linearity studies are the ability of analytical measurements such as absorbent diversity, proportion to concentration of the sample linearity experiments were performed for all active ingredients and the response was found to be linear in the study range of 2-32 PPM for both ingredients (Pantoprazole and Aceclofenac) were confirmed.

Specificity

Specificity is the ability to detect and assess the analytic in the presence of other components that may be expected to be present in the formulation. In the RP-HPLC study of standard and sample preparations, no interference due to diluents or mobile phase was observed in the analytic reaction time, indicating that the method was specific.

LOD and LOQ

The limit of detection is the lowest concentration that can be detected by instruments using a specific analytical procedure, and the limit of quantification is the lowest concentration that can be quantitatively analysed using a specific analytical procedure with acceptable precision, accuracy, and reliability. The signal-to-noise ratio for the analytical processes and instruments utilised for analysis is rigorously monitored and validated.

The LOD values for Pantaprazole and Aceclofenac were 0.92mcg/ml and 0.89 mcg/ml, respectively, and the LOQ values for AR grade Pantaprazole and Aceclofenac were 1.47 and 1.45 mcg/ml, respectively.

Robustness

Deliberate modifications are made to the approach via flow rate, mobile phase ratio, and temperature, but no discernible variations in the results were seen, and they are within the range specified by ICH. Robustness conditions such as flow rates of 0.7 ml/min and 0.9 ml/min, mobile phase concentrations of 40:50:10 and 60:30:20 for buffer acetonitrile and methanol, and temperature changes at 25^{o}C and 35^{o}C were maintained, and samples were injected in triplicate; system suitability parameters were not significantly affected, and the RSD was within the limit, indicating that the RP-HPLC method development was robust.

Accuracy

The method's accuracy was studied using recovery analysis, and it was determined by completing recovery experiments at 75%, 100%, and 125% of the target analyte concentration in the commercial forms chosen. The percentage recovery of analyte at each concentration and the mean percentage recovery for both analytes were investigated; the recovery of each concentration must fall within the permitted limit of 2%.

Accuracy Data of Pantoprazole

SL. No	Conc %	Peak Area	Amount Added mg	Amount Found mg	% Recovery	Mean Recovery %	SD	% RSD
1	75%	3653192	3.75	3.78	100.8		0.2	0.2
2	100%	5087421	5	4.98	99.6	100.18	0.6	0.09
3	125%	6298351	6.25	6.26	100.16		0.37	0.05

Accuracy Data of Aceclofenac

SL.No	Conc %	Peak Area	Amount Added mg	Amount Found mg	% Recovery	Mean Recovery %	SD	% RSD
1	75%	3699276	3.75	3.72	99.2		0.2	0.19
2	100%	4958763	5	4.96	99.2	99.63	0.6	0.58

3	125%	6148647	6.25	6.28	100.48		0.37	0.35

Precision

Precision is a measure of the degree of repeatability of procedures under normal operating conditions, and it is typically stated as the relative standard deviation (percent RSD). Precision can be accomplished at two levels: intraday precision and interday precision. Precision statistics displaying the percent RSD value for both intraday and interday experiments were less than 2%, indicating that the suggested approach is accurate and consistent.

Intraday and Inter day data of Pantaprazole and Aceclofenac

SL. No	Parameters	Inter Day		Intra Day	
		Pantaprazole	Aceclofenac	Pantaprazole	Aceclofenac
1	Retention Time	5.877	8.633	5.853	8.594
2	Avg. Peak Area	5187583	4850783	5164615	4839600
3	SD	50766	50415	52435	49355
4	% RSD	0.5	0.11	0.7	0.21

CONCLUSION

The disclosed stability indication test technique is simple, fast, robust, and reliable for estimating pantaprazole and aceclofenac in bulk and formulations. At elution time, no interference peaks were seen. System suitability parameters such as linearity, precision, accuracy, resolution, theoretical plate, and retention time of the proposed method for both drugs were checked and found to be appropriate; linearity was determined for both ingredients and a concentration range of 2-32 mcg/ml was determined. The LOD values for Pantaprazole and Aceclofenac were 0.92mcg/ml and 0.89 mcg/ml, respectively, while the LOQ values for AR grade Pantaprazole and Aceclofenac were 1.47 and 1.45 mcg/ml, respectively. Robustness conditions such as flow rates of 0.7 ml/min and 0.9 ml/min, mobile phase concentrations of 40:50:10 and 60:30:20 for buffer acetonitrile and methanol, and temperature changes at 25^{o}C and 35^{o}C were maintained, and samples were injected in triplicate; system suitability parameters were not significantly affected, and the RSD was within the limit, indicating that the RP-HPLC method development was robust.

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CONFLICT OF INTREST

There is no conflict of interest in the work presented in this manuscript.

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