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## **Estimation of bilastine and montelukast by stability indicating liquid chromatographic method in pure binary mixture and their marketed tablets**

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**Abstract**--The main objective of the current work is to develop and validate a new RP-HPLC method for estimation of Bilastine and Montelukast in bulk and in their combined tablets. A good separation of both analytes in various types of solutions was attained by using a Ascentis C18 (150 x 4.6mm, 2.7 $\mu$ m) column with a solvent or mobile phase of 0.1% OPA: Acetonitrile (50:50 v/v) at a flow rate of 1mL/min and a detection wavelength of 230nm. To test the stability of the analytes, the drug substance was put in an environment with a lot of stress, such as hydrolysis with acid and base, peroxide oxidation, and thermal degradation. At 2.27min and 2.89 min, Bilastine and Montelukast were eluted with isocratic elution. The method is expected to show a linear response from 2.5 to 15 $\mu$ g/mL for Bilastine and from 5 to 30  $\mu$ g/mL for Montelukast. Bilastine's LOD and LOQ were established to be 0.30 and 0.90  $\mu$ g/mL, while Montelukast's were 0.10 and 0.31  $\mu$ g/mL. The present method was also tested for its stability indicating nature and found to be highly specific in the presence of degradants obtained during acidic and basic stress conditions. The method was very sensitive, accurate, cost-effective, and showed stability. So, the method has a high chance of being used in the pharmaceutical industry to test Bilastine and Montelukast in the quality control department.

**Keywords**--Bilastine, Montelukast Stability indicating, C18 column, and Isocratic elution.

## Introduction

Montelukast (MTL) is a leukotriene receptor antagonist that blocks leukotriene D4 from doing its job. It is used to treat asthma and relieve seasonal allergy symptoms.<sup>1-3</sup> Bilastine (BST) is a new H1-antihistamine from the second generation that has just been approved to treat the symptoms of allergic rhinitis and urticaria by binding to H1 receptors.<sup>4-6</sup> Bilastine is an antihistamine that doesn't make you sleepy and works for a long time. When it comes to treating seasonal allergic rhino conjunctivitis (SARC), taking MTL and BST together is better than taking either drug alone.<sup>7,8</sup> The chemical structure, molecular formula and Pka value of MTL and BST were shown in figure-1.

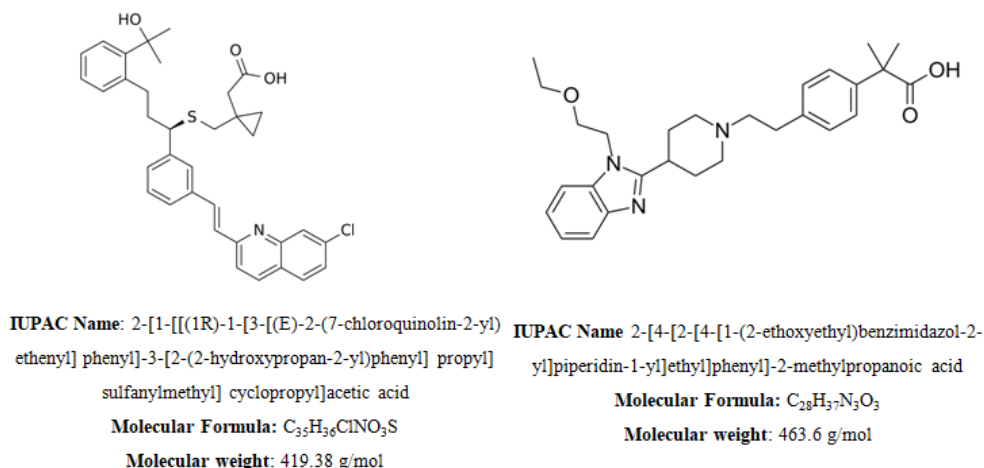


Figure -1: 1. Chemical MTL structures of and BST

A proficient analytical method is a key requirement for evaluation of a drug alone as well in combination with other drug substances simultaneously. From a wide look at the literature, it was found that there were not many UV, RP-HPLC methods published to estimate BST and MTL alone.<sup>9-13</sup> There were also a few other RP-HPLC and UV methods for analyzing MTL and BST simultaneously time in bulk mixtures and combined dosage forms.<sup>14-19</sup> There are a few problems with the reported method, such as longer retention times for either MTL or BST and gradient elution. Few of the reported methods did not have stability tests done.<sup>18</sup> For drug analysis, it should be important to have a method with a cheap solvent system, a short retention time, and a high level of sensitivity. This will cut down on the amount of time it takes to look at a sample and keep costs down. This will help improve the manufacturing of drugs by letting the quality of batches be checked in less time. In this way, efforts have been made to create a reliable, highly sensitive, and cost-effective RP-HPLC method of assessing the amount of BST and MTL as well as their stability in both blended powder and commercially available tablets. ICH Q2 guidelines were followed when validating the method that had been made.<sup>20-22</sup>

## **Materials and Methods**

### **Chemicals**

Pure drugs BST and MTL were got from Spectrum Pharma Lab, Kukatpally, Hyderabad. All HPLC rank solvents were gained from local supplier of Merck private limited. The method was established by using WATERS HPLC connected with PDA detector and Empower-2 software. In addition, 1mg sensitive balance (Denver), Ultra-sonicator, a digital pH meter (BVK enterprises), water (Milli-Q) were used for preparation of solution and mobile system.

### **Chromatographic method conditions**

An adequate and effective separation of both MTL and BST was achieved with the Ascentis C18 (150 x 4.6mm, 2.7 $\mu$ m) column, using a mobile system composition of 0.1% ortho phosphoric acid (OPA): Acetonitrile (ACN) (50:50% v/v) at a flow rate of 1mL/min and a wavelength of 230nm. Water and ACN in equal portions were used as diluents. Ambient temperatures were maintained in the auto sampling unit and column unit.

### **Preparation of standard solution**

Exactly weighed 5mg of MTL and 10mg of BST were shifted to 50mL volumetric flasks and add 30mL of diluent to this flask, sonicated for 10min. After that, the remaining flask volume was filled with the same diluent to get a solution of 100 $\mu$ g/mL of MTL and 200 $\mu$ g/mL BST which was marked as stock solution. 1mL of the ensuing solution was again diluted to 10mL to get the concentration of 10 $\mu$ g/mL of MTL and 20 $\mu$ g/mL, which was considered as the 100% level standard or standard solution.

### **Preparation of sample solution**

Tablet powder equivalent to label claim was correctly weighed and shifted to 100mL volumetric flask and add 30mL of diluent to this flask, sonicated for 10min. After that, the remaining flask volume was filled with the same diluent to get a solution of 100 $\mu$ g/mL of MTL and 200 $\mu$ g/mL BST which was marked as stock solution. 1mL of the ensuing solution was again diluted to 10mL to get the concentration of 10 $\mu$ g/mL of MTL and 20 $\mu$ g/mL of BST, which was considered as the 100% level standard or standard solution.

### **Method validation**

Validation is written proof that gives a high level of reasonable assurance about a method or process. Validation of analytical methods is done in line with Q2 of the ICH guidelines<sup>20,21</sup>.

### **System suitability test**

The system applicability of the method was tested by injecting standard solution six times repeatedly. To make sure that the stated method works more assessment on things like theoretical plates, % RSD, and tailing factors.

### **Linearity**

The linearity of an analytical procedure shows how the results of the experiment and the indicated concentrations are proportional to each other. It was done with MTL concentrations from 2.5 $\mu$ g/mL to 15 $\mu$ g/mL and BST concentrations from

5µg/mL to 30µg/mL. A liner plot was made between the concentration and the peak area to figure out the regression coefficient (R<sup>2</sup>).

### **Precision**

The procedure is believed to be precise while a close proximity is recognized among the results from the identical sample on repetitive investigation under identical conditions. The predicted method's intraday precision was performed by injecting the standard solution six times in a day, while the inter-day precision was performed by injecting the standard solution six times (two times in day for three continuous days). The %RSD values of observed peak areas and retention time (RT) were calculated.

### **Accuracy**

The standard addition technique was used to determine the accuracy of the analytical method to a significant degree. A known quantity of sample was added to different levels (50%, 100%, and 150%) of stated standard solution. At each %level, the % recovery sample concentration was assessed.

### **Specificity**

It means that an analytical method can find the drug material even if there are other ingredients in the sample. The HPLC had been each loaded with 10mL of blank solution, standard, standard treated with a placebo and forced degradation solution. It was looked into in detail how some of the other peaks interfered with the analyte peak.

### **Sensitivity**

The LOD and LOQ were determined by using standard deviation method.

$$\text{LOD} = 3.3\sigma/S$$

$$\text{LOQ} = 10\sigma/S$$

Where,  $\sigma$  – Intercept's standard deviation

S- Mean slope of linear plot

### **Robustness**

Robustness is the ability of a method to keep producing the same result even when some of its conditions are changed on intention. Some of the conditions of the method, like the flow rate ( $\pm 0.1$  mL/min), the temperature ( $\pm 5^\circ\text{C}$ ), and the detection mobile phase ratio ( $\pm 5.0$ , v/v), were changed in a slight but intentional way. By loading standard solution six times in succession, the %RSD of peak responses at each changed condition was measured.

### **Forced degradation studies:**

During the forced degradation (FD) tests, the drug substance was put under more stress than it was during the accelerated stability tests. The FD studies are used to find out how chemically stable the drug is and to make a stable formulation with good storage instructions. The ICH regulatory standards list specific types of degradation, such as acid hydrolysis, base hydrolysis, oxidative degradation, UV degradation, and thermal degradation.<sup>19</sup>

To test acid, base, and oxidative degradation, 1mL of standard stock solution was mixed with 1mL of 2N HCl, 2N NaOH, and 3% H<sub>2</sub>O<sub>2</sub>. The mixture was then refluxed

for 30 minutes at 60°C and kept aside for 24 hours. All three solutions were diluted even more until the concentrations of MTL and BST were 10µg/mL and 20µg/mL, respectively. The standard stock solution was heated for one hour at 105°C in a hot air oven. The above solution was diluted so that 10µg/mL of MTL and 20µg/mL of BST could be found in 1mL. The photo degradation solution was made by exposing a standard stock solution to UV light (200 Watt-hours/m<sup>2</sup>) or one day in a photo stability unit. The standard solution was diluted in the same way as the thermal degradation solution. 10 mL of each degradation solution was put into the HPLC instrument, and chromatograms were used to figure out how much MTL and BST had broken down. Regulatory criteria say that the most effective and appropriate way to test the stability-indicating HPLC method is to let the drug substance break down by up to 20%.

### Assay

The %purities of MTL and BST the in sample solution were determined by injecting successive injections of standard and sample solution <sup>8</sup>.

## Results and Discussion

### Method optimization

The method development was initiated by ensuring the solubility of the both MTL and BST. It was found that MTL was freely soluble in water, soluble in methanol and ACN on other side BST was freely soluble in acetone, methanol, ethanol and ACN but insoluble in water. Equal volumes of water and ACN were chosen as diluents based on the solubility of MTL and BST.

The trial and error approach was used to optimize the process. After several trials a method as mentioned in chromatographic condition of methodology was opted as optimized method The retention times of BST and MTL were noticed at 2.27min and 2.89 min respectively. The chromatogram obtained by the optimized system shown in Figure 2.

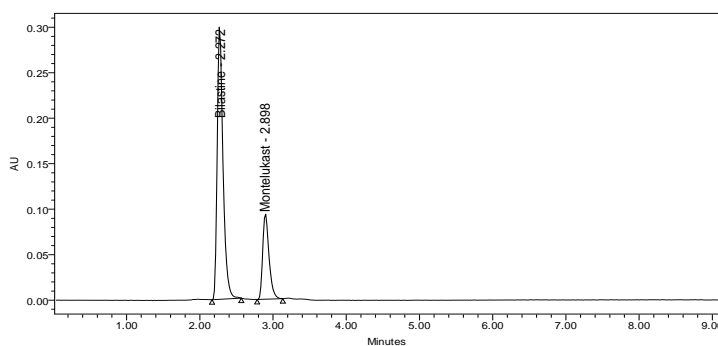


Figure No: 2. Chromatogram of the optimized method

## Method validation

### System suitability

The results were collected by injecting standard solution six times and meeting the acceptability requirements for parameters like percent RSD ( $\leq 2$ ), resolution ( $> 2$ ), tailing ( $< 2$ ), and plate count ( $> 2000$ ). In Table-1, the results are shown.

Table-1. System suitability results of standard solution

Parameters	BST			MTL			
	RT(min)	USP Plate Count	Tailing Factor	RT(min)	USP Plate Count	Tailing Factor	Resolution
Mean (n=5)	2.2154	4130.6	1.38	2.8142	5448	1.348	4.08
SD	0.004	51.52	0.012	0.008	222.37	0.02	0.09
%RSD	0.19	-	0.88	0.27	-	1.42	-

SD-Standard Deviation and %RSD-Relative Standard Deviation

### Linearity

The method has considerable linearity for BST and MTL were in the range of 2.5 $\mu$ g/mL to 15 $\mu$ g/mL and 5 $\mu$ g/mL to 30 $\mu$ g/mL. It was confirmed from linearity graph as shown in figure 3. The calculated  $R^2$  values for both BST and MTL were 0.999, which were acceptance as per ICH limit.

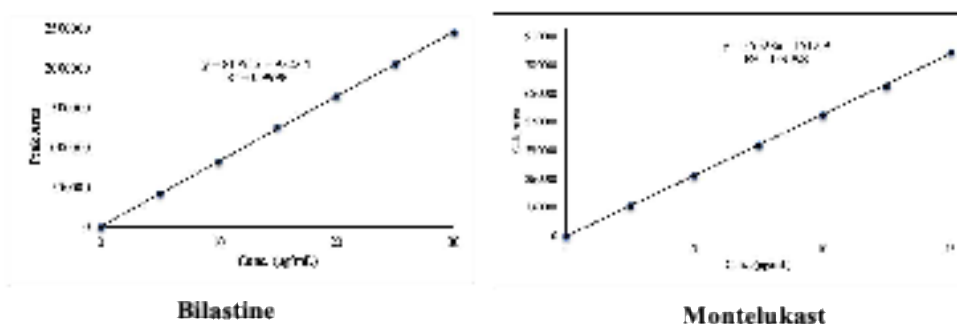


Figure – 3: Linearity curve of BST and MTL

### Accuracy

The average % recovery of the BST and MTL of sample amount in different levels of spiked stand solutions were found to be 100% $\pm$ 2. This shows that the method is accurate enough to meet the Q2 requirements of the ICH guidelines. In table 2, the results were shown.

Table-2: % Recovery of sample from various levels of standard solution

% Level	Conc. added ( $\mu\text{g/mL}$ )	Conc. recovered ( $\mu\text{g/mL}$ )	Recovery (%)	Mean Recovery (%)	Conc. added ( $\mu\text{g/mL}$ )	Conc. recovered ( $\mu\text{g/mL}$ )	Recovery (%)	Mean Recovery (%)
50%	10	9.9	99.3	100.08%	5	4.94	98.83	99.45%
	10	9.8	98.1		5	4.91	98.24	
	10	10.0	99.6		5	5.00	99.93	
100%	20	19.8	99.1		10	10.00	100.03	
	20	20.0	100.1		10	9.92	99.22	
	20	20.2	100.8		10	10.01	100.07	
150%	25	25.4	101.6		15	15.06	100.41	
	25	25.1	100.5		15	14.91	99.40	
	25	25.4	101.6		15	14.84	98.90	

### Precision

The %RSD values calculated for the peak areas of intraday and inter day precision were  $\leq 2$ . The %RSD of the MTL and BST assessed to be 0.8 and 0.6 respectively for intraday precision and 0.8 and 0.7 for inter day precision respectively.

Table -3: Precision results of MTL and BST 100% level solution

Precision	Parameter	MTL	BST
Intraday precision	Mean (n=6)	554109	1603980
	SD	4407.5	11632.1
	%RSD	0.8	0.7
Inter day precision	Mean(n=6)	539436	1574169
	SD	4046.7	9758.8
	%RSD	0.8	0.6

### Sensitivity

The LOD and LOQ values were determined as  $0.30\mu\text{g/mL}$  and  $0.90\mu\text{g/mL}$  for BST,  $0.10\mu\text{g/mL}$  and  $0.31\mu\text{g/mL}$  for MTL respectively.

### Robustness

Changes that were made on purpose to the column temperature, flow rate, and mobile phase ratio of the proposed method didn't have much of an effect on how well the system worked, and the percent RSD of the peak responses met the acceptance criteria (Table 4), which shows that the method is quite robustness.

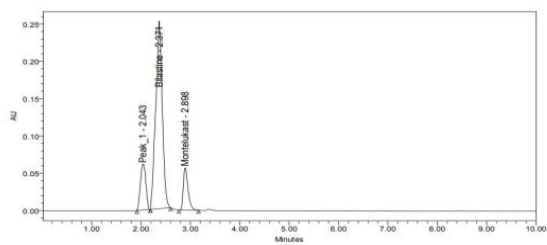
Table-4: Results of robustness of standard solution

Drug name	Peak area	Flow rate (1±0.1 mL/min)		Mobile phase (OPA:ACN) 50:50(± 5.0, v/v)		Temperature (30 ±5°C)	
		0.9 mL/min	1.1 mL/min	45:55	55:45	25°C	35°C
BST	% RSD	0.5	1.8	1.5	0.9	1.0	0.9
MTL	% RSD	0.5	1.7	1.7	1.2	1.2	1.3

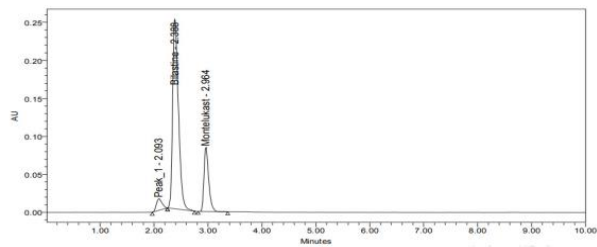
%RSD-Relative Standard Deviation

### Forced degradation

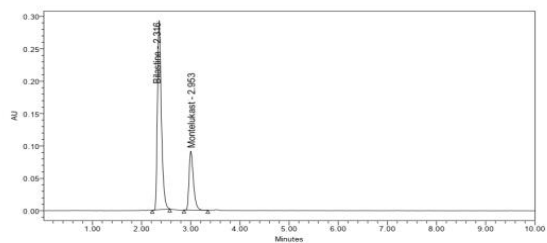
Scientists take into account up to 20 % degradation in the drug material in most analytical methods that show how stable it is. By comparing the areas of the peaks of chromatograms made from freshly made standard solution and degradation solution, the percent of BST and MTL that had degraded was found. Table-5 is a summary of the data, including the purity threshold, peak purity angle, and percent degradation. Figure 4 shows the chromatograms of a few samples that were taken during stressful situations. The fact that the purity threshold was higher than the purity angle of all generated peaks that showed the purity of drug compounds and their degradation products shows that the described method is stable. Both drugs were degraded a lot in acidic, basic pH and oxidative environments, which shows that MTL and BST are very sensitive in acid, basic and oxidation conditions. These results help to know the proper storage conditions of the manufactured drug substance and formulated product.



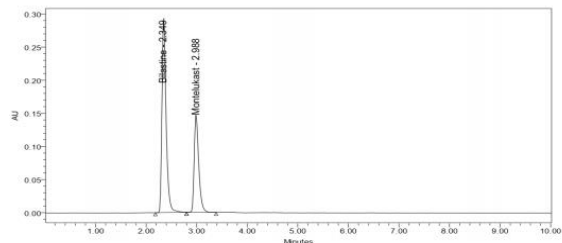
Acid degradation



Base degradation



Oxidative degradation



Thermal degradation

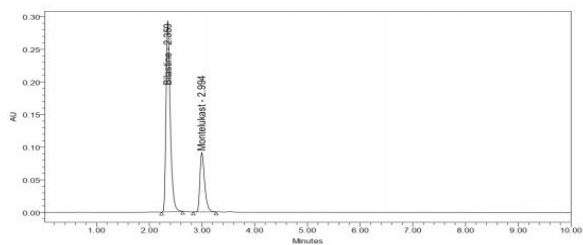


Photo degradation

Figure-4: Chromatograms of different forced degradation studies

Table 5: Results of forced degradation studies

Degradation	Peak name	RT	Area	USP Plates	USP Tailing Factor	Resolution	Purity angle	Purity threshold	% Degradation
Acid degradation	Peak-1	2.043	563465	1367	1.1	-	-	-	-
	BST	2.371	1526309	4050	1	1.7	0.261	0.309	5.43
	MTL	2.898	524246	4996	1.4	4.3	0.571	0.664	5.25
Base degradation	Peak-1	2.093	111775	1708	1.1	-	-	-	-
	BST	2.388	1540977	4389	1.4	1.7	0.324	0.499	4.53
	MTL	2.964	528190	5624	1.4	4.0	0.466	0.614	4.52
Oxidative degradation	BST	2.316	1549707	4161	1.3	-	0.140	0.293	3.98
	MTL	2.953	530787	5370	1.2	4.0	0.405	0.510	4.06
Thermal degradation	BST	2.349	1576293	3940	1.4	-	0.260	0.291	2.33
	MTL	2.988	537795	5377	1.4	4.0	0.281	0.346	2.80
Photo degradation	BST	2.359	1592836	4211	1.3		0.187	0.276	1.30
	MTL	2.990	544472	5662	1.4	4.2	0.432	0.560	1.59

## Assay

The %purity of the BST and MTL in tablet formulation (Billargic M - Bilastine (20mg) and Montelukast (10mg)) were assessed as 99.39% and 101.15% for BST and MTL respectively conventional as per ICH limit of  $100\% \pm 2$  (figure-5).

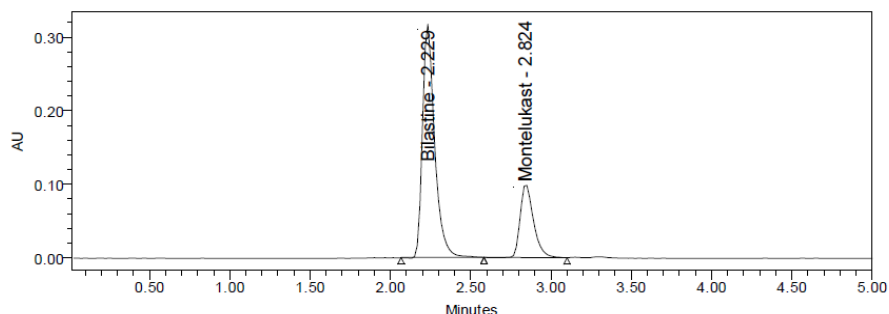


Figure 5: Chromatogram of sample solution

The current RP-HPLC method has advantages over priory reported method in terms of good sensitivity (LOD and LOQ were  $0.30\mu\text{g/mL}$  and  $0.90\mu\text{g/mL}$  for BST,  $0.10\mu\text{g/mL}$  and  $0.31\mu\text{g/mL}$  for MTL) and shorter RT (BST -2.27min and MTL -2.89 min) with economical mobile system (OPA and ACN). The cheap mobile phase was used and RT for BST and MTL were shorter than the previously described method where RT for MTL was reported in the range of 4 to 7min.<sup>19-21</sup> Apart from RT and sensitivity, in few methods gradient elution technique was used, as it is somewhat complex when compared with isocratic elution it terms of chromatographic conditions and HPLC pumping system<sup>20</sup>. The current procedure is recommended as economical, as it minimizes the elution time and utilization of mobile phase. The determination of % degradation of analytes in various forced degradation condition representing the stability- indicating character of the method.

## Conclusion

An easy, precise, accurate, specific, sensitive and isocratic RP HPLC method was created to determine BST and MTL at the same time in bulk blended powder and their combined tablets. The way the method shows how stable it is by putting the analytes through conditions that force them to be degraded. The suggested method worked well to separate BST, MTL, and their breakdown products. It was sensitive and had great resolution. The new method has excellent stability, specificity, sensitivity, a simple mobile phase, and a shorter elution time. Because of this, the suggested method is used a lot in the pharmaceutical industry's analytical research division.

## Authors' Contributions

All the authors involved with same efforts in design and structure of the work, data acquisition and analysis, both authors read the prepared manuscript and agreed for the publication.

## Conflict Of Interest

There is no conflict of interest from all the authors

**List of abbreviations**

**ACN:** Acetonitrile  
**OPA:** Ortho phosphoric acid  
**BST:** Bilastine  
**MTL:** Montelukast  
**RT:** Retention Time  
**LOD:** Limit of Detection  
**LOQ:** Limit of Quantification  
**SD:** Standard Deviation  
**RSD:** Relative Standard Deviation

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