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A New analytical method development and validation of Ambroxol, Montelukast and Levocetirizine in Tablet dosage form by RP-HPLC method

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Abstract

A simple and selective LC method is described for the determination of Ambroxol, Montelukast and Levocetirizine in tablet dosage forms. Chromatographic separation was achieved on a c18 column using mobile phase consisting of a mixture of Triethylamine Buffer:ACN:Methanol (20:30:50) with detection of 280 nm. Linearity was observed in the range 7.7-22.5 µg/ml for Ambroxol (r^2=0.999) and 1.25-3.75 µg/ml for Montelukast (r^2=0.998) and 5-15 µg/ml for Levocetirizine (r^2=0.997) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Keywords: Liquid Chromatography (LC); RSD Relative Standard Deviation; r^2 (correlation coefficient).

Introduction

A drug is a compound that exhibits action for the treatment of different diseases or to cure or diagnose the diseases. Different companies are in the progress for the production different APIs or the drugs. Analytical chemistry is divided into two types

1. Quantitative analysis: It is concerned with the exact amount present in the sample,
2. Qualitative analysis: It is concerned with the sample representation in the form of numbers.

The study of analytical chemistry provides ideal training for nearly all scientists course in quantitative analysis. In all the instrumental techniques available UV spectrophotometric technique is more simple, sensitive and precise. Different techniques are available for the analysis of samples. They are

1. Spectroscopy
2. Chromatography
3. Polarimetry
4. Electrophorosis

Spectroscopy

Measurement of electromagnetic radiation is known as spectroscopy. Most of the techniques are:

1. Infrared spectroscopy
2. Nuclear magnetic resonance spectroscopy
3. Visible spectroscopy
4. Ultraviolet spectroscopy, etc

Chromatography

The mixture of compounds is separated by chromatographic technique. Most commonly used chromatographic techniques are

1. Gas chromatography
2. Paper chromatography
3. High performance thin layer chromatography

Plan of Work

Step-1 Solubility studies.

Step-2 Determination of wavelength of Ambroxol, Montelukast and Levocetrizine.

Step-3 Method development and to optimize the mobile phase and flow rates for proper resolution and Retention time.
Step-4 Validation was developed as per the ICH (International Conference on Harmonisation).

Need For The Study

Analytical method development for pharmaceutical formulations

Quality investigation plays a very important role in quality specification establishment of chemical drugs. The number of drugs introduced into the market every year. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. Hence, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs.
Basic criteria for new method development of drug analysis:

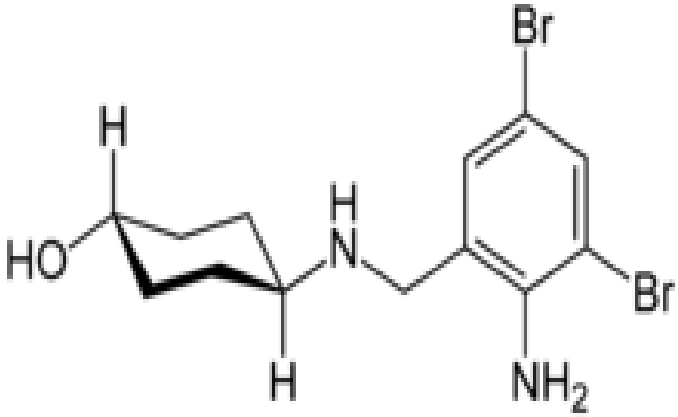
- The drug or drug combination may not be official in any pharmacopoeias.
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations.
- Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients.
- Analytical methods for a drug in combination with other drugs may not be available.
- 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.
- Analytical method development provides the support to track the quality of the product from batch to batch.
- Method development involves considerable trial and error procedures. The most difficult problem usually is where to start, what type of column is worth trying with what kind of mobile phase.
- Single dosage forms with combination of drugs are widely used today due to their advantages and their simultaneous estimation of individual component is a challenging task

Drug Profile

Ambroxol

Ambroxol is a secretolytic agent used in the treatment of respiratory diseases associated with viscid or excessive mucus. It is the active ingredient of Mucosolvan, Lasolvan or Mucoangin. The substance is a mucoactive drug with several properties including secretolytic and secretomotoric actions that restore the physiological clearance mechanisms of the respiratory tract which play an important role in the body's natural defence mechanisms. It stimulates synthesis and release of surfactant by type II pneumocytes. Surfactants acts as an anti-glue factor by reducing the adhesion of mucus to the bronchial wall, in improving its transport and in providing protection against infection and irritating agents. It stimulates synthesis and release of surfactant by type II pneumocytes [1-3].

Structure



IUPAC name

(1r,4r)-4-{[(2-amino-3,5-dibromophenyl)methyl] amino}cyclohexan-1-ol.

Chemical formula: $C_{13}H_{18}Br_2N_2O$

Molecular weight: 378.108

Mechanism of action

Ambroxol is a mucolytic agent. Excessive Nitric oxide (NO) is associated with inflammatory and some other disturbances of airways function. NO enhances the activation of soluble guanylate cyclase and cGMP accumulation. Ambroxol has been shown to inhibit the NO-dependent activation of soluble guanylate cyclase. It is also possible that the inhibition of NO-dependent activation of soluble guanylate cyclase can suppress the excessive mucus secretion, therefore it lowers the phlegm viscosity and improves the mucociliary transport of bronchial secretions.

Half-life: 7-12 h

Indication

Ambroxol is indicated as "secretolytic therapy in bronchopulmonary diseases associated with abnormal mucus secretion and impaired mucus transport. It promotes mucus clearance, facilitates expectoration and eases productive cough, allowing patients to breathe freely and deeply. Ambroxol also provides pain relief in acute sore throat. Pain in sore throat is the hallmark of acute pharyngitis.

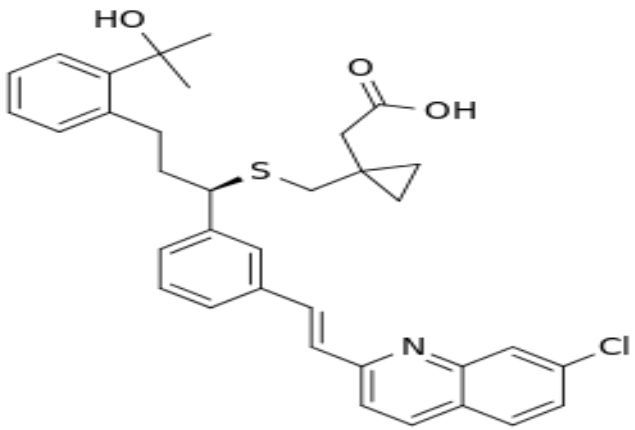
Side effects

Field tests to date have not uncovered specific contraindications of Ambroxol. However, caution is suggested for patients with gastric ulceration, and usage during the first trimester of pregnancy is not recommended.

Montelukast

Montelukast is a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. It is usually administered orally. Montelukast blocks the action of leukotriene D4 on the cysteinyl leukotriene receptor CysLT1 in the lungs and bronchial tubes by binding to it. This reduces the bronchoconstriction otherwise caused by the leukotriene, and results in less inflammation. Because of its method of operation, it is not useful for the treatment of acute asthma attacks. Again because of its very specific locus of operation, it does not interact with other allergy medications such as theophylline. Montelukast is marketed in United States and many other countries by Merck & Co. with the brand name Singulair®. It is available as oral tablets, chewable tablets, and oral granules. In India and other countries, it is also marketed under the brand name Montair®, produced by Indian company Cipla.

Structure



IUPAC name

2-[1-({[(1R)-1-{3-[(E)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl}-3-[2-(2-hydroxypropan-2-yl)phenyl]propyl]sulfanyl}methyl)cyclopropyl]acetic acid.

Molecular weight: 586.183

Chemical formula: $C_{35}H_{36}ClNO_3S$

Mechanism of action

Montelukast selectively antagonizes leukotriene D4 (LTD4) at the cysteinyl leukotriene receptor, CysLT1, in the human airway. Montelukast inhibits the actions of LTD4 at the CysLT1receptor, preventing airway edema, smooth muscle contraction, and enhanced secretion of thick, viscous mucus.

Indication

For the treatment of asthma. Montelukast is used for several conditions including asthma, exercise induced bronchospasm, allergic rhinitis, primary dysmenorrhoea (i.e. dysmenorrhoea not associated with known causes; see dysmenorrhea causes), and urticaria.

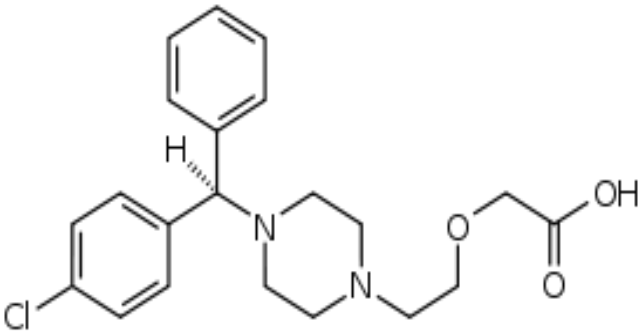
Adverse effects

The side effects include severe behavioural changes (including suicidal thoughts), angioedema, erythema multiforme, and liver problems.

Levocetrizine

Levocetirizine is an inverse agonist that decreases activity at histamine H1 receptors. This in turn prevents the release of other allergy chemicals and increased blood supply to the area and provides relief from the typical symptoms of hay fever. It does not prevent the actual release of histamine from mast cells.

Structure



IUPAC name

2-(2-{4-[(R)-(4-chlorophenyl)(phenyl)methyl] piperazin-1-yl}ethoxy)acetic acid.

Chemical formula: $C_{21}H_{25}ClN_2O_3$

Molecular weight: 388.89

Mechanism of action

Levocetirizine, the active enantiomer of cetirizine, is an anti-histamine; its principal effects are mediated via selective inhibition of H1 receptors. The antihistaminic activity of levocetirizine has been documented in a variety of animal and human models. In vitro binding studies revealed that levocetirizine has an affinity for the human H1-receptor 2-fold higher than that of cetirizine (Ki=3 nmol/L *vs*. 6 nmol/L, respectively). This increased affinity has unknown clinical relevance. In Nepal levocetirizine is available in tablet with brand name Curin manufactured by Beximco Pharma.

Indication

Levocetirizine is indicated for the relief of symptoms associated with allergic rhinitis (seasonal and perennial) in adults and children 6 years of age and older.

Availability

On 31 January 2017, the Food and Drug Administration approved Xyzal as an over-the-counter drug.

Method Development

Method development of ambroxol, montelukast and levocetrizine

Preparation of standard solution for all trails

Weigh 15 mg of Ambroxol and 10 mg of Montelukast and 2.5 mg of Levocetrizine in 100 ml of volumetric flask and make up the volume with mobile phase or Diluent upto the mark. From the above solution pipette out 1.5 ml from ambroxol and 1 ml from montelukast and 0.25 ml from Levocetrizine (2.75 ml) and transfer it to 10 ml volumetric flask make up the volume up to the diluent (27.5 µg/ml) (Table 1).

Table 1: Method development trials.

Trail	Mobile Phase	Column	PH	Wave Length	Injection Volume
Trail-1	Mixed Phosphate Buffer:Can	C18	4.0	280	20 µg/ml
Trail-2	Phosphate Buffer: Methanol:Acn	C18	4.0	280	20 µg/ml
Trail-3	Methanol:Acn:Water	C18	4.0	280	20 µg/ml
Trail-4	Mixed Phosphate Buffer:Acn	C18	4.0	280	20 µg/ml
Trail-5 (Optimized)	Triethylaminebuffer:Acetonitrile:Methanol	C18	4.0	280	20 µg/ml

Trial 1 observation

- Efficiency was less than 2000 for all the drugs. It should be more than 2000.
- Asssymetric factor was good for Ambroxol, Montelukast and Levocetrizine.
- Hence due to the unsatifactory valves of efficiency we considered trail-1 as not optimized.

Trial 2 observation

- Efficiency was not good for Ambroxol,Montelukast and Levocetrizine.
- Peak response was not good
- Base line was not proper
- Hence because of above reasons trail-2 is not optimized

Trial 3 observation

- Assymetric factor was not good for all drugs. They are more than 2.
- Efficiency of Ambroxol and Montelukast was very less.
- Peak responce of Ambroxol was not good.
- Hence trail-3 is not optimized.

Trial 4 observation

- Peak response of Lecocetrizine was very less.
- Efficiency was not good for Ambroxol.
- Hence it is not optimized.

Trial 5 observation (optimized)

- Efficiency was good for all the drugs .
- Assymetric factor was good for all the drugs.
- All suitable parameters are satisfactory.
- Hence we considered trail-5 as optimized trail (Table 2).

Validation

Validation is a documented evidence that the method which does what it is intended to it. There are 9 parameters [4-7].

- Assay
- Apecificity
- Linearity
- Accuracy
- Precision
- LOD (limit of detection)
- LOQ (limit of quantification)
- Robustness
- Ruggedness

Table 2: Optimized trail

Trail	Trail-5
Mobile phase	TriethylamineBuffer:Acetonitrile: Methanol
Ratio	20:30:50
Ph	5.0
Wavelength	280
Column	C18
Injection volume	20 µg/ml
Retention times	Ambroxol-2.276 Montelukast-3.331 Levocetrizine-5.675
Flow	1 ml

Assay

To determine the percentage of purity we will perform the Assay. We will inject 6 injections of standard and 6 injections of sample.

Preparation of standard

Weigh 15 mg of Ambroxol and 10mg of Montelukast and 2.5 mg of Levocetrizine in 100ml of volumetric flask and make up the volume with mobile phase or dupto the mark. From the above solution pipette out 1.5 ml from ambroxol and 1 ml from Montelukast and 0.25 ml from Levocetrizine (2.75 ml) and transfer it to 10 ml volumetric flask make up the volume up to the diluent (27.5 µg/ml).

Sample preparation

Weigh the 10 tablets of Lezest-M and calculate average valve for 10 tablets then calculate it equivalent to 15 mg of ambroxol and 10 mg of Montelukast and 2.5 mg of Levocetrizine. Crush the tablets in motor and pestle. Weigh the fine powder which is equivalent to standard preparation and dissolved in 100

ml volumetric flask from the above stock solution pipette out 2.75 ml and transfer intp 10 ml volumetric flask and make up the volume with mobile phase.

Acceptance criteria: 90-110%

Observation

The amount of Ambroxol, Montelukast and Levocetrizine present in the taken dosage form was found to be 98.81%, 102.56% and 98.62% respectively (Table 3).

Table 3: Results of Assay.

	Ambroxol		Montelukast		Levocetirizine	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	512118	447408.000	274517	172832.000	90705	79701
Injection-2	464544.000	448120	251182.000	171823	82349	78731
Injection-3	407777	445478.000	123262	171573	70039	79782
Injection-4	500968	449436	130097	171897	88481	79772
Injection-5	408730.000	466910	104638.000	170428.000	71321	78790
Injection-6	466211.000	470302	119277.000	170101.000	80420	79876
Average Area	460058.000	454609.000	167162.167	171442.333	80552.5	79442
Assay(%purity)	98.8155841		102.560488		98.62139598	

Linearity

Linearity is an analytical method it will determine its ability to obtain test results and are directly proportional to concentration of the analyte.

Preparation of standard for linearity (percentage in 50, 75, 100, 125 and 150)

Acceptance criteria

The relationship between the concentration of Ambroxol, Montelukast and Levocetrizine area of Ambroxol, Montelukast and Levocetrizine should be linear in the specified range and the correlation should not be less than 0.99.

Observation

The correlation coefficient for linear curve obtained between concentration *vs*. Area for standard preparations of Ambroxol, Montelukast and Levocetrizine is 0.998, 0.997 and 0.999. The relationship between the concentration of Ambroxol, Montelukast and Levocetrizine and area of Ambroxol, Montelukast and Levocetrizine is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits (Table 4, Figures 1 and 2).

Accuracy

In accuracy we will inject 3 injections of 50% and 3 injections of 100% and 3 injections of 150%.

Sample preparation

Weigh the 10 tablets of Lezest-M and calculate average valve for 10 tablets then calculate it equivalent to 15 mg of ambroxol and 10 mg of Montelukast and 2.5 mg of Levocetrizine. Crush the tablets in motor and pestle. Weigh the fine powder which is equivalent to standard preparation and dissolved in 100 ml volumetric flask from the above stock solution pipette out 2.75 ml and transfer intp 10 ml volumetric flask and make up the volume with mobile phase (Table 5).

Table 4: Linearity at various concentrations.

Preparations	Standard stock solution (ml)			Volume made up to (ml)	Concentration of solution (µg/ml)		
					Ambroxol	Montelukast	Levocetirizine
Preparation 1 (50%)	0.75	0.125	0.5	10	7.5	1.25	5
Preparation 2 (75%)	1.125	0.187	0.75	10	11.25	1.875	7.5
Preparation 3 (100%)	1.5	0.25	1	10	15	2.5	10
Preparation 4 (125%)	1.875	0.312	1.25	10	18.75	3.125	12.5
Preparation 5 (150%)	2.25	0.375	1.5	10	22.5	3.75	15

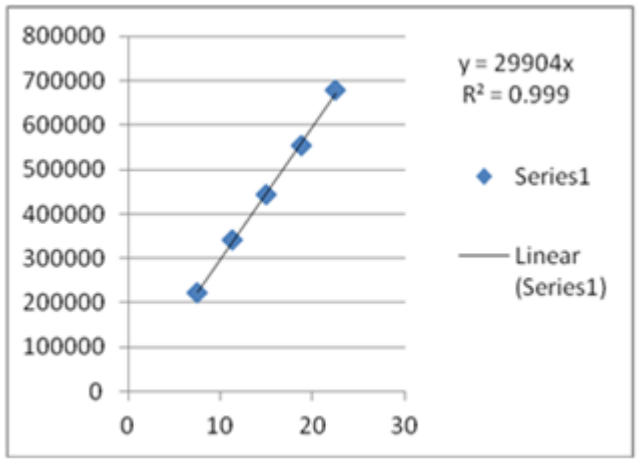


Figure 1: Linearity graph of ambroxol.

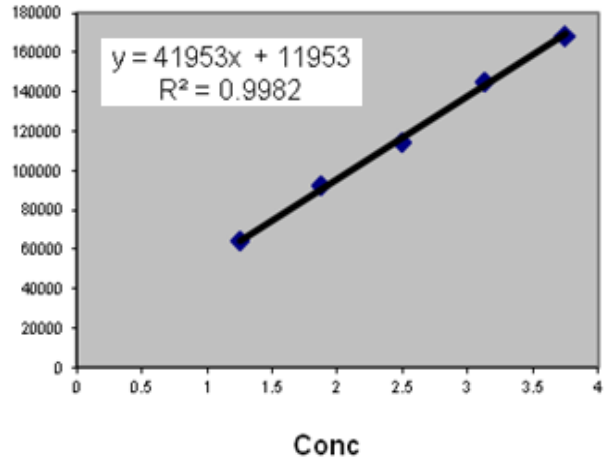


Figure 2: Linearity graph of Montelukast.

Precision

Preparation of standard for method precision

Weigh 15 mg of Ambroxol and 10 mg of Montelukast and 2.5 mg of Levocetrizine in 100 ml of volumetric flask and make up the volume with mobile phase or Diluent upto the mark. From the above solution pipette out 1.5 ml from ambroxol and 1 ml from montelukast and 0.25 ml from Levocetrizine and transfer it to 10 ml volumetric flask make up the volume up to the diluent [8-10] (Table 6).

Robustness and Ruggedness

Performing the validation by different analysts on different days (Tables 7 and 8).

Table 5: Recovery of Ambroxol, Montelukast, Levocitrezine.

Recovery level	Accuracy studies				Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	% of Mean Recovery	
Ambroxol					
50%	7.5	211804	206704.3	102.0772	101.31
	7.5	205042			
	7.5	203267			
100%	15	464586	454494	102.2578	
	15	452590			
	15	446306			
150%	22.5	687125	675686.3	99.61864	
	22.5	679922			
	22.5	660012			
Montelukast					
50%	5	55621	54658	101.3217	100.43
	5	55943			
	5	52410			
100%	10	119281	117846	102.9007	
	10	117481			
	10	116776			
150%	15	168130	167450.3	99.33284	
	15	166602			
	15	167619			
Levocetirizine					
50%	1.25	36931	35167	100.5518	101.01
	1.25	34591			
	1.25	33979			
100%	2.5	80475	78357.33	101.907	
	2.5	78429			
	2.5	76168			
150%	3.75	118022	116593.7	100.5924	
	3.75	117307			
	3.75	114452			

Table 6: Results for Method precision of Montelukast, Levocetirizine and Ambroxol.

S. No	Levocetrizine		Montelukast		Ambroxol	
	Rt	Area	Rt	Area	Rt	Area
1	2.389	94672	3.580	124265	5.308	501722.000
2	2.32	95534	3.582	123469	5.323	515056.000
3	2.34	90907	3.662	123958.000	5.223	514410.000
4	2.31	91456	3.652	124675.000	5.313	505258.000
5	2.299	94474	3.681	121289	5.323	513940.000
6	2.299	93160	3.680	124614.000	5.328	514513.000
Mean	2.326167	93367.17	3.640	123711.667	5.3030	510816.500
SD	0.031398	1701.506	0.047	1267.855	0.0399	5795.064
%RSD	**1.349755**	**1.822381**	**1.28**	**1.02**	**0.75**	**1.13**

Table 7: Result of Robustness study.

Parameter	Ambroxol		Montelukast		Levocetirizine	
	Retention time (min)	Tailing factor	Retention time (min)	Tailing factor	Retention time (min)	Tailing factor
Flow Rate						
0.8 ml/min	2.811	1.406	4.247	1.349	7.028	1.221
1.0 ml/min	2.272	1.374	3.439	1.309	5.675	1.214
1.2 ml/min	1.920	1.369	2.905	1.321	4.790	1.213
Wavelength						
278 nm	2.276	1.377	3.448	1.341	5.684	1.218
280 nm	2.276	1.372	3.447	1.317	5.679	1.213
282 nm	2.275	1.377	3.446	1.331	5.685	1.213

Table 8: Result for Ruggedness.

Drugs	% Assay		
	Ambroxol	Montelukast	Levo-cetirizine
Analyst 01	100.00	101.25	99.49
Analyst 02	101.22	99.09	100.91
%RSD	0.857989	0.759623	0.703543

Summary and Conclusion

A new method was established for simultaneous estimation of Ambroxol, Montelukast and Levocetirizine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Ambroxol, Montelukast and Levocetirizine by using X BRIDGE C18 column (4.6x50 mm), flow rate was 1 ml/min. Mobile phase ratio was (20:30:50 v/v) Triethylamine:Acetonitrile:Methanol buffer pH 5.0. detection wavelength was 280 nm. The % purity of Ambroxol, Montelukast and Levocetirizine was found to be 98.81%, 102.56% and 98.6. The Linearity study of Ambroxol, Montelukast and Levocetirizine was found in concentration range of 7.5 µg-22.5 µg, 1.25 µg-3.75 µg and 5 µg-15 µg and correlation coefficient was found to be 0.999, 0.998, 0.997. Hence the suggested RP-HPLC method can be used for routine analysis of Ambroxol, Montelukast and Levocetirizine API and Pharmaceutical dosage form.

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