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Stastical Comparision, Method Development and Validation of High Performance Liquid Chromatography for estimation of Cifixime Trihydrate and Potassium Clavunate in bulk and combine tablet dosage form

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A B S T R A C T

High performance liquid chromatography for estimation of Cifixime Trihydrate and Potassium Clavunate in their combine dosage form was developed and validated. The method was performed on Younglin Instrument with Autochro-3000 Operation software using Varian C-18 (250 X 4.6 mm i.d, 5 µm particle size column and Methanol: water: Potassium dihydrogen phosphaste Buffer (pH 3) (30:05:65, %v/v/v) as mobile phase at ambient temperature. Detection was carried out at 288 nm. Concentration range 16-80 µg/ml for Cefpodoxime Proxetil and 10-50 µg/ml for Potassium Clavunate. The percentage recovery of Cefixime Trihydrate and Potassium Clavunate Was found to be 99.57-101.16 and 98.91-103.48 respectively.. Correlation coefficient for Cefixime Trihydrate and Potassium Clavunate Sodium was found 0.998 and 0.998 respectively. The Rt values for Cefixime Trihydrate and Potassium Clavunate were found to be 5.1 min ±0.02 and 2.5 min ±0.03 respectively. The method can successfully applicable to routine analysis. And under stastical analysis ,Paired t-test is applided for comparison between developed method and reported method where, we reject the null hypothesis, because value of t is less than 0.05 so we can conclude that there is significance difference between the developed method and reported method for Cifixime Trihydrate and and Potassium Clavunate.

Keywords: Cifixime Trihydrate, Potassium Clavunate, HPLC, Mobile phase, Column

A R T I C L E I N F O

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1. Introduction

Cifixime Trihydrate is Third Generation Cephalosporin Antibiotic and it is chemically 6R,7R)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylicacid,72-(Z)-[O-(carboxy methyl) oxime] trihydrate. [3,4] Potassium Clavunate is Anti-Bacterial – Beta lactamase inhibitor and chemically it is (2R, 3Z, 5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo [3.2.0] heptane-2-carboxylic acid. Structure of Cifixime Trihydrate is illustrated in figure.1. Structure of Potassium Clavunate is illustrated in figure 2 [1,2].

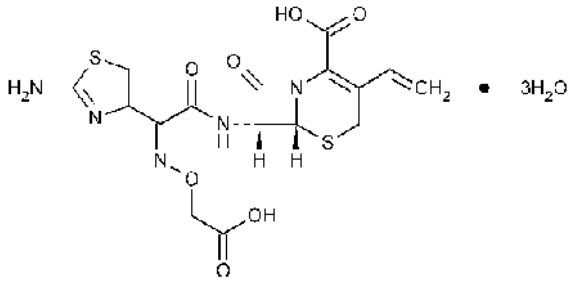

Figure 1: Structure of Cifixime Trihydrate

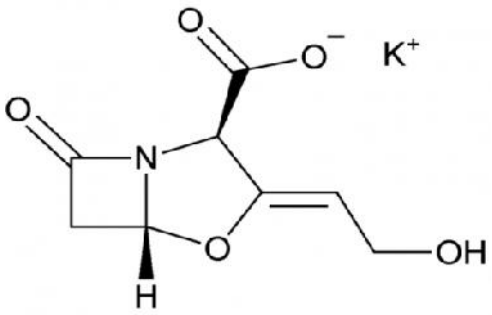

Figure 2: Structure of Potassium Clavunate

Cifixime Trihydrate is official in USP[1] and EP[2]. Potassium Clavunate is official in IP[3] and USP[4]. So far, to our present knowledge, very few HPLC method for estimation of Cifixime Trihydrate and Potassium Clavunate has been reported.[5] So an attempt was made to develop single, accurate, rapid, and precise HPLC method for the determination of Cifixime Trihydrate and Potassium Clavunate in tablet and in active pharmaceutical ingredients. [6-10]

2. Material and methods

Reagent and chemicals: Cifixime Trihydrate and Potassium Clavunate was a gift sample from Baroque pharmaceutical Limited, Khambhat. All chemicals and reagent used were analytical grade and purchased from Ranbaxy fine chemicals Limited. Combined tablet formulations (Benimax-CV) were procured from Indian market.

Preparation of standard stock solution:

An accurately weighed quantity of Cefixime Trihydrate (100 mg) and Potassium clavunate (100 mg) was dissolved in mobile phase i.e., Methanol: water: Potassium dihydrogen phosphaste Buffer (pH 3) (30:05:65, %v/v/v) in 100 ml volumetric flask and volume was made up to mark with the same solvent. Take 10 ml from above solution in 100 ml volumetric flask and diluted up to mark with mobile phase. Final strength of solution was 100 µg/ml Cifixime Trihydrate and Potassium Clavunate.

Selection of wavelength

Stock solution was scanned in UV range (200 nm- 400nm). In the spectrum of Cefixime Trihydrate & Potassium clavunate 288 nm is selected because at this wavelength both drugs absorb UV radiation as shown in figure no.3.

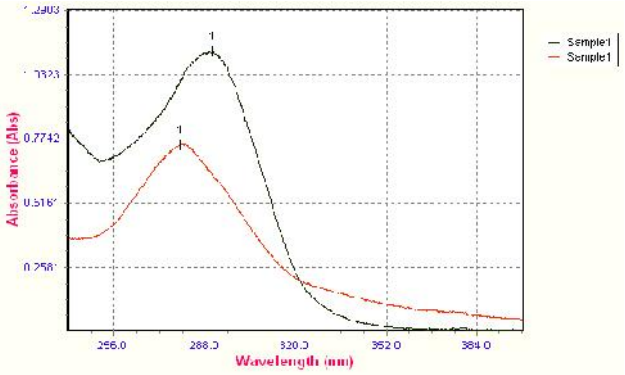

Figure 3: Overlay spectra of Cefixime Trihydrate and Potassium Clavunate.

Preparation of calibration curve:[11-12]

Calibration curves were plotted over a concentration range of 16-80 µg/ml for Cefixime Trihydrate and 10-50 µg/ml for Potassium Clavunate. Accurately measured standard stock solutions of Cefixime Trihydrate (1.6,3.2,4.8,6.4 and 8 ml) & Potassium Clavunate (1,2,3,4 and 5 ml) were transferred to a series of 10 ml volumetric flasks and the volume in each flask was adjusted to 10 ml with mobile phase. The resulting solution was injected into the column with flow rate 1 ml/min were measured at 288 nmfor Cefixime Trihydrate & Potassium Clavunate. Calibration curves were constructed for Cefixime Trihydrate & Potassium Clavunate by plotting peak area versus concentration at 288 nm.

Analysis of commercial formulation:

Weigh and powder 20 tablets. Accurately weighed quantity equivalent to 50 mg of Cefixime Trihydrate and 31.25 mg of Potassium clavunate was transferred into 100 ml volumetric flask and dissolved in mobile phase with vigorous shaking. The solution was sonicated for 20 minutes and the volume was made up to mark with the same solvent. The solution was filtered through whatman

filter paper No. 41. 10 ml of filtrate was transferred in to 100 ml volumetric flask and volume was made up to mark with methanol to get the concentration of 500 µg/ml Cefixime Trihydrate & 312.5 µg/ml Potassium clavunate. 1 ml of filtrate was transferred in to 10 ml volumetric flask and volume was made up to mark with methanol to get the concentration of 50 µg/ml Cefixime Trihydrate & 31.25 µg/ml Potassium clavunate.

3. Results and discussion

The method was validated by establishing linearity, accuracy, interday and intraday precision of measurement of sample application. The limit of detection and limit of quantification were also determined. Chromatogram of Cefixime Trihydrate and Potassium clavunate in active pharmaceutical ingredients is shown in figure no 4,5 and 6. Chromatogram of Cefixime Trihydrate and Potassium clavunate in Tablet dosage form is shown in figure no 7.

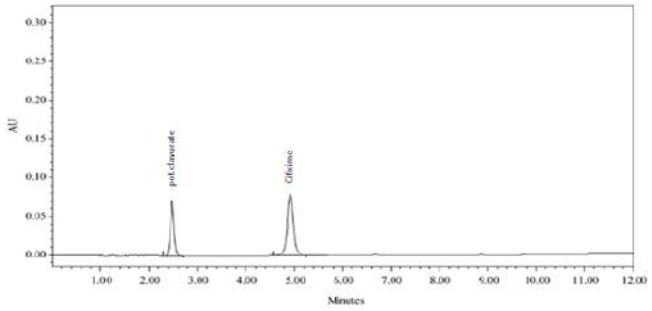

Figure 4: Chromatogram of Cefixime Trihydrate & Potassium Clavunate in standard solution.

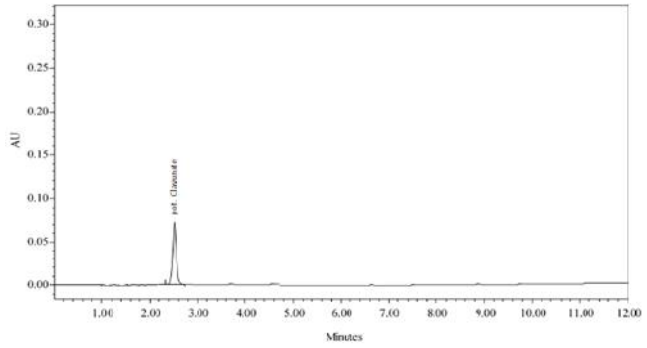

Figure 5: Chromatogram of Potassium Clavunate in standard solution

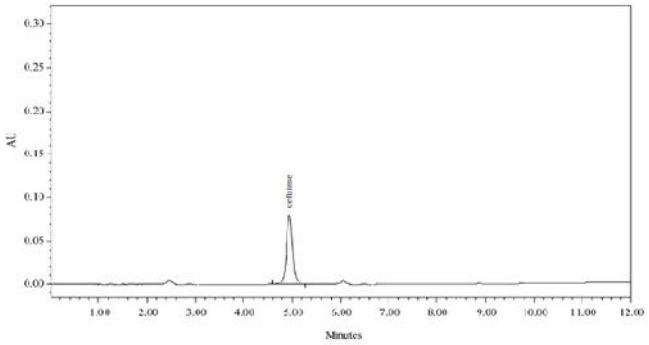

Figure 6: Chromatogram of Cefixime Trihydrate in standard solution

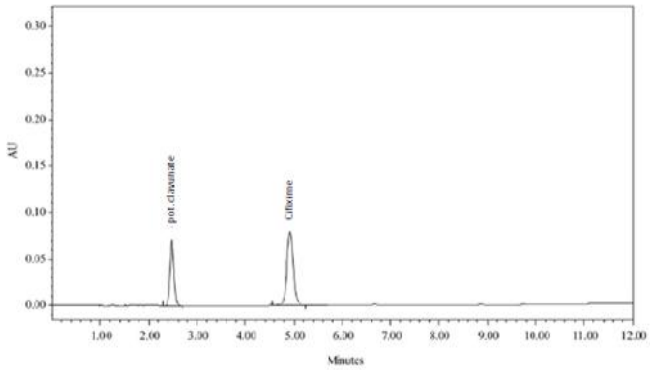

Figure 7: Chromatogram of Cefixime Trihydrate & Potassium Clavunate in sample solution.

Linearity calibration curve:

Calibration curve were found to be linear in the range of 16-80 µg/ml of Cefixime Trihydrate and 10-50 µg/ml of Potassium clavunate. Five concentration points were assayed in triplicate. Both Cefixime Trihydrate and Potassium clavunate showed good linearity in tested range. The regression coefficient (R2) Value for Cefixime Trihydrate and Potassium clavunate were found to be 0.998 and 0.998 respectively. Linear regression data for the calibration plots (n=6) are illustrated in figure no 8 and 9 respectively [11-12].

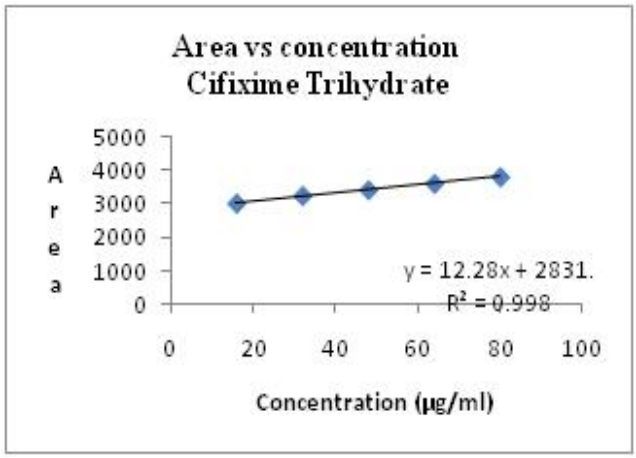

Figure 8: Calibration graph of Cefixime Trihydrate

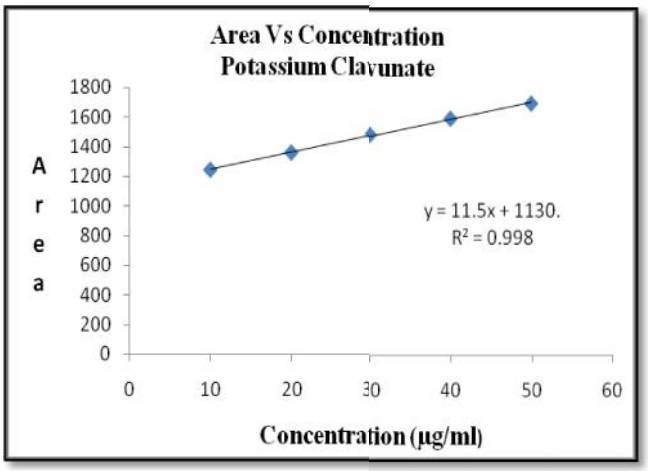

Figure 9: Calibration graph for Potassium Clavunate

Accuracy:

Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets (Cefixime Trihydrate 48 µg/ml and Potassium Clavunate 30 µg/ml) with three different concentrations of standards at 80 %, 100 % and 120 % Cefixime Trihydrate (38,48,57 µg/ml) and Potassium Clavunate (24,30,36 µg/ml). The % recovery and %RSD were calculated and found to be within the limit as shown in table no III.

Precision: [11-12]

Intraday precision was found by analysis of standard drug at six times on the same day. While interday assay precision was carried out on six different day. The RSD was found to be less than 2 for both interday precision and intraday precision. Result for the interday precision and intraday precision is shown in table no IV. respetively.

LOD and LOQ:

Limit of Detection and Limit of quantitation are calculated based on calibration Curve and the results are shown in table no V. [**11-12]**

Robustness:

Robustness data clearly shows that the proposed method is robust at small but deliberate change. Results of robustness are shown in table VI. [11-12]

Application of proposed method to the Pharmaceutical dosage form: The proposed method was applied successfully to the tablet dosage form and results obtained are shown in table no VII.

System Suitability Parameters [11-12]

Statistical analysis of parameters required for system suitability testing of the HPLC method. Data related to system suitability parameters are shown in table no VIII.

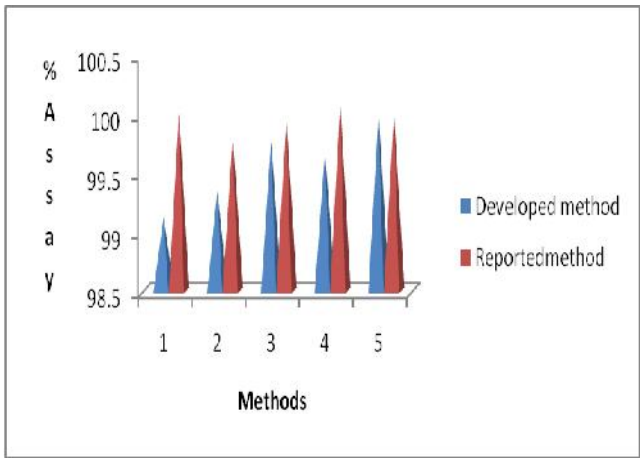

Figure 10: Stastical Comparision between reported method and developed method for Cifixime Trihydtare.

Interpretation:

p value 0.045585987 < 0.05 so, we reject the null hypothesis, and conclude that there is significance difference between the all developed methods for Cifixime Trihydrate.

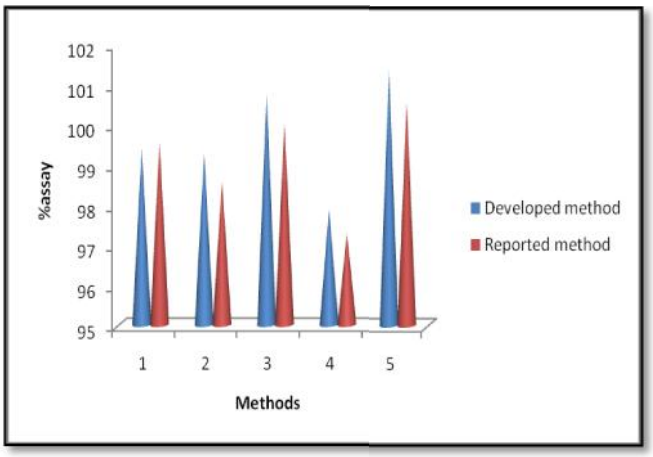

Figure 11: Stastical Comparision between reported method and developed method for Potassium Clavunate.

Interpretation: p value 0.00019704 < 0.05 so, we reject the null hypothesis, and conclude that there is significance difference between the all developed methods for Potassium Clavunate.

Discussion:

- The results indicated that the proposed method was simple, sensitive, accurate and precise for simultaneous estimation of Cefixime Trihydrate and Potassium Clavunate in tablet dosage form by HPLC.
- Separation of drugs was carried out using Methanol: water: Potassium dihydrogen phosphaste Buffer (pH 3) (30:05:65, %v/v/v) mobile phase with wavelength 288nm. The Rt values for Cefixime Trihydrate and Potassium Clavunate were found to be 5.1 min ±0.02and 2.5 min ±0.03respectively.
- The drug response with respect to peak area was linear over the concentration range 16-80 µg/ml for Cefpodoxime Proxetil and 10-50 µg/ml for Potassium Clavunate. The percentage recovery of Cefixime Trihydrate and Potassium Clavunate Was found to be 99.57-101.16 and 98.91-103.48 respectively.
- The % RSD values for intraday precision and interday precision study were ≤2.0% conforming that the method was sufficiently precise. The limit of detection and limit of quantification were found to be 01.41µg/ml and 2.87 µg/ml for Cefixime Trihydrate and Potassium Clavunate 0.91 µg/ml and 1.81 µg/ml.
- The %RSD values of robustness study were were ≤2.0%, conforming that the proposed method was found to be robust enough to withstand such deliberate changes and allow routine analysis of sample.
- The system suitability parameters were also performed and were found within acceptable range.
- This method can be successfully employed for simultaneous estimation of Cefixime Trihydrate and Potassium Clavunate.

Table 1: Detail of HPLC instrument

Component	Brand/Model/software	Manufacturer/ Supplier
HPLC	Younglin	--
Operation software	Autochro-3000 Operation software	--
Column	Varian C-18 (250 X 4.6 mm i.d, 5 µm particle size	--
Injector	Microliter syringe Injector (Rheodyne)	--
Detector	Dual wavelength UV Detector	--
Filter	Ultipore N_{66} Nylon 6,6 Filter membrane, (0.45µ and 0.2µ)	pall Life sciences

Table 2: Optimized chromatographic conditions

S.N	Parameter	Chromatographic conditions
1	Stationary Phase	Varian C-18 (250 X 4.6 mm i.d, 5 µm particle size)
2	Mobile Phase	Methanol: water: Potassium dihydrogen phosphaste Buffer (pH 3) (30:05:65, %v/v/v)
3	Mode of Elution	Isocratic
4	Temperature	25ºC
5	Flow rate	1 ml/min
6	Injection Volume	10 µl
7	Wavelength of detection	288 nm

Table 3: Recovery data for Cefixime Trihydrate and Potassium clavunate

Level*	Concentartion (µg/ml)		Spike solution		Area of spike solution		% Assay	
	Cifi	Pot.clv	Cifi	Pot.clv	Cifi	Pot.clv	Cifi	Pot.clv
80%	48	30	38	24	3308	1403	101.16	98.91
100%	48	30	48	30	3412	1485	98.57	102.90
120%	48	30	57	36	3540	1545	100.24	100.24

*= Average result of three replicate samples

Table 4: Data of Interday Precision and Intraday precision

Concentration(µg/ml)		Area		% Assay	
Cefixime Trihydrate	Potassium Clavunate	Cefixime Trihydrate	Potassium Clavunate	Cefixime Trihydrate	Potassium Clavunate
Interday Precision					
48	30	3418	1480	99.6	101.4
Intraday precision					
48	30	3415	1473	99.1	99.4

Table 5: Results of LOD and LOQ

Parameter	Compound	Result
Detection limit	Cefixime Trihydrate	1.44 µg/ml
	Potassium Clavunate	0.91 µg/ml
Quantitation limit	Cefixime Trihydrate	2.87 µg/ml
	Potassium Clavunate	1.87 µg/ml

Table 6: Data of robustness

Chromatographic factor	level	Retention time		Tailing factor	
		Cefixime Trihydrate	Potassium Clavunate	Cefixime Trihydrate	Potassium Clavunate
Flow rate	0.8ml/min	5.18±0.03	2.25±0.02	0.96±0.05	0.99±0.02
	1.2ml/min	5.05±0.01	2.14±0.05	0.97±0.03	0.97±0.04
Methanol: water: Potassium dihydrogen	(20:05:75, %v/v/v)	5.07±0.02	2.13±0.04	0.96±0.05	0.98±0.01
	(30:05:65, %v/v/v)	5.15±0.04	2.06±0.06	0.99±0.02	0.98±0.03

phosphaste Buffer pH 3					
Detection wavelength	260nm	5.22±0.05	2.28±0.06	0.98±0.04	0.98±0.03
	270nm	5.10±0.04	2.03±0.03	0.96±0.03	0.98±0.01

Table 7: Application of the method to the determination of tablet dosage form

Formulation	Cefixime Trihydrate			Potassium Clavunate		
	Amount taken(µg/ml)	Amount found(µg/ml)	% Amount found (n=3)	Amount taken(µg/ml)	Amount found(µg/ml)	% Amount found (n=3)
Benimax-CV	50	49.54	99.12±0.35	31.25	31.11	99.55±0.12

*= Average result of six replicate samples

Table 8: Data of system suitability parameters.

Parameter	RT*	AUC*	No. of theoretical plates*	Tailing factor*	Resolution
Cefixime Trihydrate	5.1 min ±0.02	13492.6±3871.45	8389.21±90.52	0.98±0.02	5.4
Potassium Clavunate	2.5 min ±0.03	358238.2±572.86	7668.48±139.80	0.97±0.01	

*=Average result of six replicate samples

Table 9: For Cefixime Trihydate

	Developed method	Reported method
Mean	99.695	99.965
Variance	0.0767	0.015566667
Observations	4	4
Pearson Correlation	0.635724084	
Hypothesized Mean Difference	0	
df	3	
t Stat	-2.456237661	
P(T<=t) one-tail	0.045585987	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.091171973	
t Critical two-tail	3.182446305	

Table 10: For potassium Clavunate

	Developed method	Reported method
Mean	99.805	99.0925
Variance	2.4033	2.166091667
Observations	4	4
Pearson Correlation	0.999927578	
Hypothesized Mean Difference	0	
df	3	
t Stat	17.68629068	
P(T<=t) one-tail	0.00019704	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.00039408	
t Critical two-tail	3.182446305	

Table 11: Summary of validation parameter

Parameter	Cefixime Trihydrate	Potassium Clavunate
Wavelength	288 nm	288 nm
Range	16-80 µg/ml	10-50 µg/ml

Linearity	0.998	0.998
%RSD (Interday pricision)	0.48	0.91
%RSD (Intraday pricision)	0.21	0.90
LOD	1.41 µg/ml	0.91 µg/ml
LOQ	2.87 µg/ml	1.81 µg/ml

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