International Research Journal of Pharmaceutical and Applied Sciences (IRJPAS)
Available online at **www.irjpas.com**
Int. Res J Pharm. App Sci., 2013; 3(5):161-167

Research Article

DEVELOPMENT AND VALIDATION OF LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF CIPROFLOXACIN AND TINIDAZOLE IN COMBINED DOSAGE FORM

Ravi Varma.A*, Dr.J.V Shanmukha Kumar, Dr.S.Mutth Reddy
Department Of Chemistry, Kl University,Guntur, Andhra Pradhesh, India

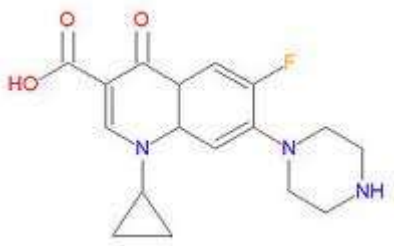
Corresponding Author: : Ravi Varma.A, Email: ravivarma.athota@gmail.com

Abstract: An isocratic, reversed phase-liquid-chromatographic method was developed for the quantitative determination of Ciprofloxacin and Tinidazole in combined-dosage form. A Inertsil C18 (150*4.6*5µ) column with mobile phase containing water pH 2.4 adjusted with ortho phosphoric acid: aceto nitrile in the ratio of (850: 150, v/v) was used. The flow rate was 1.0 mL/min, column temperature was 30°C and effluents were monitored at 275 nm. The retention times of Ciprofloxacin and Tinidazole were 2.419min and 5.119min, respectively. The correlation co-efficient for ciprofloxacin and tinidazole was found to be 0.99 and 0.99, respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Recovery of Ciprofloxacin and Tinidazole in formulations was found to be in the range of 97-103% and 97-103% respectively confirms the non-interferences of the excipients in the formulation. Due to its simplicity, rapidness and high precision. The method was successfully applied to the estimation of Ciprofloxacin and Tinidazole in combined dosage form.

Keywords: RP-HPLC, Ciprofloxacin and Tinidazole

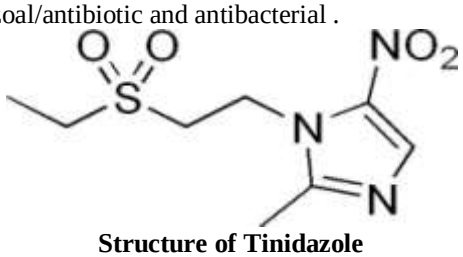
INTRODUCTION

Ciprofloxacin is a broad spectrum antibiotic active against gram +ve and gram –ve bacteria. It functions by inhibiting DNA gyrase[1,2], a type 2 topoisomerase and type IV, enzymes necessary to separate bacterial DNA,there by inhibiting cell division[2,4]. Ciprofloxacin is chemically 1-cyclopropyl-6-fluoro- 4-oxo- 7-(piperazin-1-yl)-quinoline -3-carboxylic acid[5,7]. Literature survey reveals that no method has been reported for estimation of ciprofloxacin satisfactorily. In The present investigation an attempt has been made to develop a simple accurate, reproducible and economical spectrophotometric method for the estimation of ciprofloxacin in tablet dosage



Structure of ciprofloxacin

Tinidazole (TNZ) is a 1-[2-(ethyl sulphonyl) ethyl] – 2-methyl – 5- nitro – 1H- imidazole, derivative used as antiprotozoal/antibiotic and antibacterial .



Structure of Tinidazole

MATERIAL AND METHODS

Instrumentation: The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, Waters Empower2 software and A Inertsil C18 (150*4.6*5µ) column.

Chemicals and Reagents Ciprofloxacin and Tinidazole was a gift sample by Dr. Reddy's Laboratories Ltd., Hyderabad. Acetonitrile of HPLC grade was purchased from E. Merck (India) Ltd., Mumbai. Orthophosphoric acid of AR grade was obtained from S.D. Fine Chemicals Ltd., Mumbai and milli Q water.

HPLC Conditions: The mobile phase consisting of water (pH 2.4 adjusted with orthophosphoric acid) and Acetonitrile (HPLC grade) were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 850:150v/v was pumped into the column at a flow rate of 1.0ml/min. The column temperature was 30°C. The detection was monitored at 275nm and the run time was 8min. The volume of injection loop was 10µl prior to injection of the drug solution the column was equilibrated for at least 15 min. with the mobile phase flowing through the system.

Preparation Of Standard Solution

Ciprofloxacin: Accurately weighed quantity, 500.0 mg of Ciprofloxacin was transferred into 100ml of volumetric flask and adds 30ml of water and sonicate for 15 min. make up the volume with water. Transferred above solution 5ml into 50ml volumetric flask and diluted to the mark with water.

Tinidazole: Accurately weighed quantity, 600mg of Tinidazole was transferred into 100ml of volumetric flask and

adds 30ml of water and sonicate for 15mins make up the volume with water. Transferred above solution 5ml into 50ml volumetric flask and diluted to the mark with water.

Preparation Of Sample Solution:

Accurately weighed 1382mg of sampel. Transfer the sampel powder into 100ml of volumetric flask added 25ml of water and sonicated for 30mins and make up the volume with water and filtered through the 0.45µm filter paper Transfer above solution 5ml into 50 ml volumetric flask and make up the volume with water.

Fig. 1: Standard chromatogram for arterolane and Ciprofloxacin

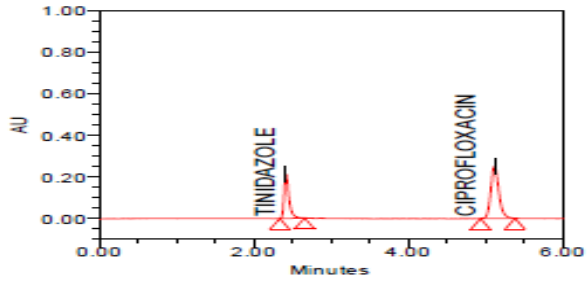
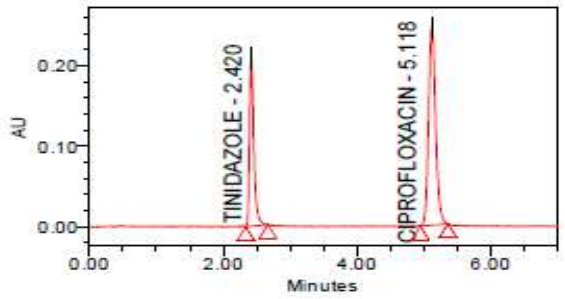


Fig.2: Formulation chromatogramfor arterolane and Tinidazole



METHOD VALIDATION

System Suitability Studies: The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table 1). The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method**.**

Table1: System Suitability Parameters

Parameters	Tinidazole	Ciprofloxacin
Correlation Coefficient	0.99	0.99
Regression Equation	y = 16616x	y = 19288x
LOD	3.3135	1.432
LOQ	11.0450	4.775
Theoretical plates	7857	11834
Tailing	1.544	1.175

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc

Accuracy And Precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained, added recoveries of standard drugs were found to be accurate (Table-3&4). The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated. The chromatograms of three different levels shown in Fig 3, 4 &5. From the data obtained, the developed RP-HPLC method was found to be precise (Table-2).

Table 2 : Precision Studies

SNO	Sample Wt	Area Tiz	Area Cipro	%Assya(tin)	%Assya(c ipro)
1	1382.10	1751534	885902	99	100
2	1382.10	1751873	885287	99	100
3	1382.10	1750277	885405	99	100
4	1382.10	1751697	885611	99	100
5	1382.10	1750177	885142	99	100
6	1382.10	1755696	885196	99	100

Fig. 3: Accuracy Chromatograms-50% of Ciprofloxacin andTinidazole

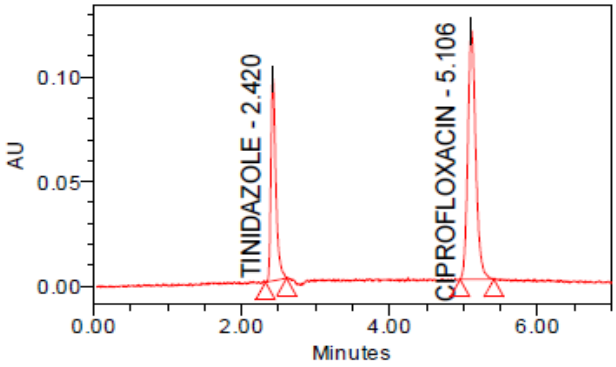


Fig. 4: Accuracy Chromatograms-100% of Ciprofloxacin And Tinidazole

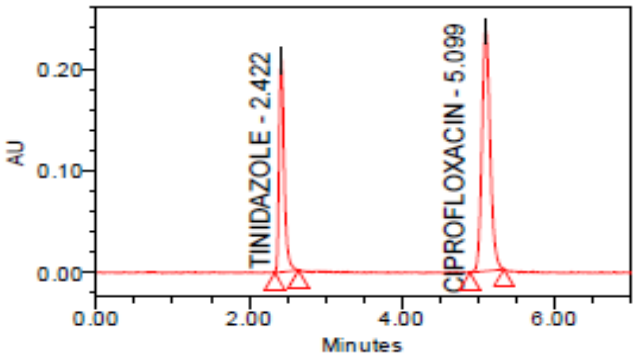


Fig. 5: Accuracy Chromatograms-150% of Ciprofloxacin And Tinidazole

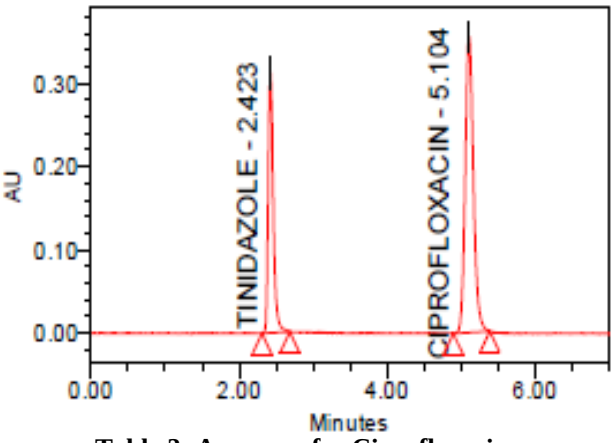


Table 3: Accuracy for Ciprofloxacin

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	mean
50%	691.05	878434	247.500	247.66	100	100
50%	691.05	878147	247.500	247.58	100	
50%	691.05	878452	247.500	247.67	100	
50%	691.05	878298	247.500	247.63	100	
50%	691.05	878729	247.500	247.75	100	
50%	691.05	878624	247.500	247.72	100	
100%	1382.10	1755785	495.000	495.02	100	100
100%	1382.10	1752475	495.000	494.09	100	
100%	1382.10	1755958	495.000	495.07	100	
150%	2073.20	2639499	742.518	744.17	100	100
150%	2073.20	2633402	742.518	742.46	100	
150%	2073.20	2639219	742.518	744.10	100	
150%	2073.20	2637602	742.518	743.64	100	
150%	2073.20	2636033	742.518	743.20	100	
150%	2073.20	2633365	742.518	742.45	100	

Table 4: Accuracy for Tinidazole

Spiked level	Sample weight	Sample area	µg/ml added	µg/ml found	% recovery	mean
50%	691.05	442375	300.000	298.82	100	100
50%	691.05	442448	300.000	298.87	100	
50%	691.05	442232	300.000	298.72	100	
50%	691.05	442755	300.000	299.08	100	
50%	691.05	442099	300.000	298.63	100	
50%	691.05	442510	300.000	298.91	100	
100%	1382.10	885535.00	600.000	598.17	100	100
100%	1382.10	885476.00	600.000	598.13	100	
100%	1382.10	885372.00	600.000	598.06	100	
150%	2073.20	1326300	900.022	895.90	100	99
150%	2073.20	1325407	900.022	895.30	99	
150%	2073.20	1323006	900.022	893.68	99	
150%	2073.20	1322683	900.022	893.46	99	
150%	2073.20	1327047	900.022	896.41	100	
150%	2073.20	1324760	900.022	894.86	99	

Linearity And Range

The linearity of the method was determined at five concentration levels. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 16616x (R^2=0.99) for Ciprofloxacin and y = 19288x (R^2=0.99) for Tinidazole The results shows that an excellent correlation exists between areas and concentration of drugs within the concentration range indicated above. The overlay chromatograms of Linearity for Ciprofloxacin and Tinidazole shows in Fig 6 and the results for calibration curves are given in Fig 7&8.

Fig. 7: Linearity Curve for Ciprofloxacin

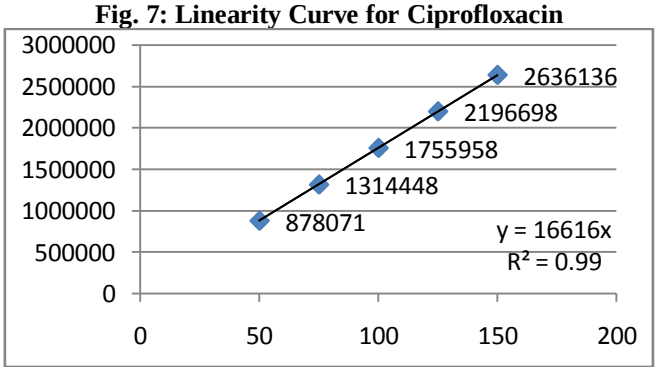


Fig. 8: Linearity Curve for Tinidazole

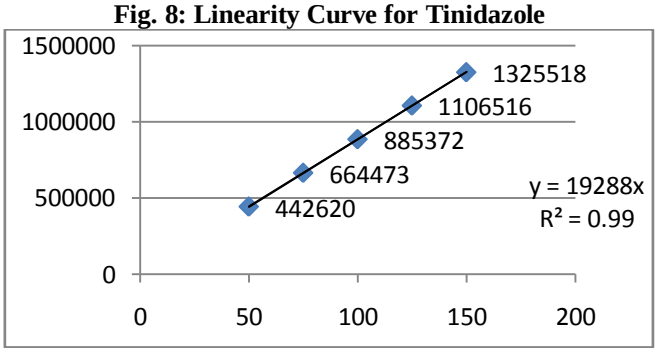
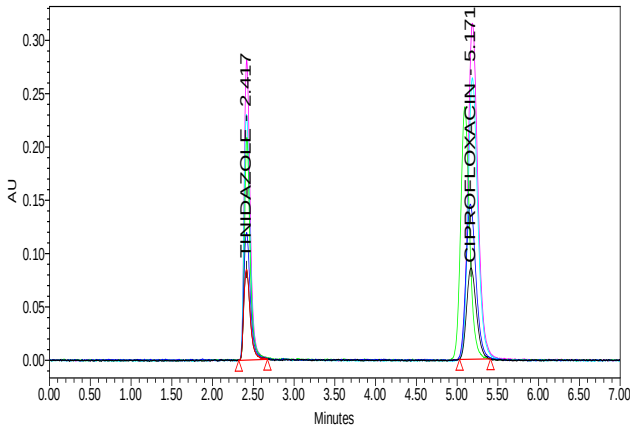
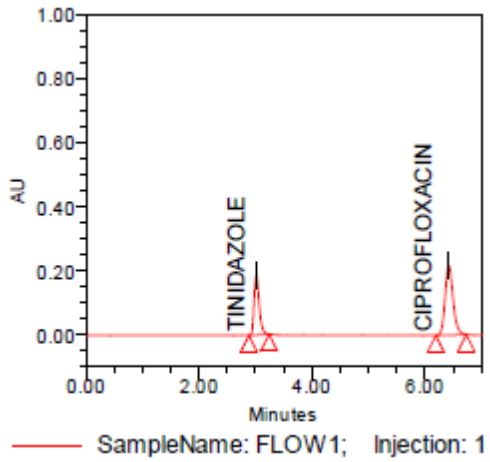


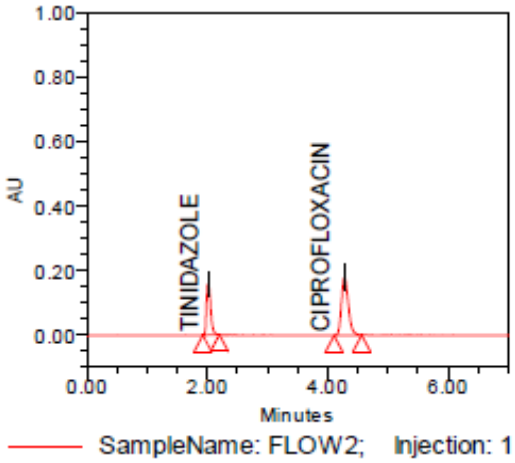
Fig. 9: Overlay chromatograms of Linearity for Ciprofloxacin And Tinidazole

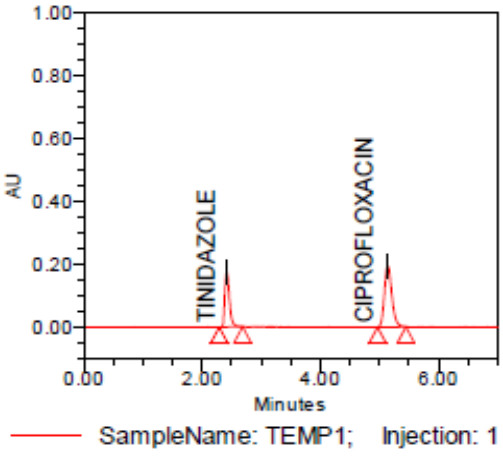


Robustness

Robustness of the method was determined by making slight changes in the chromatograph conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP HPLC method developed, are rugged and robust (Table-5&6).







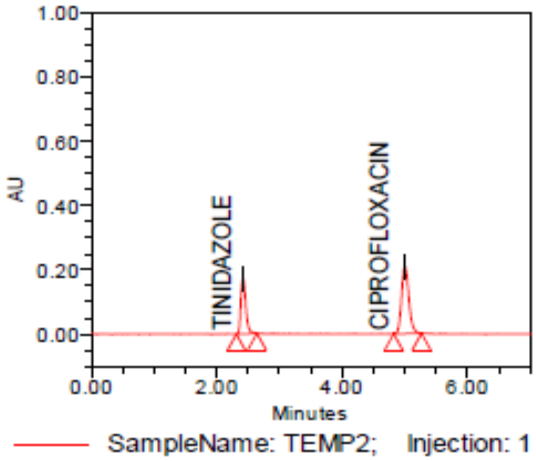


Table5:Robustness for Ciprofloxacin

	SAMPEL NAME	INJ	NAME	RT	AREA	USP TAILING	USP PLATECOUNT	S/N
1	TEMP-1	1	Ciprofloxacin	5.142	1701816	1.233	7910	155.091
2	TEMP-2	1	Ciprofloxacin	5.004	1685623	1.162	8997	134.276
3	FLOW-1	1	Ciprofloxacin	6.422	2134067	1.179	10165	153.365
4	FLOW-2	1	Ciprofloxacin	4.280	1436936	1.275	6874	126.561

Table6:Robustness for Tinidazole

	SAMPEL NAME	INJ	NAME	RT	AREA	USP TAILING	USP PLATECOUNT	S/N
1	TEMP-1	1	Tinidazole	2.417	955394	1.438	4643	138.200
2	TEMP-2	1	Tinidazole	2.420	946579	1.318	4859	109.687
3	FLOW-1	1	Tinidazole	3.016	1178935	1.356	5373	131.716
4	FLOW-2	1	Tinidazole	2.012	774370	1.454	4227	111.749

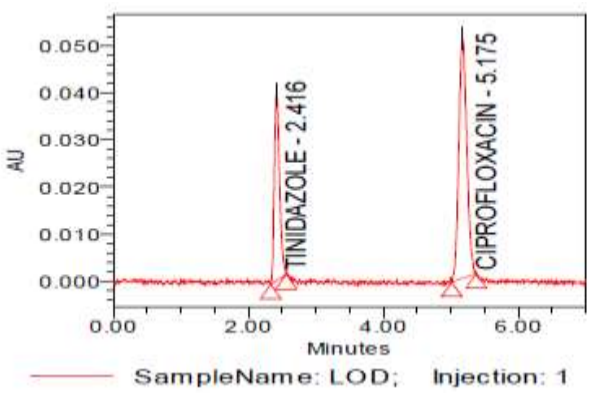
LOD&LOQ: Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of ciprofloxacin and tinidazole. Relative standard deviation (σ) method was applied, the LOQ and LOD values were predicted using following formulas (a) and (b). Precision was established at these predicted levels and the results are tabulated in Table-5.

(a) LOQ = 10 σ / S

(b) LOD = 3.3 σ / S

Where σ = residual standard deviation of response

S = slope of the calibration curve.



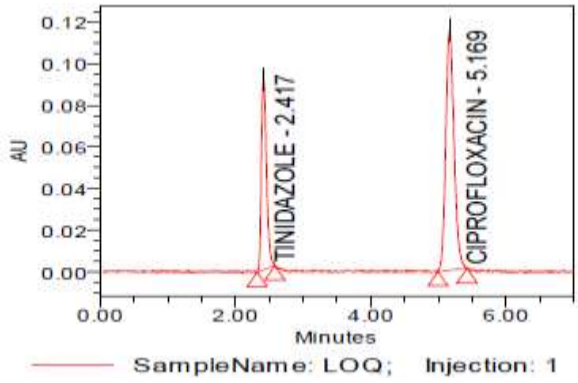


TABLE 7: LOD AND LOQ FOR Ciprofloxacin and Tnidazole

	Sampel name	inj	Name	Rt	Area	Usp Tailing	Usp Plate Count
1	LOD	1	Cipro	5.175	418524	1.104	9394
2	LOQ	1	Cipro	5.169	979109	1.162	8556
1	LOD	1	Tind	2.416	213319	1.338	4642
2	LOQ	1	Tind	2.417	481423	1.343	5035

RESULTS AND DISCUSSION

System suitability results were given by table1 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1 so we can say system is suitable for analysis method specificity was concluded by fig:1 and fig:2 those figures are Ciprofloxacin and Tinidazole standard chromatogram and other one is formulation they were not observed placebo and excipients peaks interference with standard and analytic peak so it proves method is selective. The result given in table 2 says that the method precision passed for both Ciprofloxacin and Tinidazole studies. The method accuracy was evaluated by recovery studies. Ciprofloxacin and Tinidazole recovery was founded 100% as per ICH 97%-103% and also percentage RSD was very low so method is accurate shown in table 3&4. Linearity calibration curve was given below fig: 7&8 and plot the graph three different concentrations versus areas to construct the linear regression equation and to calculate the value of correlation coefficient. Linear correlation was found to be Y= 16616 for Ciprofloxacin and y = 19288 for Tinidazole Method robustness results were given by table 5&6, LOQ and LOD Rsults were given by table 7.

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Ciprofloxacin and Tinidazole in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Ciprofloxacin and Tinidazole in pure and its pharmaceutical dosage forms.

ACKNOWLEDGEMENT

Author thankful to department of chemistry in KL University, Guntur and Rainbow pharma training lab, Kukatpally, for providing instruments and analytical support and analytical support.

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