IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Simultaneous Estimation Of Losartan Potassium And Chlorthalidone In Tablets By RP-HPLC Method

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ABSTRACT

A simple, precise, accurate and rapid **RP-HPLC** method has been developed and validated for the **simultaneous estimation of losartan potassium and chlorthalidone in pharmaceutical dosage form**. The process was carried out on C_{18} column using a mobile phase of phosphate buffer **pH 4.0,** acetonitrile and methanol in the ratio of **20:30:50 v/v/v** at a flow rate of 1.0mL/min.The detection of Lamda max by using uv–visible spectroscopy losartan potassium and chlorthalidone was carried out at a wavelength selection of **400nm-200nm.** Losartan potassium for **234nm**, Chlorthalidone for **228nm**, Methanol using a solvent.The calibration curves were linear range of **4-20µg**/mL for losartan potassium and 2-10µg/mL for chlorthalidone. **The retention time of Chlorthalidone and Losartan potassium was found to be 2.96min and 10.41 min,** respectively. Results of the analysis were validated statistically by recovery studies. It can be successfully used to estimate the drug contents in the raw material and marketed formulation.

Key-words: Losartan potassium(LOS), Chlorthalidone(CHL) , RP-HPLC.

INTRODUCTION

Losartan potassium chemically, 2-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl) benzyl] imidazole-5-methanol mono potassium salt and chlorthalidone 2-chloro-5-(1-Hydroxy-3-oxoisoindolin-3-yl) benzene sulphonamide. The combination is useful in treatment of anti hypertensive disease. Literature survey reveals that various spectrophotometric and HPLC methods were reported for the individual determination of losartan potassium and chlorthalidone in pharmaceutical dosage forms. No method has been developed for the estimation of these drugs simultaneous. The present work describes a simple, precise and accurate HPLC method for the simultaneous estimation of losartan potassium and chlorthalidone in tablet dosage form.

MATERIALS AND METHODS

Reagent:

Standard bulk drug sample Losartan Potassium and Chlorthalidone of were provided by M/s. surian Pharmaceuticals Chennai. A tablet of combined dosage form was procured from the local market. All other reagents used were of analytical grade. Shimadzu RP-HPLC-ILC-2030, model and Chromatogram were recorded using program having following specifications wavevelength. Quantitative HPLC was performed on Shimadzu HPLC system of LC-20AT pump, 20A UV-Visible absorbance detector, Shimadzu Win Chrome software with hypersis ODS C_{18} column. Sample injection via a Rheodyne syringe. . Acetonitrile and methanol of HPLC grade were obtained from M/s. Qualigens Chennai.

EXPERIMENT

Method 1: Employing simultaneous equations

Chromatographic conditions: The mobile phase used in this study was a mixture of phosphate buffer pH 4.0, acetonitrile and methanol in the ratio of 50:20:30 v/v/v. The run time and the flow rates were 12 min and 1 mL/min, respectively. The mobile phase was filtered before use through 0.45µ membrane filter and degassed for 15min. The eluents were monitored at 224nm. The injection volume was 20µL.

Preparation of stock solution: Standard solution of the pure drug was prepared by dissolving accurately weighed 100mg of losartan potassium and chlorthalidone in a 100mL volumetric flask using 25mL of diluents, acetonitrile:methanol:Phosphate buffer in the ratio of 20:30:50 v/v/v. Then the volume made up to the mark with the same solvent and obtains the concentration of 1mg/mL.

ANALYSIS OF TABLET FORMULATION

This method was applied to determine losartan potassium and chlorthalidone samples. For analysis of tablet formulation, 20 tablets were weighed and average weight was determined and these were powdered. Sample solution was prepared by dissolving powdered tablets weighed equivalent to 50mg of losartan potassium and 25mg of chlorthalidone in 50mL volumetric flask. Then the drugs were dissolved by using 25mL of diluents and the volume was made up to the mark with diluents. The solution was filtered through Whatmann filter paper No.41 and further diluted with diluents to get a final concentration of 8µg/mL of losartan potassium and 4µg/mL of chlorthalidone. 20µL of the standard and sample solution were injected, respectively into HPLC system under chromatographic conditions and the chromatograms were recorded. The amount of drug present in tablet formulation was calculated by comparing the mean peak area ratio from the standard. The results are given in Table-1.

Method validation: The method was validated in terms of linearity, accuracy, intra-day inter-day precision, reproducibility, and specificity, limit of detection (LOD) and limit of quantification (LOQ). Linearity was determined on standard solution by analyzing different concentration and the calibration curve was plotted. Accuracy of the method was ascertained by recovery studies by adding a known quantity of standard drug to the pre-analyzed sample and the contents were analyzed by the proposed method. The intra-day and inter-day precision was determined by analyzing on the same day and on three different days over a period of two weeks. The intra-day and inter-day in the peak area ratio of the drug solution to that of internal standard was calculated in terms of percentage relative standard deviation and the results are shown in Table-2. Specificity was carried out by injecting placebo solution. Robustness of the method was evaluated by performing the assay with variations in wavelength, pH and flow rate. The chromatographic parameters were validated by system suitability parameters and the values are given in Table-2.

RECOVERY STUDIES

To study accuracy, reproducibility and precision of the proposed methods, recovery studies were carried out by standard addition method. Results of recovery studies were found to be satisfactory and presented in Table 3. Precision of the method was determined by performing Intra Day (n = 3) and Inter Day (n = 3) refer the results in Table-3

RESULTS AND DISCUSSION

The retention time of chlorthalidone and losartan potassium was found to be 2.96 and 10.41 min, respectively. A typical chromatogram of Losartan Potassium and Chlorthalidone range of 4-20µg/mL for Losartan Potassium and 2-10µg/mL for Chlorthalidone, with correlation of 0.999 for Losartan Potassium and 0.999 for Chlorthalidone, respectively. The high percentage of recovery of the drugs indicates that the method is highly accurate. Recovery data from the study is reported in Table-3. There was good repeatability of proposed method with high percentage RSD 1.1750 for Losartan Potassium and 0.8469 for Chlorthalidone. No interfering peaks were found in the chromatogram indicating that the excipients in tablet formulations did not interfere with the estimation of the drug and the peak response was due to individual drug components only. The LOD and LOQ for Losartan Potassium were found to be 0.3701 and 1.1215. The LOD and LOQ for Chlorthalidone were found to be 0.0944 and 0.2863.

Linearity:

Calibration curves were prepared for both the drugs at the selected analytical wavelengths are summarized in Table1.This shows that Losartan Potassium and Chlorthalidoneobeys Beer's law in the concentration range of 4-20 µg/ml and 2-10 µg/ml

Accuracy:

The accuracy of the method was determined by investigating the recovery of Losartan and Chlorthsalidone three levels ranging from 50, 75 &100% of the nominal concentration by standard addition technique. The results as shown in Table-3 indicate excellent recoveries.

Precision &repeatability:

The precision and repeatability of the method was studied by repeating the proposed method three times in a day and the average percentage, RSD values of the results were tabulated, and when the experiment was repeated on three different days the average percentage RSD values for determination was tabulated in Table-4 . The results confirm the intra day and inter day precision of the method.

CONCLUSION:

The proposed RP- HPLC mrthod was found to be simple, rapid, specific, precise and accurate for the estimation of Losartan Potassium and Chlorthalidone in tablet dosage forms. Hence, it can be easily and conveniently adopted for routine qulity control analysis. High percentage recovery shows that the method is free from the interference of excipients used in the formulation.

ACKNOLEDGEMENT:

The authors are thankful to the management and the Principal of Adhiparasakthi College of pharmacy for providing the necessary facilities to carry out the research work.

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TABLE-1: ASSAY OF COMBINED TABLET DOSAGE FORM

Drug	Sample No.	Label claim (mg/tablet)	Amount estimated* (mg/tablet)	% Amount found
Losartan Potassium	1	25	24.91	99.67
	2	25	24.62	100.42
	3	25	24.77	99.11
Chlorthalidone	1	12.5	12.52	100.17
	2	12.5	12.71	101.67
	3	12.5	12.44	99.56

TABLE II: SYSTEM SUITABILITY PARAMETERS

Parameter	Losartan Potassium	Chlorthalidone
Linearity (µg/mL)	4-20	2-10
Slope	1123450.29	1264484.92
Correlation coefficient	0.9991	0.9990
Theoretical plates (N)	3530	2502
Tailing factor	1.08	1.12
Percentage recovery	99.29	100.35
% RSD	1.1747	0.8469
LOD (µg/mL)	0.3701	0.0944
LOQ (µg/mL)	1.1215	0.2863

TABLE III: RECOVERY STUDY.

Drug	**Label claim (mg)**	**Amount added (mg)**	**Amount recovered (mg)**	**Recovery (%)**
Losartan Potassium	25	8	8.0363	100.45
Chlorthalidone	12.5	3.2	3.2493	101.53
Losartan Potassium	25	6.4	6.4470	100.72
Chlorthalidone	12.5	4	3.9806	99.51
Losartan Potassium	25	9.6	9.5471	99.44
Chlorthalidone	12.5	4.8	4.8800	101.68

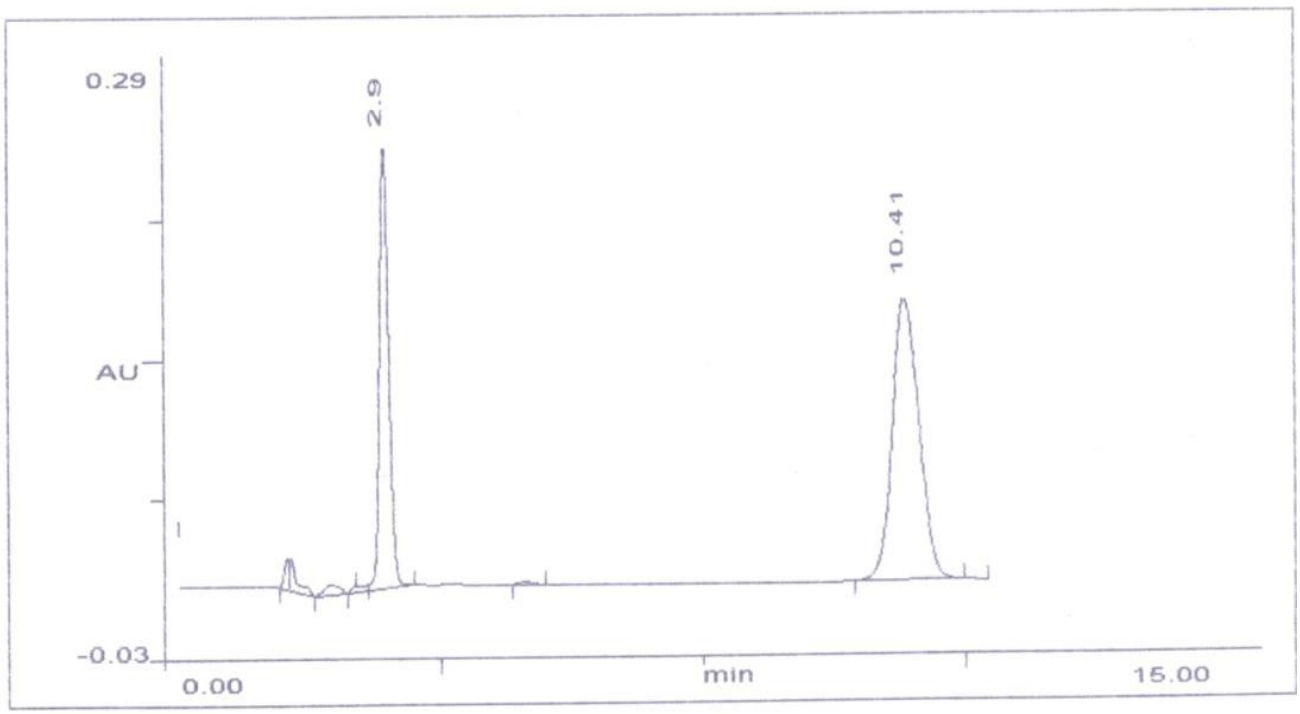
TABLE IV: RESULTS OF INTRA DAY& INTER DAY STUDIES (n = 3)

S.No	Method	INTRA DAY %AMOUNT FOUND*		INTERDAY %AMOUNT FOUND*		% RSD-1		% RSD2	
		LOS	CHL	LOS	CHL	LOS	CHL	LOS	CHL
1	Simultaneous Equation	99.45 100.93 101.18	100.55 99.88 100.46	99.45 101.44 100.71	100.5 98.91 99.69	0.691 1.0727 0.2298	0.691 0.643 0.714	0.69 0.64 1.054	0.70 0.204 0.855

*Each reading is an average of six replicates

Phosphate buffer **pH 4.0:** Acetonitrile :Methanol(**20:30:50 v/v/v)**

Fig.I – Precision- Chromatogram for Chlorthalidone & Losartan Potassium at 224 nm (2µg/mL ,4µg/mL)

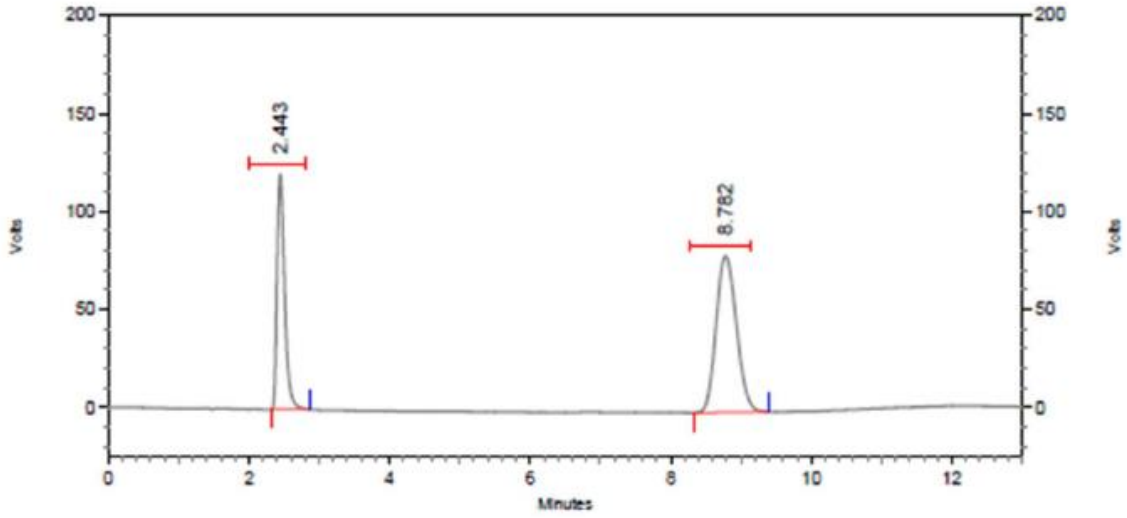


RT- 2.9- CHL-Chlorthalidone. (4µg/mL)
RT- 10.41- LOS-Losartan Potassium.(8µg/mL)

Fig.I- Chromatogram for Chlorthalidone combine Losartan Potassium Tablet Dosage Form.

Phosphate buffer **pH 4.0:** Acetonitrile :Methanol(**20:30:50 v/v/v)**

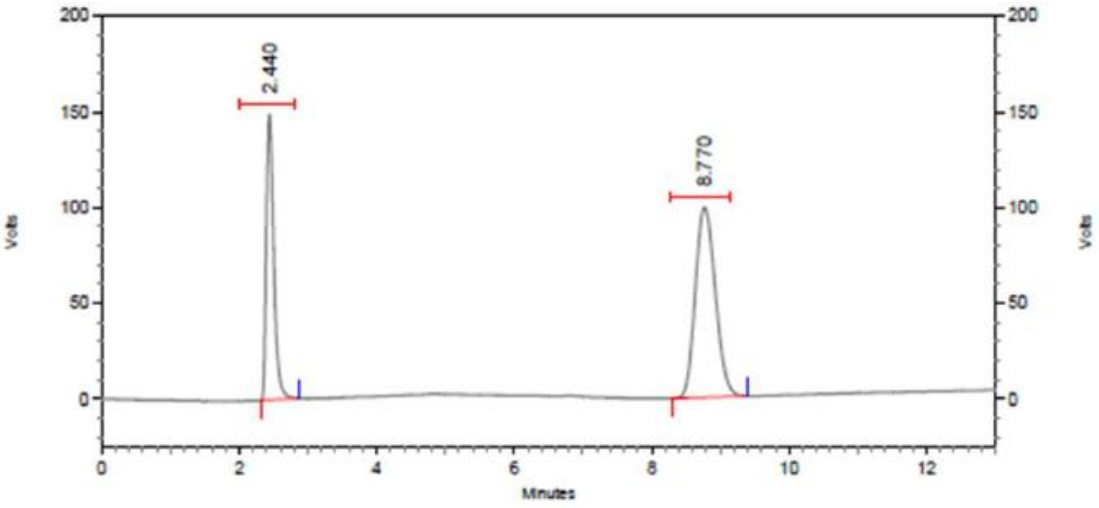
Fig.II - Linearity Chromatogram for Chlorthalidone and Losartan Potassium at 224 nm (2µg/ml,4µg/ml)



Retention Time	Area	Area %	Theoretical plates (USP)	Resolution (USP)	Asymmetry
2.443	1151053	37.95	2435	0.00	1.47
8.807	1881907	62.05	4212	17.18	1.16
	3032960	100.00			

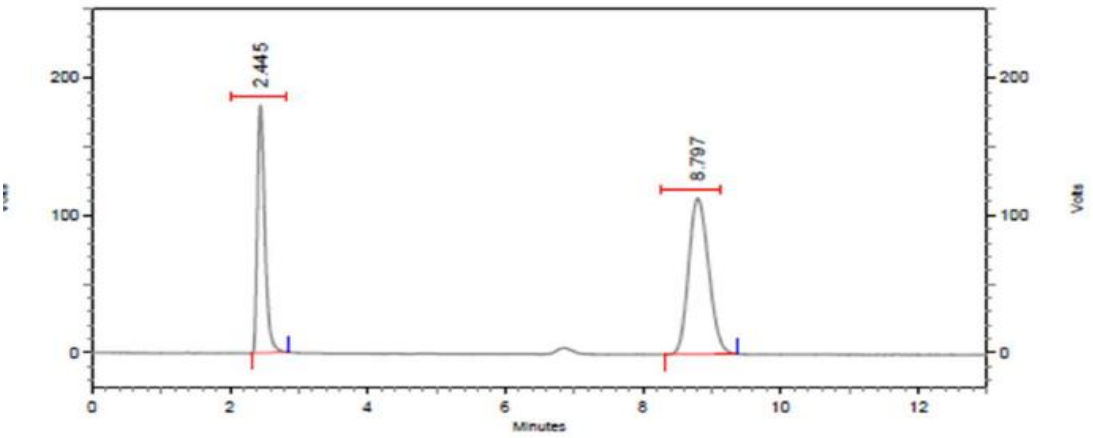
Phosphate buffer **pH 4.0:** Acetonitrile :Methanol(**20:30:50 v/v/v)**

Fig.III - Linearity Chromatogram for Chlorthalidone and Losartan Potassium at 224 nm (10µg/ml,20 µg/ml)



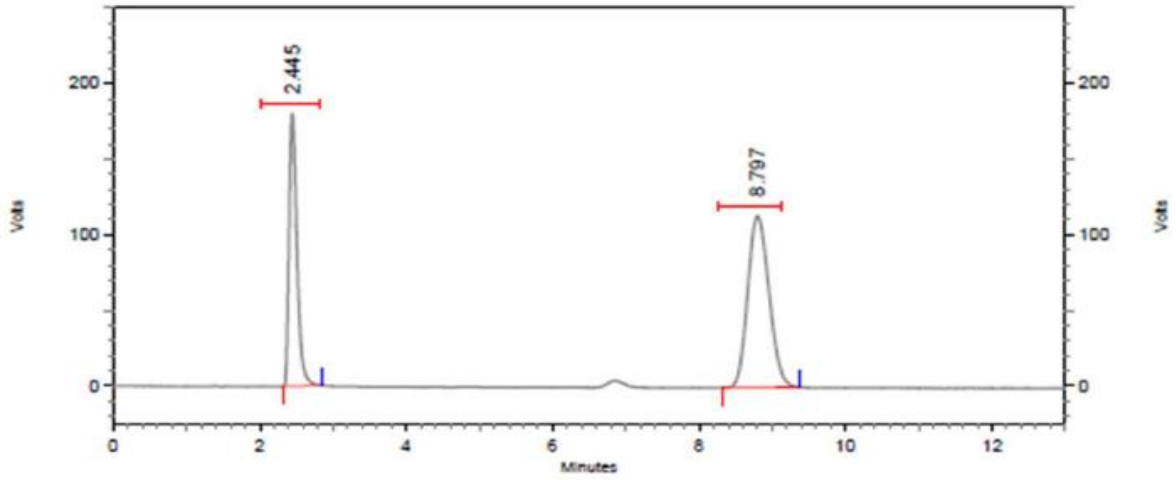
Retention Time	Area	Area %	Theoretical plates (USP)	Resolution (USP)	Asymmetry
2.440	1123535	35.83	2501	0.00	1.46
8.770	2011841	64.17	4159	17.13	1.15
	3135376	100.00			

Fig-IV- Chromatogram for Recovery Analysis (Recovery -1)



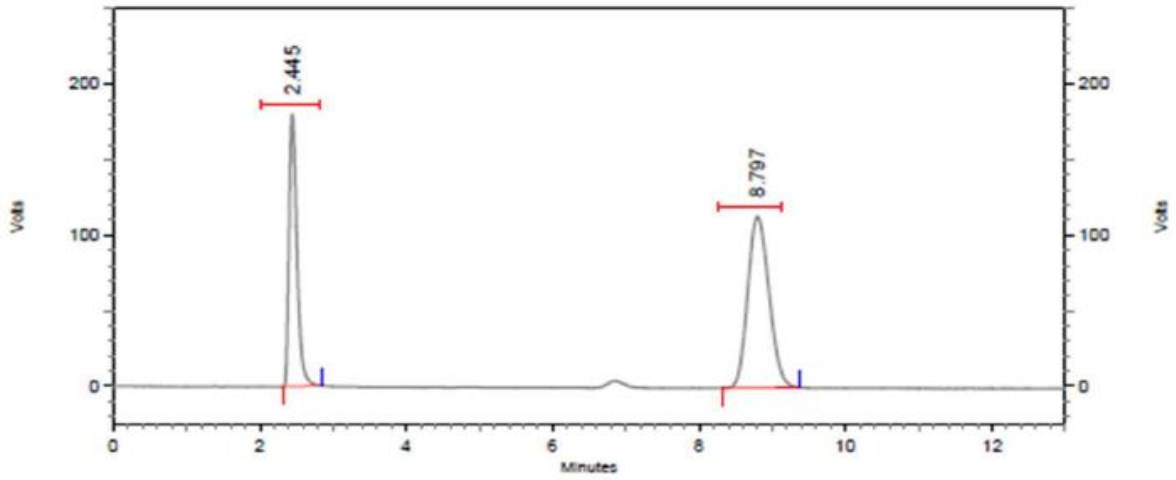
Retention Time	Area	Area %	Theoretical plates (USP)	Resolution (USP)	Asymmetry
2.445	1359088	37.19	2519	0.00	1.48
8.797	2295482	62.81	4177	17.18	1.16
	3654570	100.00			

Fig-V- Chromatogram for Recovery Analysis (Recovery -2)



Retention Time	Area	Area %	Theoretical plates (USP)	Resolution (USP)	Asymmetry
2.445	1359088	37.19	2519	0.00	1.48
8.797	2295482	62.81	4177	17.18	1.16
	3654570	100.00			

Fig-VI- Chromatogram for Recovery Analysis (Recovery -3)



Retention Time	Area	Area %	Theoretical plates (USP)	Resolution (USP)	Asymmetry
2.445	1359088	37.19	2519	0.00	1.48
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*Each reading is an average of six replicates

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*Each reading is an average of six replicates

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*Each reading is an average of six replicates

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*Each reading is an average of six replicates

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