

**DEVELOPMENT AND VALIDATION OF ESTIMATION OF CHONDROITIN AND
IBUPROFEN BY U.V. SIMULTANEOUS METHOD IN DOSAGE FORMS**

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ABSTRACT

Development and validation of the UV simultaneous method for the estimation of Chondroitin and ibuprofen in pharmaceutical dosage forms was presented. The method development involved selecting appropriate solvent systems, determining suitable analytical wavelengths, and optimizing other experimental parameters such as diluent selection and instrumental conditions. A UV-Visible spectrophotometer was utilized for method development and subsequent analysis. The selection of wavelengths was based on the absorption maxima of chondroitin and ibuprofen, ensuring optimal sensitivity and specificity for both compounds.

Method validation was conducted following International Conference on Harmonization (ICH) guidelines to assess various parameters including linearity, accuracy, precision, specificity, and robustness. Calibration curves were constructed for both chondroitin and ibuprofen over a concentration range suitable for pharmaceutical analysis. The method exhibited excellent linearity, with correlation coefficients exceeding 0.99 for both compounds, indicating a strong linear relationship between concentration and absorbance. Accuracy and precision were evaluated through recovery studies and intraday/interday variability assessments, respectively. The results demonstrated satisfactory accuracy and precision, with recovery values within acceptable limits and low relative standard deviations, indicating the reliability and reproducibility of the method. Specificity was confirmed by analyzing placebo samples, which showed no interference at the retention times of chondroitin and ibuprofen.

Keywords: Ibuprofen, Chondroitin, Glycosaminoglycan, UV-Visible spectrophotometer, ICH, NSAID, HPLC

INTRODUCTION

Chondroitin, a glycosaminoglycan¹, and ibuprofen, a nonsteroidal anti-inflammatory drug (NSAID)², are commonly used in combination for the management of osteoarthritis and related conditions³. The concept of drug treatment, which was earlier “right drug for right person” is now changing from “right does for the right person” to “right time of the does for the right person”⁴.

The term "hydrotropy" has been used to designate the increase in solubility of various substances due to the presence of large amounts of additives⁵. Various hydrotropic agents such as sodium salicylate, sodium benzoate, urea, nicotinimide, sodium citrate and sodium acetate have been used to enhance the aqueous solubility of a large number of drugs⁶. Maheshwari and his associates have analyzed a large number of poorly water-soluble drugs by titrimetric and spectrophotometric analyse⁷. Process validation is establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality characteristics⁸. For validation of analytical methods applicant should follow characteristics or parameters needed for validation according to ICH Q2A and ICH Q2B⁹.

It is necessary to find the concept of each drug either in bulk or single or combined dosage forms for purity testing. It is also essential to know the concentration of the drug and its metabolites in biological fluids after taking the dosage for treatment. The scope of developing and validating and analytical method is to ensure a suitable method for a particular analyte more specific, accurate and precise¹⁰.

The aim and objective of the present work is to develop new simple, sensitive and Validated RP-HPLC method for the simultaneous estimation of Chondroitin and Ibuprofen in marketed formulation. The developed analytical method was validate according to ICH guideline.

MATERIALS AND METHODS

The proposed work was carried out on a Labindia UV-Visible Spectrophotometer (Model: UV-3000+), which possesses a double beam double detector configuration with matched 1 cm quartz cells. Reference standard of Chondroitin and Ibuprofen was a generous gift from Pharmaceutical Company. Sodium acetate and Sodium Benzoate obtained from Merck Chemical Division,

Mumbai. Commercial tablets of CHO and IBU, IBU Dycerin-A Tab (Glenmark) were procured from the local drug market. Label claim of CHO and IBU in tablet is 2 and 50 mg respectively. Reverse Osmosis Water was used throughout the study. Chondroitin and Ibuprofen combination recently launched in the market for the treatment of Anti Inflammatory, Analgesic in the strength of 50:100 mg. till date there is no method for the spectrophotometric estimation of Chondroitin and Ibuprofen in combination by using hydrotropic agent. Following are the marketed formulation to be estimated by using hydrotropic phenomenon.

Identification of drug

Solubility: Solubility of CHO and IBU was determined at $25\pm 1^\circ\text{C}$. Accurately weighed 10 mg CHO and IBU was added in different 10 ml volumetric flask containing different solvent and placed at mechanical shaker for 8 hrs. After 8 hrs filter both solution were filtered through Whatman filter paper No. 41. The filtrates were diluted suitably and analyzed by spectrophotometrically against water.

Enhancement of solubility was more than 60 to 70 % for CHO and IBU respectively in mixed hydrotropic solution. The enhancement of solubility of CHO and IBU was due to the hydrotropic solubilization phenomenon. Results of solubility in different solvent for both the drug were shown in table no. 6.2.

Table 6.2: Solubility of drug in different solvents

S. No.	SOLVENTS	SOLUBILITY	
		CHO	IBU
1	Water	-	-
2	Hot water	-	-
3	Cold water	-	-
4	2M Sodium acetate	+	+
5	8M Urea	-	-
6	2M Sodium Citrate	-	-
7	2M Sodium Benzoate	+	+
8	2M Sodium acetate: 2M Sodium Benzoate (1:1)	+	+
9	2M Urea:2M Sodium acetate (1:1)	+	+
10	2M Sodium Citrate:8M Urea (1:1)	+	+

Selection of solvent system: CHO and IBU and IBU were scanned in various hydrotropic agent in the spectrum mode over the UV range (200-400) and 2M Sodium acetate: 2M Sodium Benzoate (1:1) was found to be most appropriate because:

- Both drugs are soluble in it (60 and 70 %)
- Both drugs are stable
- Both drugs exhibit good spectral characteristics
- Sodium acetate: Sodium Benzoate solutions have no interference with the λ_{\max} of both drugs AS compare to urea solution.

Establishment of stability profile: Stability of both drugs were observed by dissolving CHO and IBU in Sodium acetate: Urea (2M:8M) solution used as solvent. Solution of CHO and IBU was prepared in the conc. of 5 $\mu\text{g/ml}$ and 10 $\mu\text{g/ml}$ respectively and scanned under time scan for 30 min. Spectra of both drugs under time scan shows that of both drugs are stable in mixed hydrotropic solution.

Linearity range and calibration graph:

Selection of wavelength for linearity: Solutions of 5 $\mu\text{g/ml}$ of CHO and 10 $\mu\text{g/ml}$ IBU were prepared separately. Both the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of CHO and IBU was observed at 242.0 nm and 278.0 nm, respectively. CHO and IBU showed linearity in the concentration range of 10-50 $\mu\text{g/ml}$ and 10-15 $\mu\text{g/ml}$ at their respective maxima. Calibration curve was plotted, absorbance versus concentration. To study the linearity of CHO and IBU, the selected wavelengths were 242.0nm and 278.0 nm respectively.

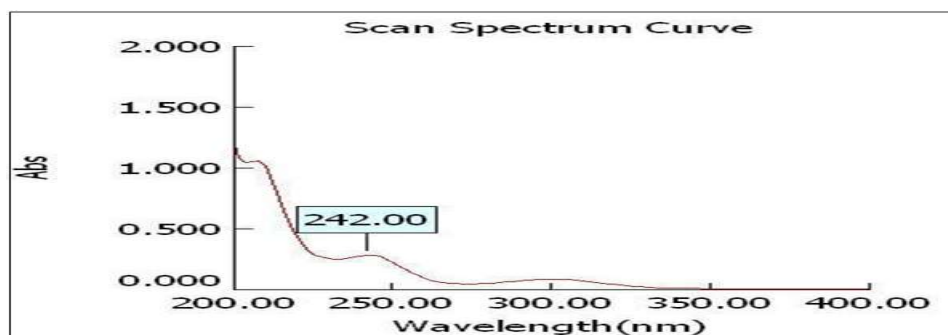


Figure 6.1: Determination of λ_{\max} of CHO

Table 6.3: Linearity of IBU at $\lambda_{max} = 242.0$ nm

Standard Conc. ($\mu\text{g/ml}$)	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Mean
0	0	0	0	0	0	0
10	0.205	0.204	0.205	0.206	0.205	0.205
20	0.405	0.406	0.405	0.405	0.406	0.405
30	0.625	0.624	0.625	0.624	0.626	0.625
40	0.815	0.815	0.816	0.815	0.812	0.815
50	1.012	1.013	1.015	1.016	1.016	1.014

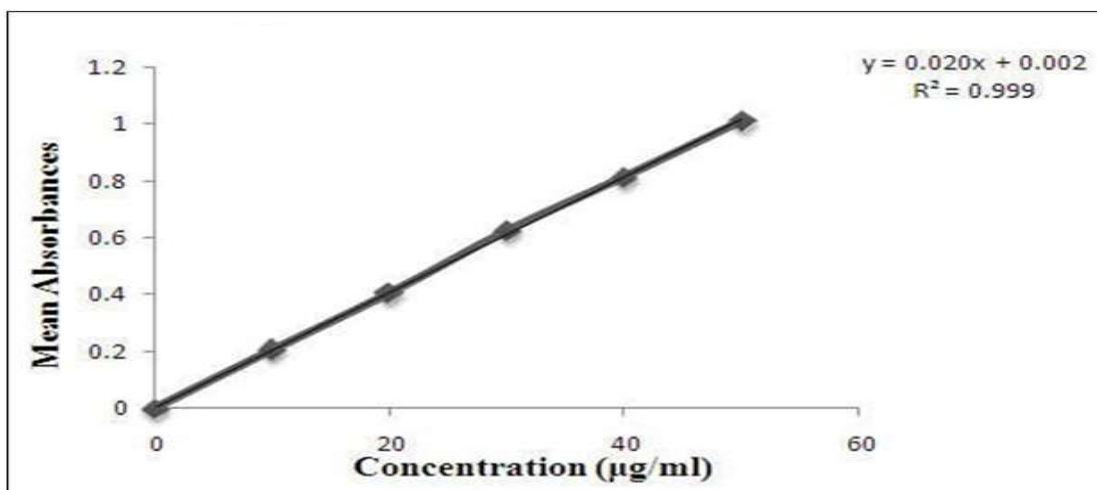


Figure 6.2: Calibration curve of CHO

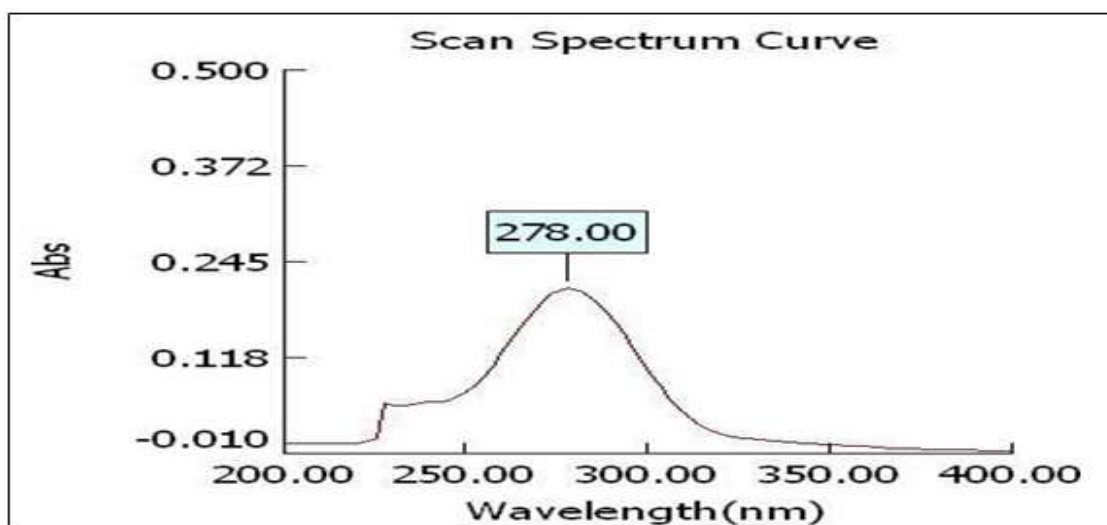
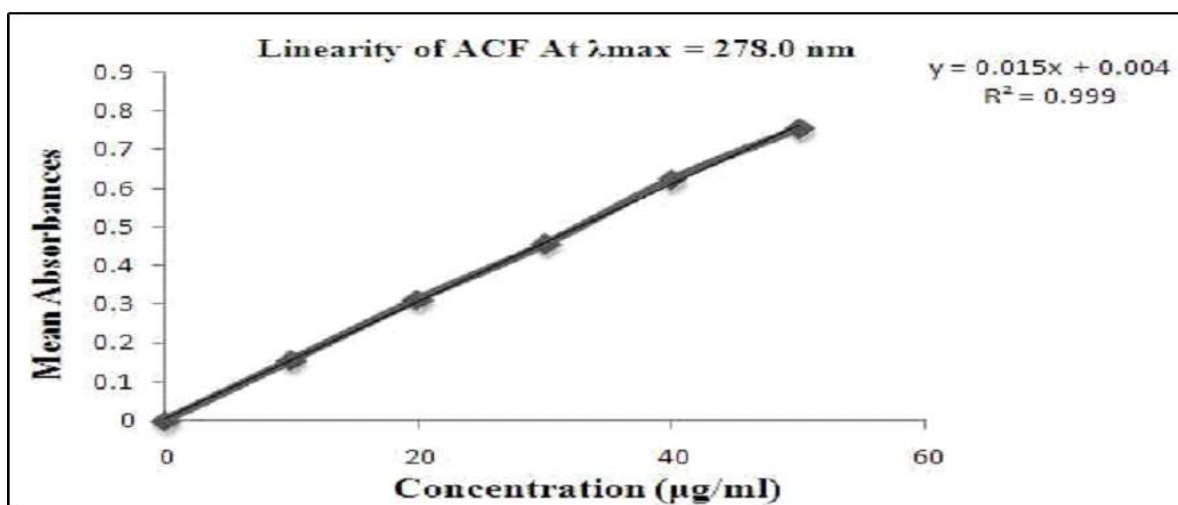


Figure 6.3: Linearity of IBU at 278.0 nm Table 6.4: Linearity of IBU at 278.0 nm

Table 6.4: Linearity of IBU at 278.0 nm

Standard Conc. ($\mu\text{g/ml}$)	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Mean
0	0	0	0	0	0	0
10	0.154	0.155	0.156	0.157	0.158	0.156
20	0.315	0.316	0.314	0.312	0.315	0.314
30	0.458	0.457	0.456	0.454	0.455	0.456
40	0.624	0.622	0.624	0.625	0.624	0.624
50	0.754	0.755	0.756	0.754	0.754	0.755

**Figure 6.4: Calibration curve of IBU****Method I (Simultaneous equation method)**

Study of overlay spectra: Working standard solution from the standard stock solution prepared in concentration $5 \mu\text{g/ml}$ of CHO and $10 \mu\text{g/ml}$ of IBU were scanned in the spectrum mode over the range of 200-400 nm against RO Water as blank and the overlain spectra of the two were recorded. DIA showed an absorbance peak at 242.0 nm, whereas IBU at 278.0 nm. The overlain spectra also showed absorptive points at 285.0 nm. Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method.

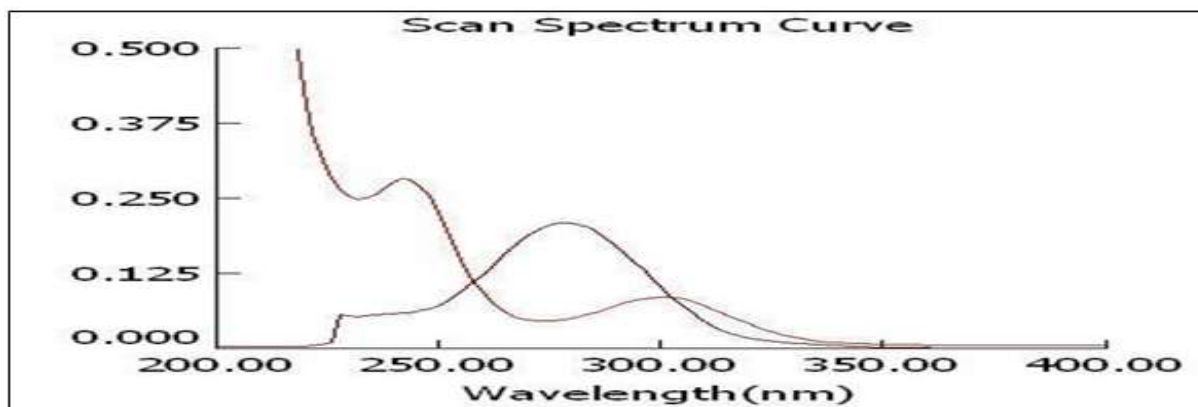


Figure 6.5: Overlay Spectra of CHO and IBU

Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 242.0 nm and 278.0 nm that are λ_{\max} of CHO and IBU respectively. The absorbances were measured at the selected wavelengths and absorptivities ($A^{1\%, 1\text{cm}}$) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations.

Validation of simultaneous equation method

A1: Linearity: Linearity of both drugs was established by response ratios of drugs. Response ratio of drug was calculated by dividing the absorbance with respective concentrations. Then a graph was plotted between concentration and response ratio.

Table 6.5: Response Ratio of CHO and IBU

S. No.	CHO			IBU		
	Conc. ($\mu\text{g/ml}$)	ABS	Response Ratio	Conc. ($\mu\text{g/ml}$)	ABS	Response Ratio
1.	0	0	0	0	0	0
2.	10	0.205	0.0205	10	0.156	0.0156
3.	20	0.405	0.0203	20	0.314	0.0157
4.	30	0.625	0.0208	30	0.456	0.0152
5.	40	0.815	0.0204	40	0.624	0.0156
6.	50	1.014	0.0203	50	0.755	0.0151

B1: Accuracy: The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e., 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of CHO and IBU to pre analyzed tablet solutions. The resulting solutions were then re-analyzed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels.

Table 6.6: Recovery study of CHO

Level of Recovery (%)	CHO			IBU		
	80	100	120	80	100	120
Amount Present	10	10	10	10	10	10
	10	10	10	10	10	10
	10	10	10	10	10	10
Amount of Std. Added	8	10	12	8	10	12
	8	10	12	8	10	12
	8	10	12	8	10	12
Amount Recovered	7.99	10.00	11.98	8.01	9.98	11.95
	7.96	9.96	11.97	7.99	9.95	11.93
	8.00	9.99	11.96	8.02	9.94	11.98
% Recovery	99.76	100.0	99.921	100.125	99.8	99.583
	99.361	99.6	99.845	99.875	99.5	99.417
	100.110	99.9	99.591	100.25	99.4	99.833
Mean % Recovery	99.760	99.900	99.888	100.083	99.567	99.611

C. Precision: Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week. The results are shown in tables.

C1-1: Repeatability:**Table 6.8: Results of analysis Data of Tablet Formulation**

Drug	Label Claim	Amount found	Label claim(%)	S.D.	% RSD
CHO	50 mg	49.5	99.00	0.112	0.145
IBU	100 mg	99.56	99.56	0.115	0.132

C1-2: Intermediate Precision:**C1-2.1: Day-to-Day Variation:****Table 6.9: Intra-day & Inter-day Precision of CHO**

Intra-day Precision			Inter-day Precision		
Label Claim			Label Claim		
	CHO%	IBU%		CHO%	IBU%
After 1hr	99.10	99.50	First day	98.51	99.02
After 2hr	99.05	99.12	Second day	98.41	98.50
After 3hr	99.00	99.05	Third day	98.12	98.01
Mean	99.050	99.22	Mean	98.347	98.51
SD	0.050	0.242	SD	0.203	0.505
% RSD	0.050	0.244	% RSD	0.206	0.513

Analysis of tablet sample:

Twenty marketed tablets of CHO and IBU were weighed and ground to a fine powder; amount equal to 10mg of CHO was taken in 10 ml volumetric flask. The IBU present in this amount of tablet powder was 20mg. Then 4 ml of Sodium acetate and Sodium Benzoate solution was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with hydrotropic solution. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with RO Water to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from simultaneous equation method. The procedure was repeated for five times.

Table 6.11: Analysis of Tablet Formulation of CHO and IBU

Drug	Label claim	Amount found	Label claim (%)	S.D.	% RSD
CHO	50 mg	49.85	99.7	0.116	0.132
IBU	100 mg	99.65	99.65	0.136	0.145

RESULTS AND DISCUSSION

Based on the solubility, stability and spectral characteristics of the drugs, 2M Sodium acetate and 2M sodium benzoate (50:50% w/v) was selected as hydrotropic agent. Presence of hydrotropic agent does not show any significant interference in the spectrophotometric assay thus further confirming the applicability and reproducibility of the developed method.

The developed methods were found to be linear. The values of mean percent recoveries were found and shown in **Table 7.2** and results of validation were shown in **Table 7.3**. The mean percent label claims of tablets by the proposed methods were close to 100, indicating the accuracy of the proposed method and low values of standard deviation, percent coefficient of variation and standard error further validated the proposed method.

Table 7.1: Results of Linearity of CHO and IBU by Simultaneous equation methods

PARAMETER	CHO	IBU
Working	242 nm	278 nm
Beer's law limit ($\mu\text{g/ml}$)	10-50	10-50
Correlation Coefficient (r^2)*	0.999	0.999
Slope (m)*	0.020	0.015
Intercept (c)*	0.002	0.004

Table 7.2: Results of Recovery Studies on Marketed Formulations

Recovery Level %	% Recovery (Mean \pm SD) *	
	CHO	IBU
80	99.750	100.083
100	99.800	99.567
120	99.778	99.611

Table 7.3: Results of validation (% RSD)

PARAMETER		DIA	ACF
Precision (%RSD)	Repeatability	0.145	0.132
	Intra-day Precision	0.050	0.244
	Inter-day Precision	0.206	0.513

CONCLUSION

Modern medicines for human use are required to comply with specific standards and regulation set forth by the concerned authorities. The efficacy and safety of medicinal products can only be assured by analytical monitoring of its quality. Pharmaceutical analysis is an art and science of determining the concentration of drug constituents present in marketed formulation. It is considered as an application of procedures necessary to determine and estimate the identity, strength, quality and purity of drug. Therefore, the quality control laboratory is considered as the backbone of the Pharma industries with ever-increasing need for the development of analytical techniques for drug formulation. In the present study, a successful attempt was made for the Spectrophotometric quantitative estimation of three new antihypertensive marketed combinations by using hydrotropic agent. The method was developed by experimentation based on thorough literature survey and ascertained by statistical parameters of sampling. The entire work was performed on Labindia UV/VIS double beam-double detector spectrophotometer (Model-3000+ series). The result obtained shows the developed method to be precise, simple, rapid and accurate. Thus, these can be used for routine analysis of Chondroitin and Ibuprofen in bulk drug and tablet dosage form instead of processing of extraction using organic solvent separately. It may reduce the large total concentration of hydrotropic agents necessary to produce modest increase in solubility by employing combination of agents in lower concentration. Spectrophotometric precluding the use of organic solvents and thus avoids the problem of residual toxicity, error due to volatility, pollution, cost etc.

It was thus, concluded that the proposed method is new, simple, accurate, safe, free from pollution, precise and can be successfully employed in the routine analysis. The simplicity, rapidity reproducibility and economy of the proposed methods completely fulfill the objective of this research work

CONFLICTS OF INTERESTS

There are no conflicts of interests

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