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## DETERMINION of DICLOFENAC SODIUM IN TABLETS FORMULATIONS by DERIVATIVE SPECTROPHOTOMETRY

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### ABSTRACT

A new sensitive, simple, rapid, precise, accurate, economical and reproducible derivative spectrophotometric method has been developed. It requires no prior separation for the estimation of Diclofenac Sodium (Dic.Na) in tablets pharmaceutical form. First order spectrophotometric derivative method was adopted to eliminate spectral interference. The first order derivative absorption wavelength of (Dic.Na) was found to be 255 nm, dissolved in NaOH medium (1M). This wavelength was selected for the analysis of (Dic.Na) at 255 nm, obeyed Beer-Lambert's law in the concentrations range of 0.5 - 32  $\mu\text{g/mL}$ . Regression analysis showed a good correlation coefficient  $R^2 = 0.9999$ . The limit of detection (LOD) and limit of quantification (LOQ) were to be 0.05 and 0.15  $\mu\text{g/mL}$  respectively. The proposed method has been successfully applied in analyzing (Dic.Na) in some Syrian trademark drugs. All studied samples showed that the drug level was in conformity with the United State Pharmacopeia (USP) legislation.

**KEYWORDS:** Diclofenac sodium, Spectrophotometric, Absorption, 1DS Derivative spectrophotometry.

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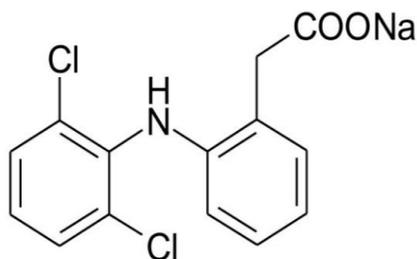
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## 1. INTRODUCTION

(Dic.Na) or 2-[(2, 6-dichlorophenyl) amino] phenyl[1] belongs to the family of (NSAID) or cyclo-oxygenase (cox) inhibitors. It is an effective anti-inflammatory, analgesic and antipyretic agent. It is commonly used in the treatment of acute and chronic pain, rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and sport injuries[2,3]. The pharmacological effects of this drug are thought to be related to the inhibition of the conversion of arachidonic acid to prostaglandins, which are the mediators of the inflammatory process. It is employed mainly in oral formulations, and to some extent for intramuscular injection and topical formulation[1].

Different analytical methods have been employed for the quantification of (Dic.Na) in pharmaceutical formulations, in tablet dosage form widely marketed along all the non-steroidal anti-inflammatory drug (NSAID) until this date. These methods include UV spectrophotometry[4,5,6], Kinetic spectrophotometry[7], spectrofluorometric methods[8,9], atomic absorption spectrometric method[10], Voltammetry[11], Voltammetry and gas chromatography[12] Polarography[13], FT-Raman Spectroscopy [14,15], HPLC[16,17,18], FTIR and HPLC[19]. In the field UV-VIS spectrophotometry, the analysis of pharmaceutical without prior separation step is always a difficult task due to spectral peaks interference with excipients. Although the derivative spectroscopic approach continues to be a promising tool to solve this problem[20].

Therefore, the need for a fast, low cost and selective method is obvious, especially for the routine quality control analysis of pharmaceutical formulations containing (Dic.Na) without interference with the tablet's excipients. The spectrophotometric method is based on derivative spectroscopy technique provides high sensitivity, precision and accuracy of analysis. Thus, it offers practical and economic advantages over other techniques and which has proved advantageous in masking spectral interferences excipients. (Dic.Na) chemical structure[21] is presented in **Figure (1)**.



**FIGURE (1): CHEMICAL STRUCTURE OF DICLOFENAC SODIUM**

## 2. Materials and Chemicals

### 2.1. Apparatus

All spectral measurements were carried out using a T80+ UV/V spectrophotometer PG instrument Ltd (UK) connected to computer, quartz cells 1 cm, ultrasonic bath (Daihan, USA), analytical balance TE64 Sartorius (Germany) sensitivity 0.01 mg, Germany digital pipettes (Isolab).

## 2.2. Chemicals

Methanol from Euro lab (UK), purity 99.8%. (Dic.Na) from Cipla Pvt. Ltd., Mumbai, purity 99.60% (India). NaOH from Merck Germany, purity 98%, and double distilled water was used.

## 3. Selection of common solvent

After assessing, the solubility of the drug in different media, methanol was selected as a common solvent (Dic.Na) for the stock solution.

## 4. Stock standard preparation

Stock solution of (Dic.Na) was prepared by dissolving accurately 100 mg (100.4 mg after taking the purity in consideration) of drug in 100 mL volumetric flask with methanol, this gave a stock solution of 1000 µg/mL.

## 5. Calibration curve

To construct the calibration (Dic.Na) curve, for each concentration ten standard solutions were prepared and measured the absorbance five times for each solution.

## 6. Samples preparation

Four Syrian trademark products of (Dic.Na) tablets with different doses were studied.

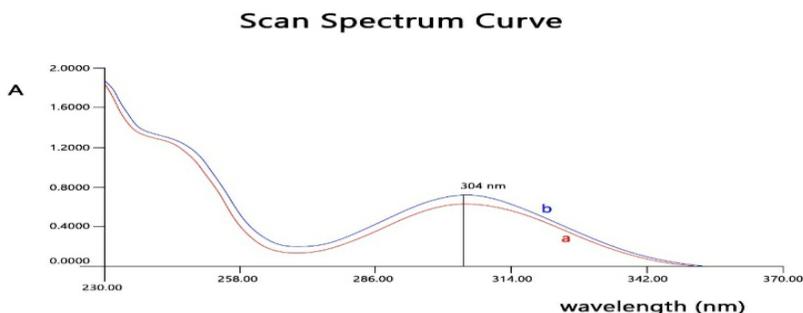
- Twenty tablets of (Dic.Na) 25 mg/tab, **Diclofenac Hama Pharma**, were weighed accurately. Then 20 tablets were ground to fine powder and average weight was calculated. A quantity of tablet powder equivalent to 25 mg of (Dic.Na) was accurately weighed and transferred into 50 mL volumetric flask and methanol was added up to volume, to produce sample solution of 0.5 mg/mL. The content was ultrasonicated for 20 min, then filtered through a paper filter (Whatman 3, England). We have directly transferred from the filtrate solution 1.2 mL into 25 mL volumetric flask, the volume was made up to mark with NaOH 1 M to give concentration of 24 µg/mL of (Dic.Na).
- Twenty tablets of (Dic.Na) 50 mg/tab, **DICLOFENC HUMAN**, were weighed accurately. Then 20 tablets were ground to fine powder and average weight was calculated. A quantity of tablet powder equivalent to 50 mg of (Dic.Na) was accurately weighed and transferred into 50 mL volumetric flask and methanol was added up to volume, to produce sample solution of 1 mg/mL. The content was ultrasonicated for 20 min, then filtered through a paper filter (Whatman 3, England). We have directly transferred from the filtrate solution 0.6 mL into 25 mL volumetric flask, the volume was made up to mark with NaOH 1 M to give concentration of 24 µg/mL of (Dic.Na).
- Twenty tablets of (Dic.Na) 75 mg/tab, **Diclofenac Alyousef 75**, were weighed accurately. Then 20 tablets were ground to fine powder and average weight was calculated. A quantity of tablet powder equivalent to 75 mg of (Dic.Na) was accurately weighed and transferred into 100 mL volumetric flask and methanol was added up to volume, to produce sample solution of 0.75 mg/mL. The content was ultrasonicated for 20 min, then filtered through a paper filter (Whatman 3, England). We have directly transferred from the filtrate solution

0.8 mL into 25 mL volumetric flask, the volume was made up to mark with NaOH 1 M to give concentration of 24  $\mu\text{g/mL}$  of (Dic.Na).

- Twenty tablets of (Dic.Na) 100 mg/tab, **Voltaraze 100**, were weighed accurately. Then 20 tablets were ground to fine powder and average weight was calculated. A quantity of tablet powder equivalent to 100 mg of (Dic.Na) was accurately weighed and transferred into 100 mL volumetric flask and methanol was added up to volume, to produce sample solution of 1 mg/mL. The content was ultrasonicated for 20 min, then filtered through a paper filter (Whatman 3, England). We have directly transferred from the filtrate solution 0.6 mL into 25 mL volumetric flask, the volume was made up to mark with NaOH 1 M to give concentration of 24  $\mu\text{g/mL}$  of (Dic.Na).

## 7. Results and Discussion

Zero spectrophotometric spectra at the same concentrations 12  $\mu\text{g/mL}$  for both standard (Dic.Na) and product sample (Dic.Na), shown in **Figure (1)**, an absorption increasing due to excipients as (starch, lactose, talc, magnesium stearate and polyvinyl povidone generally used in manufacturing).



**FIGURE (2): ZERO SPECTROPHOTOMETRIC SPECTRA, (DIC.NA) [12]  $\mu\text{G/ML}$  FOR BOTH. A: STANDARD (DIC.NA) AND B: PRODUCT SAMPLE (DIC.NA).**

So, the developed method for derivative spectrophotometric determination of (Dic.Na) in tablets formulations was found to be simple and convenient for the routine analysis. Practically no interference with tablets excipients was observed, proved by the products recoveries as presented later. The method is accurate, simple, rapid, precise, reliable, sensitive, reproducible and economical. For projected method, we used easily available and cheap solvent like methanol and media as NaOH. Derivative spectrophotometric analysis method of (Dic.Na) does not required any expensive and satisfactory apparatus in contrast to reported chromatographic and other hyphenated techniques. First order absorption spectrum of the standard drug 12  $\mu\text{g/mL}$  (Dic.Na) solution was recorded within a wavelength range of 230 – 370 nm against NaOH 1 M and methanol as a required proportion. The first derivative spectrum of (Dic.Na) was used to determine (Dic.Na) at 255 nm, **Figure (3)**.

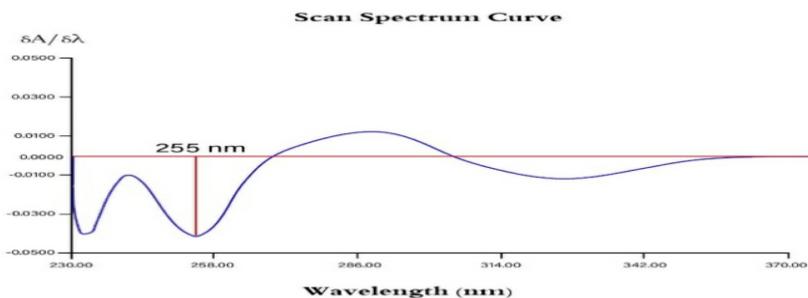


FIGURE (3): FIRST DERIVATIVE SPECTRA, (DIC.NA) [12]  $\mu\text{G/ML}$ .

## 8. Method's validation

The validity of the proposed method was assessed by accuracy (reported as recovery percentage), precision (reported as RSD%), linearity (evaluated by regression equation), limit of detection LOD and limit of quantification LOQ.

### 8.1. Linearity

The concentrations linearity of (Dic.Na) was in the range 0.5 - 32  $\mu\text{g/mL}$  at  $\lambda_{\text{max}} = 255 \text{ nm}$  by  $^1D_{255}$ , as seen as **Figure (4)** and **Figure (5)**.

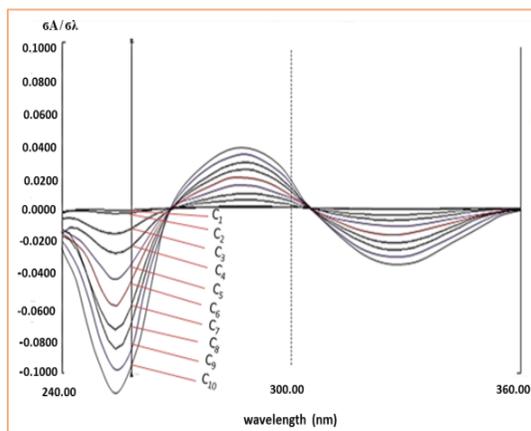


FIGURE (4): SPECTRA OF (DIC.NA):  $C_1: 0.5 \mu\text{G/ML}$ ,  $C_2: 1 \mu\text{G/ML}$ ,  $C_3: 4 \mu\text{G/ML}$ ,  $C_4: 8 \mu\text{G/ML}$ ,  $C_5: 12 \mu\text{G/ML}$ ,  $C_6: 16 \mu\text{G/ML}$ ,  $C_7: 20 \mu\text{G/ML}$ ,  $C_8: 24 \mu\text{G/ML}$ ,  $C_9: 28 \mu\text{G/ML}$ ,  $C_{10}: 32 \mu\text{G/ML}$ .

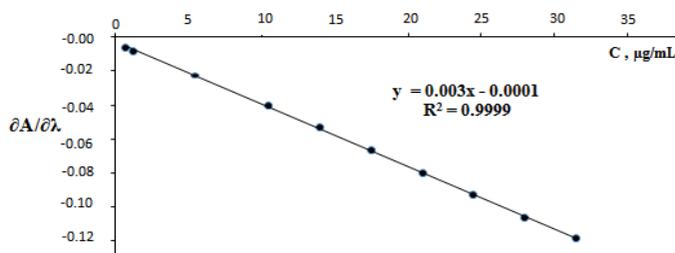


FIGURE (5): CALIBRATION CURVE FOR (DIC.NA).

n = 5 measurements for each concentration.

## 8.2. Limit of detection LOD and limit of quantification LOQ

LOD and LOQ were calculated and presented in **Table (1)**, using **Equation (1)** and **Equation (2)** [22]:

$$LOD = \frac{3.3 \times SD}{m} \quad LOD = \frac{3.3 \times SD}{m} \quad (1)$$

$$LOQ = \frac{10 \times SD}{m} \quad LOQ = \frac{10 \times SD}{m} \quad (2)$$

Where SD is the standard deviation of y-intercepts (a) of regression lines, and (m) is the slope of the equation of calibration curve,  $y = a + m x$ .

**TABLE (1): STATISTICAL DATA FOR CALIBRATION GRAPHS.**

Method	Analyte	Selected wavelength nm	Linearity rang $\mu\text{g/mL}$	Correlation coef. ( $R^2$ )	LOD $\mu\text{g/mL}$	LOQ $\mu\text{g/mL}$
DS	(Dic.Na)	$^1D_{255}$	0.5 - 32	0.9999	0.05	0.15

## 8.3. Accuracy

To determine the accuracy in addition to precision of the proposed method, five replicate determinations were carried out on five different concentrations of standards (Dic.Na). The accuracy and precision results are presented in **Table (2)**.

**TABLE (2): ACCURACY AND THE ADDITION OF PRECISION FOR DETERMINATION OF (DIC.NA) BY THE PROPOSED METHOD**

Method	Theoretical Concentration n $\mu\text{g/mL}$	$\bar{x}$ Observed Concentration n $\mu\text{g/mL}$	SD $\mu\text{g/mL}$	Precision on RSD %	Accuracy y %
$^1DS$ $\lambda = 255$ nm	4.95	4.92	0.13	2.64	99.39
	9.00	8.92	0.20	2.24	99.11
	18.00	17.81	0.28	1.57	98.94
	36.00	35.70	0.33	0.92	99.17
	72.00	72.36	0.39	0.54	100.50

$\bar{x}$ : mean of five replicated determinations,

Accuracy (%) = (observed concentration/theoretical concentration) x 100,

Precision (RSD %) = (standard deviation/mean concentration) x 100.

## 8.4. Precision

Precision was determined after repeating the (Dic.Na) determination three times at the same day (Intra-day) as presented in **Table (3)**, and repeated the (Dic.Na) determination in three different days (Inter-day) as presented in **Table (4)**.

**TABLE (3): ACCURACY AND ADDITION TO PRECISION FOR (DIC.NA) IN INTRA-DAY**

Drug	Theoretical concentration ( $\mu\text{g/mL}$ )	*Observed concentration ( $\mu\text{g/mL}$ )			Precision RSD (%)			Accuracy (%)		
		T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
(Dic.Na)	16	16.02	15.99	16.01	0.12	0.19	0.19	100.12	99.94	100.06
	20	19.96	19.98	20.04	0.10	0.20	0.20	99.80	99.90	100.20

	24	23.96	23.99	24.03	0.33	0.37	0.37	99.92	99.96	100.12
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T: three different times in the same day.

Precision (RSD %) = (standard deviation/mean concentration)  $\times$  100.

Accuracy (%) = (observed concentration/theoretical concentration)  $\times$  100.

\* Five separate determinations were performed and calculated the mean.

**TABLE (4): ACCURACY AND ADDITION TO PRECISION FOR (DIC.NA) IN INTER-DAY**

Drug	Theoretical concentration ( $\mu\text{g/mL}$ )	*Observed concentration ( $\mu\text{g/mL}$ )			Precision RSD %			Accuracy %		
		D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>
(Dic.Na)	16	16.01	16.02	16.04	0.19	0.19	0.31	100.06	100.12	100.25
	20	20.01	20.02	19.99	0.25	0.25	0.29	100.05	100.10	99.95
	24	23.97	24.06	24.01	0.37	0.42	0.45	99.87	100.25	100.04

D: three different days.

Precision (RSD %) = (standard deviation/mean concentration)  $\times$  100.

Accuracy (%) = (observed concentration/theoretical concentration)  $\times$  100.

\* Five separate determinations were performed and calculated the mean.

## 8.5. Robustness

The robustness of an analytical procedure is a measurement of its capacity to maintain unaffected results by a very small variation of some parameters; and provides an indication of its reliability during normal usage.

The studied variables parameters as slit range, scan speed and the wavelength which are performed at a concentration of 16.00  $\mu\text{g/mL}$  of (Dic.Na). The obtained results in table 5 showed no significant differences.

**TABLE (5): ROBUSTNESS TEST FOR (DIC.NA) AT  $\lambda_{MAX} = 255 \text{ NM}$**

16.00 $\mu\text{g/mL}$ of Diclofenac Sodium.						
Raw sample	parameter	Deviation	$\bar{x}$ ( $\mu\text{g/mL}$ )	SD ( $\mu\text{g/mL}$ )	Per %	RSD %
(Dic.Na) At 255 nm	Slit range	2	16.05	0.05	100.31	0.31
		1	16.09	0.04	100.56	0.25
	Scan speed (Fast)	Fast	16.10	0.06	100.62	0.37
		Slow	16.12	0.03	100.75	0.19
	Wave length	+ 2 nm	16.07	0.05	100.44	0.31
		- 2 nm	16.02	0.04	100.13	0.25

## 8.6. Specificity

In general, the UV-Vis. Spectrophotometry is considered nonspecific, but in our case Dic.Na tablets excipients as starch, lactose, talc, magnesium stearate and polyvinyl povidone could be considered specific as the resulted recoveries of the studied products were around 100%.

## 9. RECOVERY

To check the accuracy of the developed method and to study the interference of formulation additives. Recovery studies were carried out by standard additions

method at three different levels 80%, 100% and 120% of the sample diluted value. The recovery was studied by three addition standards for every product. **Table (6)** presents the recoveries results for the four Syrian tablets products where RSD% doesn't exceeded 1.09%.

**TABLE (6): RECOVERIES OF (DIC.NA) TABLETS FOR FOUR SYRIAN PRODUCTS.**

Product	Sample $\mu\text{g/mL}$	Added $\mu\text{g/mL}$	Total Found $\mu\text{g/mL}$	Recovery %	SD %	RSD %	Recovery Average %
Diclofenac Hama Pharma (Dic.Na) 25 mg/tab	14.04	11.20	25.18	99.46	1.08	1.09	99.89
	14.04	14.00	28.02	99.86	0.10	0.10	
	14.04	16.80	30.90	100.36	0.69	0.69	
DICLOFENAC HUMAN 50 (Dic.Na) 50 mg/tab	13.98	11.20	25.18	100.00	1.08	1.08	99.96
	13.98	14.00	27.99	100.07	0.09	0.09	
	13.98	16.80	30.75	99.82	0.69	0.69	
(Dic.Na) Diclofenac Alyousef (Dic.Na) 75 mg/tab	12.06	9.60	21.69	100.31	0.93	0.93	100.19
	12.06	12.00	24.10	100.33	0.11	0.11	
	12.0	14.40	26.45	99.93	0.19	0.19	
Voltaraze 100 (Dic.Na) 100 mg/tab	10.04	8.00	18.02	99.75	0.33	0.33	99.98
	10.04	10.00	20.06	100.20	0.18	0.18	
	10.04	12.00	22.04	100.00	0.11	0.11	
Range of Recovery Average %		99.46 – 100.36					

## 10. Application

The developed first derivative method was applied for quantitative determination for (Dic.Na) in four different Syrian tablets trademarks. The samples were prepared as described in the section of samples preparation and analyzed. Quantitative analysis was done by using calibration curves. The obtained results are summarized in **Tables (7-10)** for five different batches, for each studied Syrian trademark: (Dic.Na) 25, 50, 75, 100 mg/tab.

**TABLE (7): RESULTS OF DICLOFENAC HAMA PHARMA, (DIC.NA) 25 MG/TAB, FOR FIVE DIFFERENT BATCHES.**

No. of batches	Result dose mg/tab.	SD mg/tab.	RSD %	Per %
1	24.96	0.21	0.84	99.84
2	25.11	0.12	0.48	100.44
3	25.18	0.25	0.99	100.72
4	24.97	0.22	0.88	99.88
5	25.14	0.14	0.56	100.56
Range mg/tab	24.96 – 25.18			
Range Per %	99.84 – 100.72			

**TABLE (8): RESULTS OF DICLOFENAC HUMAN, (DIC.NA) 50 MG/TAB, FOR FIVE DIFFERENT BATCHES.**

No. of batches	Result dose mg/tab	SD mg/tab	RSD %	Per %
1	50.20	0.29	0.58	100.40
2	50.25	0.43	0.86	100.50

3	49.92	0.20	0.40	99.84
4	50.22	0.47	0.94	100.44
5	49.85	0.41	0.82	99.70
<b>Range mg/tab</b>	<b>49.85 – 50.22</b>			
<b>Range Per %</b>	<b>99.70 – 100.50</b>			

**TABLE 9: RESULTS DICLOFENC ALYOUSEF, (DIC.NA) 75 MG/TAB, FOR FIVE DIFFERENT BATCHES.**

No. of batches	Result dose mg/tab	SD mg/tab	RSD %	Per %
1	74.76	0.93	1.24	99.68
2	75.39	0.30	0.40	100.52
3	75.26	0.35	0.46	100.35
4	74.89	0.63	0.84	99.85
5	75.12	0.30	0.40	100.16
<b>Range mg/tab</b>	<b>74.76 – 75.39</b>			
<b>Range Per %</b>	<b>99.68 – 100.52</b>			

**TABLE 10: RESULTS OF VOLTARAZE 100, (DIC.NA) 100 MG/TAB, FOR FIVE DIFFERENT BATCHES.**

No. of batches	Result dose mg/tab	SD mg/tab	RSD %	Per %
1	100.28	0.31	0.31	100.28
2	99.69	0.72	0.72	99.69
3	100.17	0.41	0.41	100.17
4	99.88	0.89	0.89	99.88
5	100.24	0.41	0.41	100.24
<b>Range mg/tab</b>	<b>99.69 – 100.28</b>			
<b>Range Per %</b>	<b>99.69 – 100.28</b>			

According to USP legislation, the tablets must contain not less than 90 percent and not more than 110 percent of labeled amount of (Dic.Na). Therefore, the obtained results are in conformity with USP legislation[21].

## 11. CONCLUSION

The proposed method is simple, precise, accurate and rapid for the determination of (Dic.Na) in raw material and tablet dosage forms. This method can be adopted as an alternative to the existing spectrophotometric methods. Analysis of authentic samples containing (Dic.Na) showed no interference from the common additives and excipients. Hence, recommended procedure is well suited for the assay and evaluation of drugs in pharmaceutical preparations. It can be easily and conveniently adopted for routine quality control analysis.

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