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Validation of a capillary electrophoresis method for the determination of cephradine and its related impurities

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Abstract

A method validation is reported for the determination of the β-lactamic antibiotic cephradine and its main impurity, cephalexin, by micellar electrokinetic chromatography (MEKC) with UV photometric detection. The validation was carried out in compliance with the analytical performance parameters required by the USP XXII. The capillary electrophoresis (CE) method was statistically compared with the HPLC method used in quality control laboratories; the analysis of variance showed no significant difference between the results obtained by the two methods, demonstrating CE to be a suitable alternative technique to HPLC in routine quality control.

1. Introduction

Capillary electrophoresis (CE) is a relatively new but a very promising technique, which was first developed in biological (proteins and nucleic acids) separations, as an evolution of classical electrophoresis. Its importance has grown in recent years in different fields, mainly owing to its advantages over the more commonly used chromatographic techniques, such as high efficiency and selectivity in a short run time, reduced use of organic solvents and ease and low cost of operation.

Although in the last few years many studies of CE applications, using both free solution capillary electrophoresis (FSCE) and micellar electrokinetic chromatography (MEKC), in pharmaceutical analysis have been published [1–4], there have been few investigations on validated methods for routine analysis [5–8].

Pharmaceutical quality control needs analytical methods for the determination of the active component and its related impurities in a matrix which might be relatively complex. We found two interesting separation conditions that have been reported [7,8] for the quantitative analysis of a mixture of cephalosporins by MEKC. When such conditions were applied to the analysis of our bulk products (a powder containing 65% cephradine and 35% arginine or 70% cephradine and (30% sodium carbonate), it was found the migration time was not reproducible on repeated injections. A mixed micellar system was then investigated.

The method reported in this paper for the determination of cephradine and cephalexin was validated following the analytical performance parameters required by the USP XXII; it shows linearity in the range of concentrations consid-

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ered, with a correlation coefficient of 0.999, good precision under repeatable conditions [repeatability (R.S.D. of seven successive injections of a 1 mg/ml standard solution of cephradine) = 0.37%], good day-to-day reproducibility (expressed by the R.S.D. obtained in a recovery trial, which was performed by analysing three pairs of sample solutions at different concentrations on three days; R.S.D. = 0.88%) for UV response, good precision of migration time (R.S.D. = 0.4%) and perfect correspondence with HPLC data, demonstrating CE to be a valuable alternative in pharmaceutical quality control.

2. Experimental

2.1. Instrumentation

Experiments were carried out on a P/ACE System 2050 instrument (Beckman Instruments, Fullerton, CA, USA) equipped with a 30-kV power supply, a UV spectrophotometric detector connected to a data collection system and able to perform both hydrodynamic injection and voltage injection. The detection wavelength was 214 nm.

Separations were performed in a fused-silica capillary (57 cm × 0.075 mm I.D., 50 cm effective length) (Beckman), thermoregulated at 25°C by a liquid coolant (Beckman), with a voltage of 20 kV applied. Hydrodynamic injection was performed at 0.5 p.s.i. for 5 s (corresponding to an injection volume of about 5 nl).

2.2. Standards and reagents

Working standards of cephradine monohydrate and cephalexin were prepared by purification of our products and assayed against the corresponding USP standard.

Disodium hydrogenphosphate was purchased from Merck (Merck-Bracco, Milan, Italy), boric acid from Sigma (Sigma Aldrich, Milan, Italy) and sodium dodecyl sulphate (SDS) and polyoxyethylene lauryl ether (Brij 35) from Merck.

Water used to prepare standard solutions, sample solutions and run buffer was obtained from a Millipore system (Millipore, Vimodrone, Milan, Italy).

Working standard solutions of cephradine and cephalexin were prepared dissolving 50 mg of cephradine monohydrate (potency 93.3%, cephalexin content 2.52%) in 50 ml of deionized water; sample solutions were prepared by dissolving 50 mg of sample powder in 50 ml of deionized water; run buffer solution was prepared by dissolving 122 mg of boric acid, 284 mg of disodium hydrogenphosphate, 1.442 g of SDS and 100 mg of Brij 35 in 100 ml of deionized water.

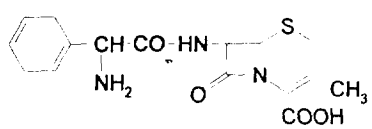
3. Results and discussion

3.1. Separation of cephradine by MEKC

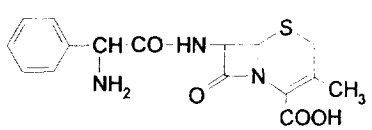
In order to develop a method for the determination of cephradine, we first tried to resolve a cephradine–cephalexin mixture (cephalexin being the main impurity of cephradine) by FSCE. Using 0.1 *M* borate buffer we could not achieve complete separation either by varying the buffer pH in the range of 6–10 or modifying the applied voltage from 10 to 30 kV.

The great similarity between the two cephalosporin structures (Fig. 1) is responsible for their very similar electrophoretic mobilities, making it very difficult to achieve a satisfactory separation in the FSCE operating mode.

We then applied the conditions suggested by



Cephradine



Cephalexin

Fig. 1. Structures of cephradine and cephalexin.

Nishi et al. [7] for the separation of nine cephalosporins by MEKC, i.e., 0.02 *M* borate–phosphate buffer (pH 9) containing 0.15 *M* SDS. Under these conditions, the selectivity was very good.

A very good separation of cephradine and cephalexin was also obtained applying the conditions reported by Sciacchitano et al. [8], who studied the separation of five cephalosporins by MEKC, using a higher concentration (0.2 *M*) of SDS in the buffer (0.02 *M* buffer).

The separation of such similar compounds when a micellar buffer is employed is achieved by the specific partitioning of the analytes between the hydrophobic micelles and the aqueous run buffer (chromatographic effect), resulting in an increased difference in relative mobilities, which improves the separation.

Such high SDS concentrations in the buffer (0.1–0.2 *M*) resulted, of course, in very good selectivity with good repeatability of migration time on repeated injections when a cephradine standard solution was analysed, as shown in Fig. 2. Similar conditions are unsuitable, however, for routine analysis when our products (cephradine buffered with arginine and cephradine buffered with sodium carbonate) are to be analysed.

When repeated injections of a buffered cephradine solution were made, a dramatic drift of the migration times was noticed, with a corresponding broadening of peak width. There was no similar problem when a cephradine standard solution was injected several times. This effect on migration time was interpreted as being due to some interaction between a component of either the buffer (SDS) or the sample (carbonate or arginine) or both and the silica capillary surface, interfering with the separation process. For this reason, the column was rinsed three times between each run with 0.1 *M* NaOH, water and buffer solution to protect the capillary surface from modifications, in compliance with the study of Smith et al. [9], although the rinsing procedure was not effective in improving the reproducibility of migration times, as shown in Fig. 3.

To obtain reproducibility of migration times, fresh buffer should be used in each run, making in impossible to use the autosampler in a routine analysis sequence. We sought to overcome the poor reproducibility by searching for a suitable mixed micellar system (ionic plus non-ionic surfactants) in order to decrease the SDS concentrations without losing selectivity.

Cephradine and cephalexin and all the related impurities were baseline resolved (the resolution factor between cephradine and cephalexin is 2.05), as shown in Fig. 4, when using 0.02 *M* borate–phosphate buffer containing 0.05 *M* SDS and 0.1% Brij 35. The repeatability of migration times is good for up to ten injections, after which the buffer in the run vials must be replaced with some of the buffer previously prepared and stored for this purpose.

3.2. Method validation

We validated our CE method in compliance with the analytical performance parameters required by the USP XXII for HPLC method validation. The following parameters were evaluated: selectivity, migration time precision, area precision, linearity and range, accuracy and comparison with HPLC results for cephradine, precision, linearity, detection limit and quantification limit for cephalexin.

Selectivity

Selectivity is correlated with the resolution factor, defined as

$$R_s = 2 \cdot \frac{t_2 - t_1}{W_2 + W_1}$$

where t = migration time of peaks 1 and 2 and W = width of the peaks measured on the baseline in time units. The resolution factor for cephradine and cephalexin, as already mentioned, was 2.05.

Accuracy and reproducibility

Accuracy and reproducibility were measured by recovery trails, according to the following method. Six standard solutions (St) and six sample solutions (S) of the same batch, two for each of concentrations $a/2$, a and $3/2a$, where a

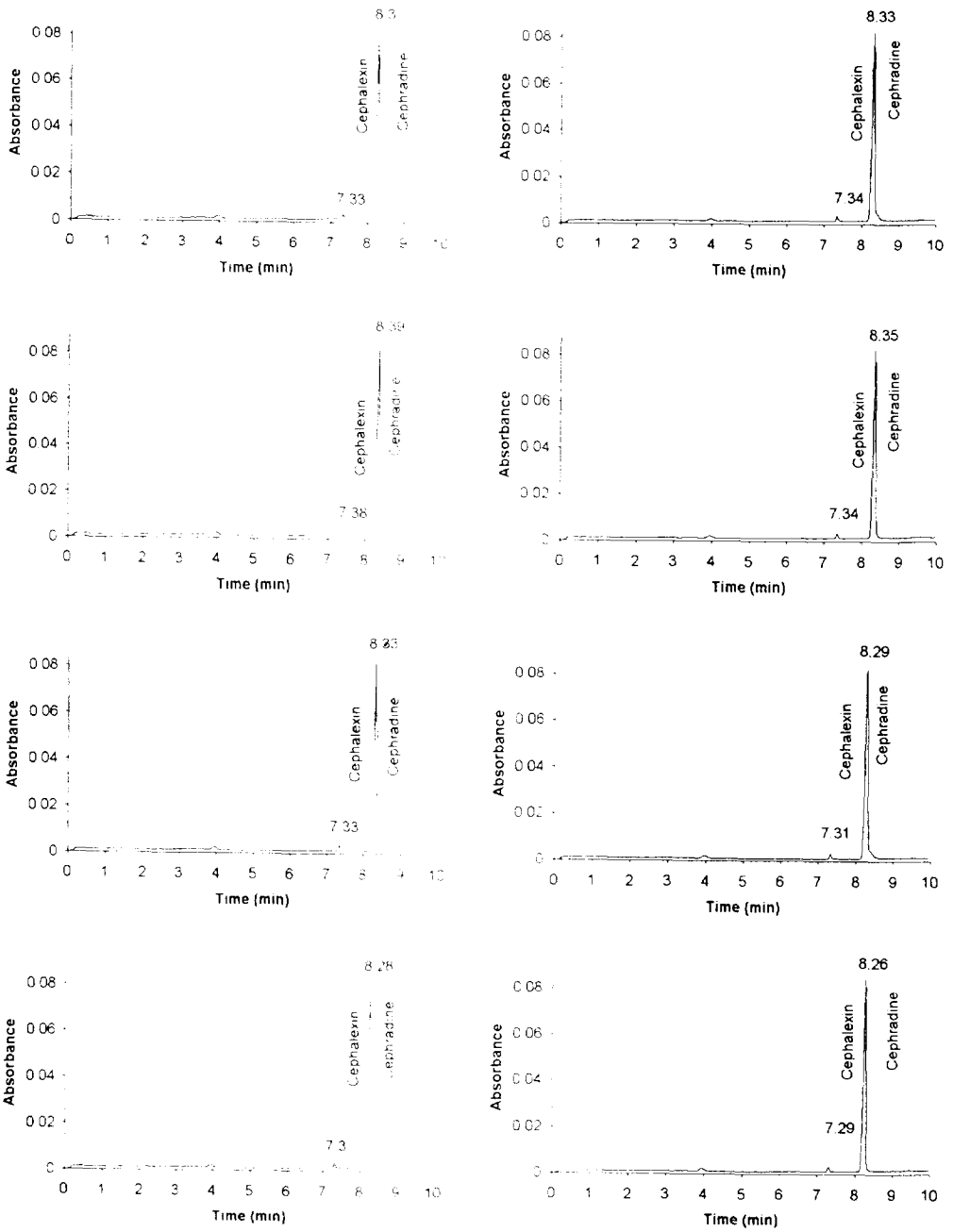


Fig. 2. Chromatogram for repeated injections of cephradine standard solution (1 mg/ml). CE conditions: 0.02 *M* borate–phosphate buffer (pH 9) containing 0.15 *M* SDS, 20 kV, 214 nm.

is the working concentration (1 mg/ml), were prepared on three days and analysed by three analysts with different buffer solutions, in the following orders: $a/2$, St_1–S_1–St_2–S_2; a, St_3–S_3–St_4–S_4; and $3/2a$, St_5–S_5–St_6–S_6. The results are summarized in Table 1 together with the results of the recovery trial.

Accuracy, defined as

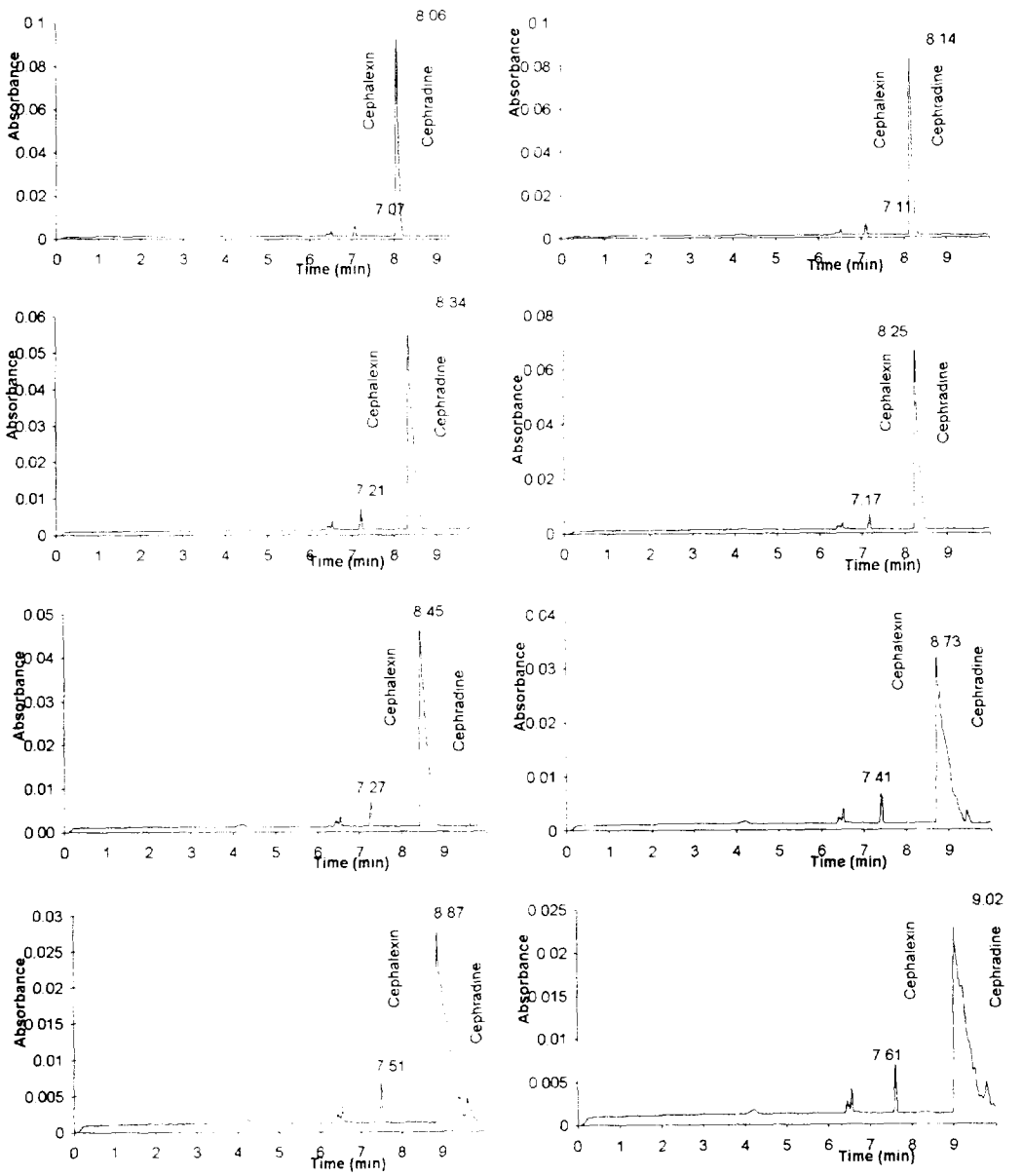


Fig. 3. Chromatogram for repeated injections of cephradine sodium carbonate solution (1 mg/ml), showing the migration time drift and the progressive broadening of cephradine peak. Conditions as in Fig. 2; rinsing between each run.

$$\text{Accuracy} = M \pm (\text{S.D.}/\sqrt{n})t$$

is 70.2 ± 0.31, where M is the mean potency value from recovery testing and Student's t is

$$t(0.05,17) = 2.11$$

The validation was made analysing 18 samples by the t-test so the Student's t is relative to $18 - 1 = 17$ determinations ($P = 0.05$).

The statistical analysis of the means and of the variances showed the homogeneity of the values within the confidence limits of 95%.

The reproducibility is defined by the relative standard deviation (R.S.D.) characteristic of the

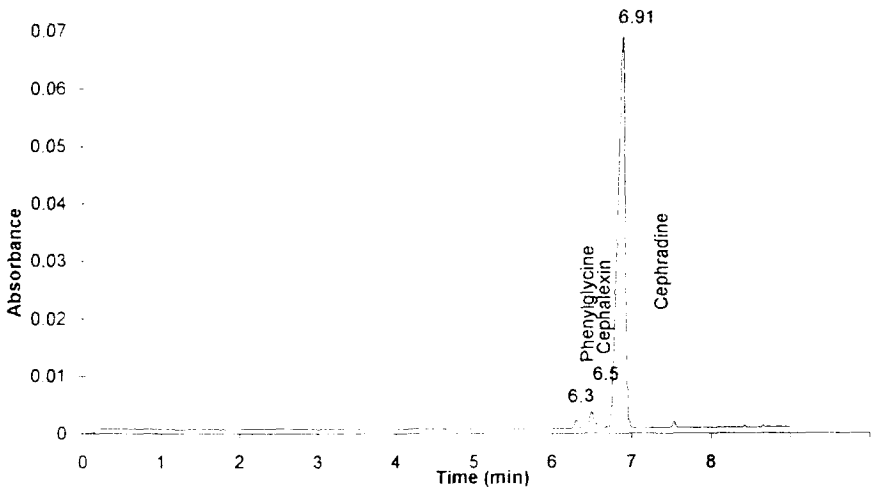


Fig. 4. Chromatogram for a chosen batch of cephradine (1 mg/ml). CE conditions: 0.02 *M* borate–phosphate buffer containing 0.05 *M* SDS and 0.1% Brij 35.

method; the R.S.D. was obtained from the recovery trial and was found to be 0.88%.

Linearity and repeatability

To evaluate the linearity, three solutions of cephradine were prepared and analysed. A solution having the working concentration (1 mg/ml) was prepared; this solution was named *a*. Three solutions having concentrations of 1.5*a*, *a* and *a*/2 were prepared.

Each of these solutions was injected seven times starting from the least to the most concentrated. Successively, the area values obtained were analysed using EXCEL software to evaluate the correlation coefficient (*r*), standard deviation (S.D.) within solutions, sensitivity (slope), intercept, limit of detection (LOD) and limit of quantification (LOQ). The same operations as used to evaluate cephradine linearity were repeated for cephalexin, but the concentrations of the solutions were in this case 2.52% of the working concentration *a* (1 mg/ml of cephradine), 1.26% *a* and 3.75% of *a*. The results are given in Table 2.

The detection limit [signal-to-noise ratio (S/N) = 3] for cephalexin is 0.526 μg/ml and the quantification limit (S/N = 10) is 1.752 μg/ml. Detection limit is defined by LOD = S.D. (area

Table 1
Reproducibility, accuracy and results of recovery tests

Sample concentration	Potency (%)					Average recovery (%)
	Day 1	Day 2	Day 3	Average	R.S.D. (%)	
a/2	70.91	69.18	70.27	70.1	1.0476	99.8
a/2	70.10	69.27	70.79			
a	69.98	70.37	70.88	70.3	0.7221	100.2
a	69.50	70.63	70.62			
3/2*a*	70.90	69.59	69.68	70.3	0.8623	100.1
3/2*a*	69.86	70.53	70.90			
Pooled				70.2	0.8773	100.03

Reproducibility = 0.88%; accuracy = 70.2 ± 0.31%.

Table 2
Repeatability and linearity regression data for cephradine and cephalexin

Product	Repeatability		LOD (μg/ml)	LOQ (μg/ml)	Linearity			
	Area R.S.D. (%)	Migration time R.S.D. (%)			Slope	Correlation coefficient	Range (mg/ml)	Intercept
Cephradine	0.37	0.4			8.99	0.9996	0.5–1.5	0.091
Cephalexin	0.56		0.526	1.752	15.33	0.9993	0.01–0.04	−0.001

corresponding to the lower concentration of the range − blank area) · 3/slope. The quantification limit is defined by LOQ = S.D. (area corresponding to the lower concentration of the range − blank area) · 10/slope.

The R.S.D. of the areas was calculated for the three concentrations. The repeatability value which is characteristic of the method is that relative to solution *a* and was found to be 0.37% for cephradine and 0.56% for cephalexin.

The R.S.D. of the migration time was measured for 23 repeated injections of cephradine buffered with sodium carbonate and was found to be 0.4%. Fresh buffer was used after each sequence of ten injections.

Comparison with HPLC results

Nine batches of cephradine buffered with sodium carbonate were analysed by both the traditional HPLC method and MEKC. In both methods a working standard solution (1 mg/ml) was injected first, followed by a sequence of a maximum of five sample solutions (1 mg/ml) and the series of analyses was repeated twice. The results are reported in Table 3.

From these data, the mean and the R.S.D.

Table 3
Comparison of HPLC and CE assays for cephradine buffered with sodium carbonate, with results of the variance analysis

Day	Batch No.	HPLC Mean potency (%)	CE				Difference, HPLC–CE (%)
			Determination I	Determination II	Mean potency (%)	Difference, I − II (%)	
1	057/4	73.46	72.96	74.05	73.51	−1.48	−0.05
2	064/4	74.17	72.39	72.23	72.31	0.22	1.86
2	065/4	73.20	72.47	71.71	72.09	1.05	1.11
2	066/4	73.57	72.23	71.80	72.02	0.60	1.55
2	067/4	72.49	73.23	72.80	73.02	0.59	−0.53
3	055/4	75.25	74.04	73.49	73.77	0.75	1.49
3	058/4	71.80	71.17	71.22	71.20	−0.07	0.61
3	059/4	74.0	72.22	73.56	72.89	−1.84	1.11
3	060/4	70.40	69.21	70.37	69.79	−1.66	0.61
Mean		73.15			72.29		0.86
S.D.		1.43			1.23		
R.S.D.		1.95			1.70		
Variance		2.0336			1.5128		
F (calc.)		0.74					
F (tab.)		4.45					
t (calc.)		1.370					
t (tab.)		2.11					

were calculated and an analysis of variance was performed, showing no significant difference (at the 95% confidence limits) between the results obtained with the two methods, with t (calculated) $< t$ (tabulated) and F (calculated) $<$ F (tabulated).

4. Conclusions

CE was evaluated to determine its suitability for β-lactam antibiotic analysis in routine quality control. The method reported for the determination of cephradine by MEKC was validated in compliance with the USP XXII analytical performance parameters. All required statistical parameters were respected. The resolution between the main peak and the related impurities was greater than 2. The detector response was linear in the considered range (correlation coefficient 0.999). The R.S.D. was less than 2% (0.4% for both areas and migration times).

The values obtained with our CE method and those obtained by the validated HPLC method were not significantly different. This validation demonstrates that CE may be a valuable alternative technique to HPLC in pharmaceutical quality control.

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