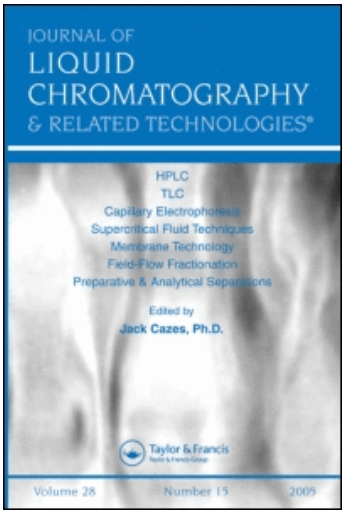

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Quantitation of Terfenadine, Pseudoephedrine Hydrochloride, and Ibuprofen in a Liquid Animal Dosing Formulation Using High Performance Liquid Chromatography

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QUANTITATION OF TERFENADINE, PSEUDOEPHEDRINE HYDROCHLORIDE, AND IBUPROFEN IN A LIQUID ANIMAL DOSING FORMULATION USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract

Stability-indicating assay methods based on high performance liquid chromatography have been developed for the quantitation of terfenadine, pseudoephedrine hydrochloride, and ibuprofen when combined in an aqueous 0.5% w/v Tween 20 and 0.5% methylcellulose animal dosing formulation. Because of the diversity of this drug mixture two separate chromatographic systems were required for the assays. A reversed phase system using a 3-μm Spherisorb ODS-2 column was used to assay for terfenadine and ibuprofen. An ion-exchange system using a 10-μm Partisil SCX column was used to assay for pseudoephedrine hydrochloride. The methods are accurate and precise with relative standard deviations over the concentration ranges of interest of 2% or less.

INTRODUCTION

Terfenadine is a newly introduced widely prescribed antihistamine (Fig. 1) with unique non-sedating properties (1). Very little has been published in the literature on the liquid chromatography (HPLC) of this important drug (2,3) or its major degradation product, MDL 9917 (Fig.1). In contrast, many chromatographic procedures for pseudoephedrine hydrochloride (4-11) and ibuprofen (12) have been published.

This report describes rapid stability-indicating assays for the quantitative determinations of terfenadine, pseudoephedrine hydrochloride, and ibuprofen when combined in an aqueous animal dosing formulation containing 0.5% w/v Tween 20 and 0.5% w/v methylcellulose.

MATERIALS AND METHODS

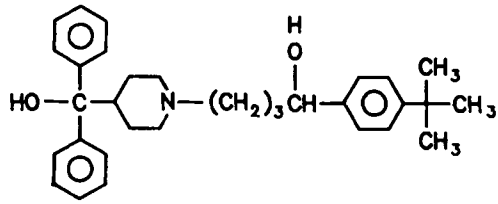
Apparatus: The liquid chromatograph consisted of a pump[1]; an automatic sampler[2], a variable wavelength UV detector[3], and a laboratory data system[4]. Depending on the analyte either a 3 µm Spherisorb ODS-2 (100 x 4.6 mm)[5] or a 10 µm Partisil SCX (250 x 4.6 mm)[6] column was used. For both column systems a precolumn filter was also used[7].

Chemicals and Reagents: All chemicals and reagents were either USP-NF or ACS reagent grade. The water and acetonitrile were of HPLC grade. Reference standards for terfenadine and its known degradation product, MDL 9917, were obtained in-house.

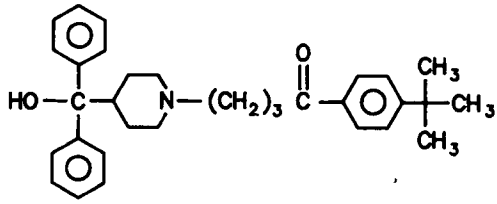
Formulation

The animal dosing formulation was a water base containing 0.5% w/v Tween 20, 0.5% w/v methylcellulose, and various levels of the drugs. The drug levels

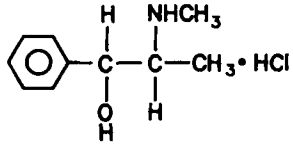
Terfenadine



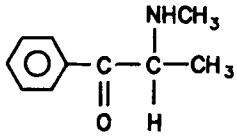
MDL 9917
(oxidation product
of Terfenadine)



Pseudophedrine
Hydrochloride



Oxidation product of
Pseudophedrine



Ibuprofen

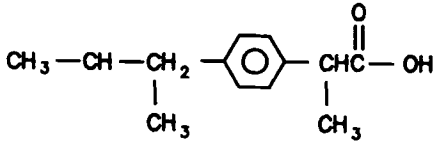


FIGURE 1. Drugs and potential degradation products.

ranged from 0.3-30 mg/mL terfenadine, 0.6-60 mg/mL pseudoephedrine hydrochloride, and 3-300 mg/mL ibuprofen.

Chromatographic Conditions

The assays for both terfenadine and ibuprofen were performed using the 3-µm Spherisorb ODS-2 column and a mobile phase of 60/40 v/v acetonitrile/water, made 0.012M in sodium phosphate buffer (ca pH 2.3) and 0.021M in sodium perchlorate. The assay for pseudoephedrine hydrochloride was performed using a 10-µm Partisil SCX column and a mobile phase of 50/50 v/v acetonitrile/water, made 0.024M in sodium phosphate buffer (ca pH 2.3). A flow rate of 1.5 mL/min, a detector wavelength of 210 nm at 0.32 AUFS, ambient temperature, and an injection volume of 20 µL were used for all the assays.

Mobile Phase Preparation: For the terfenadine and ibuprofen assays, 400 mL of water, 1.0 g sodium dihydrogen phosphate monohydrate, 0.5 g phosphoric acid (85%), and 3.0 g sodium perchlorate monohydrate were mixed until all the ingredients were dissolved, then 600 mL of acetonitrile were added. The solution was mixed and degassed prior to use. For the pseudoephedrine hydrochloride assay, 500 mL of water, 2.0 g sodium dihydrogen phosphate monohydrate and 1.0 g phosphoric acid (85%) were mixed until all the ingredients were dissolved, then 500 mL of acetonitrile were added. The solution was mixed and degassed prior to use.

Standard Solution Preparation

Approximately 30 mg terfenadine, 60 mg of pseudoephedrine hydrochloride and 300 mg of ibuprofen were accurately weighed and transferred to a 100-mL volumetric flask. The drugs were dissolved in and diluted to volume with methanol to

obtain the stock standard solution. Five mL of this solution were further diluted to 50 mL with the terfenadine-ibuprofen mobile phase to obtain the standard solution used for both the terfenadine and pseudoephedrine hydrochloride assays. Five mL of this standard solution were further diluted to 50 mL with the same mobile phase to give the ibuprofen standard solution.

Suitability Test Solution for Terfenadine:

Approximately 1 mg of MDL 9917 was transferred to a 50 mL volumetric flask. To this flask 5 mL of the standard stock solution were added and the solution was diluted and brought to volume with the terfenadine-ibuprofen mobile phase.

Assay Procedure:

A 5 mL sample of the well mixed formulation was obtained. This sample was serial diluted on the basis of label claim to approximate levels of terfenadine and pseudoephedrine hydrochloride of 30 µg/mL and 60 µg/mL, respectively. The dilutions were made with methanol as a solvent except for the last dilution which was made with the terfenadine-ibuprofen mobile phase. This sample solution was then used for both the terfenadine and pseudoephedrine hydrochloride assays. For the ibuprofen assay this sample solution was further diluted on the basis of label claim with the same mobile phase to an approximate 30 µg/mL level of ibuprofen.

System Suitability:

For each analyte a two part system suitability test was performed encompassing chromatographic resolution and system precision criteria (13). For

terfenadine, using the proper chromatographic conditions the system suitability test solution for terfenadine was injected and a resolution of greater than 5.0 for the ibuprofen and terfenadine peaks and greater than 3.0 for the terfenadine and MDL 9917 peaks were required for the system to possess adequate resolution. For ibuprofen, using the proper chromatographic conditions the ibuprofen standard solution was injected and a resolution of greater than 2.5 was required between the pseudoephedrine and ibuprofen peaks for the system to possess adequate resolution. For pseudoephedrine hydrochloride, using the proper chromatographic conditions, the pseudoephedrine standard solution was injected and a resolution greater than 3.0 was required between the pseudoephedrine and terfenadine peaks for the system to possess adequate resolution.

The system precision tests for all three drugs were performed similarly. With the chromatographic system operating under the conditions described for the particular drug, the appropriate standard solution was chromatographed five times. The relative standard deviation of the analyte's peak areas (or heights) was calculated and must be less than 2.0% for the system to possess adequate precision.

RESULTS AND DISCUSSION

The three drugs differ greatly in structure and lipophilicity as shown in Figure 1. The development of a single isocratic chromatographic stability indicating assay for all three drugs would therefore be unlikely. Several attempts were made using short chain alkyl, cyano, and ion-exchange bonded phase column packings. Under none of these conditions was a separation obtained which could resolve the three drugs from each other and the various degradation products of the drugs. A divide and conquer approach was therefore taken. Terfenadine and ibuprofen were found to chromatograph well using the

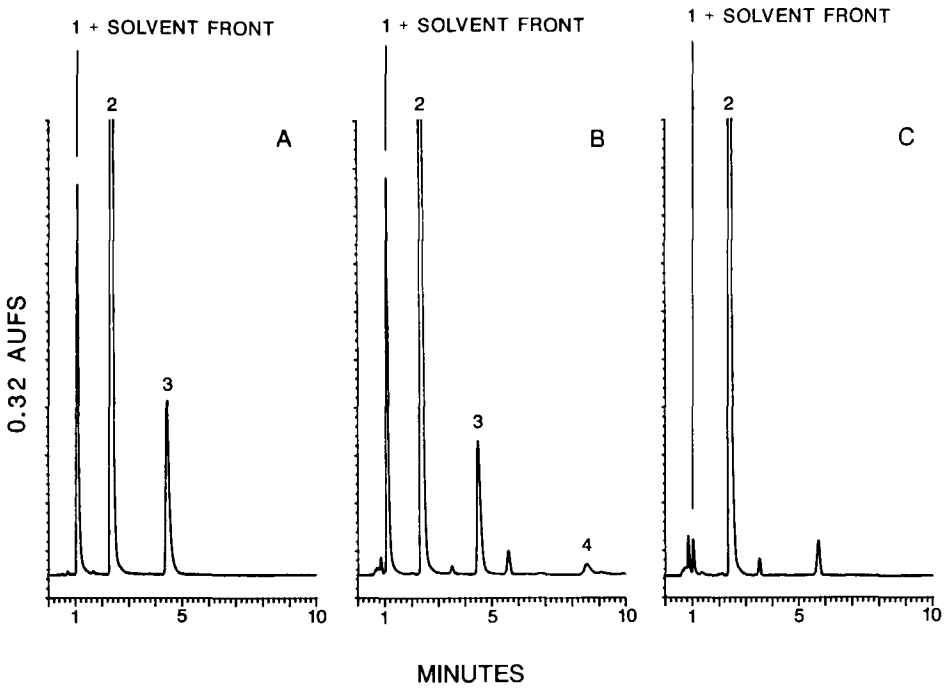


FIGURE 2. Chromatograms of A) a terfenadine standard solution, B) a stressed sample of 0.3 mg/mL terfenadine in the formulation vehicle treated with 0.1M HCl and held at 90°C for 16 hrs, and C) a stressed formulation vehicle control sample (no terfenadine) treated with 0.1M HCl and held at 90°C for 72 hrs. Peak 1 = pseudoephedrine, peak 2 = ibuprofen, peak 3 = terfenadine, and peak 4 = MDL 9917.

3-μm Spherisorb ODS-2 column and prescribed mobile phase. Using this system, as expected, pseudoephedrine eluted in the solvent front ($k' \approx 0$) of the chromatogram (Figure 2). Attempts to chromatograph pseudoephedrine using reversed phase systems both with and without ion-pairing techniques were made. Without ion-pairing techniques pseudoephedrine gave very poor peak shapes. Using ion-pairing with alkyl sulfonates in the mobile phase the pseudoephedrine peak shape was greatly improved but ibuprofen also eluted as a very late peak which would interfere with successive injections of pseudoephedrine samples. Fortunately using the ion-exchange mode with the Partisil SCX column and the

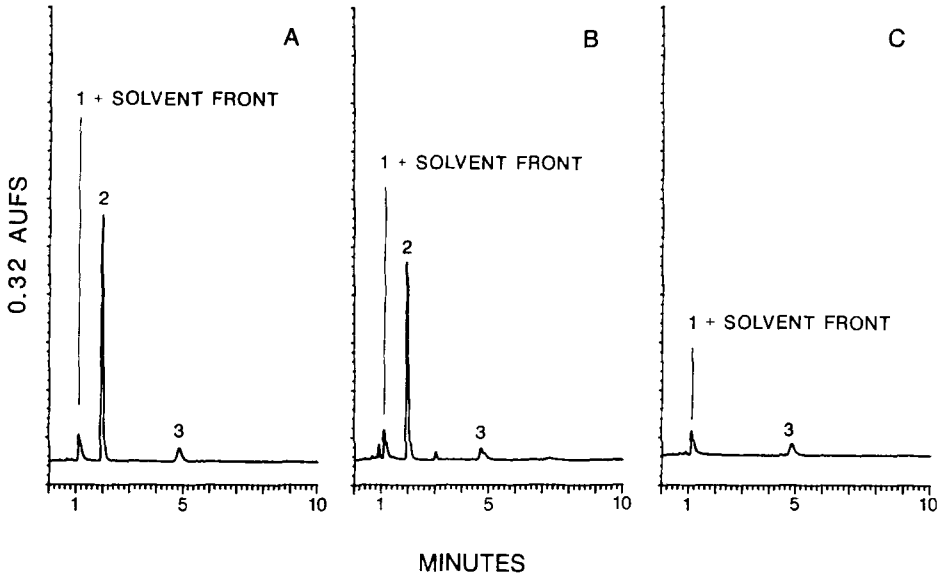


FIGURE 3. Chromatograms of A) and ibuprofen standard solution, B) a stressed sample of 3.0 mg/mL ibuprofen in the formulation vehicle treated with 0.1M HCl and held at 90°C for 72 hrs, and C) a stressed formulation vehicle control sample (no ibuprofen) treated with 0.1M HCl and held at 90°C for 72 hrs. For peak identity see Figure 2.

conditions described ibuprofen elutes in the solvent front, terfenadine is retained but well separated from pseudoephedrine, pseudoephedrine gives a well shaped peak (Figure 4), and the oxidation product of pseudoephedrine (Figure 1) is later eluting and well separated from pseudoephedrine. Although it might appear that terfenadine could also be assayed by this ion-exchange system, this is precluded as terfenadine coeluted with its degradation product, MDL 9917.

The drugs were formulated in a ratio of approximately 1:2:10 terfenadine: pseudoephedrine hydrochloride:ibuprofen. Even though the terfenadine and ibuprofen assays use the same chromatographic conditions, an additional dilution step is necessary for the ibuprofen assay because of the order of magnitude difference in concentrations. A detection wavelength of 210 nm was

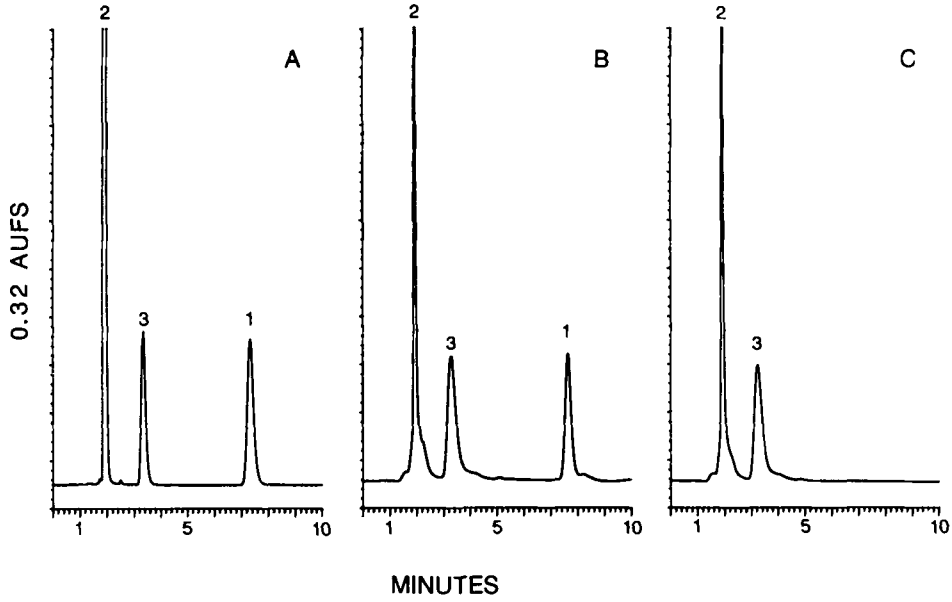


FIGURE 4. Chromatograms of A) a pseudoephedrine hydrochloride standard solution, B) a stressed sample of 0.6 mg/mL psuedoephedrine hydrochloride in the formulation vehicle treated with 0.1M HCl and held at 90°C for 72 hrs, and C) a stressed formulation vehicle control sample (no pseudoephedrine hydrochloride) treated with 0.1M HCl and held at 90°C for 72 hrs. For peak identity see Figure 2.

chosen for all three drugs as this allowed good sensitivity and quantitation for all of the drugs without changing instrument parameters.

Method Recovery and Precision

Using the outlined procedures recovery studies were performed at both the lowest and highest drug levels of interest. The recovery studies at the lowest level were performed over 3 days using two different columns. On each of the three days, samples of the formulation were prepared at the 80, 100, and 120% of a 0.3 mg/mL terfenadine, 0.6 mg/mL pseudoephedrine hydrochloride, and 3.0

mg/mL ibuprofen level and assayed. The results listed in Table I show good recoveries with relative standard deviations no greater than 2.3%. Likewise to show the applicability for the highest drug levels of interest a sample was prepared at 30 mg/mL terfenadine, 60 mg/mL pseudoephedrine hydrochloride, and 300 mg/mL ibuprofen and analyzed five times. The results also listed in Table I show good recoveries with relative standard deviations no greater than 1.4%.

Stability Indicating Ability

To demonstrate the stability indicating abilities of the assays for the drugs, synthetic samples of the formulation were prepared, from methanol or acetonitrile stock solutions of the drugs, at the lowest drug levels of

TABLE I

RECOVERIES FOR DRUGS AT THE ASSAY LEVEL EXTREMES

Drug	Approximate Prepared Level (mg/mL)	% Recovery (mean value)		Relative Standard Deviation (%)	
		Peak Area	Peak Height	Peak Area	Peak Height
Terfenadine	0.3*	100.7	101.3	1.5	2.3
	30.0**	100.2	100.3	0.7	0.3
Pseudoephedrine Hydrochloride	0.6*	100.4	100.3	1.4	0.5
	60.0**	99.9	99.7	1.1	0.8
Ibuprofen	3.0*	100.1	100.2	0.5	0.6
	300.0**	99.6	100.3	1.4	1.0

* n=9
** n=5

interest (0.3 mg/mL terfenadine, 0.6 mg/mL pseudoephedrine hydrochloride, and 3.0 mg/mL ibuprofen) using either water, 0.1M hydrochloric acid, or 0.1M sodium hydroxide as a solvent. Similarly for each drug, "control" samples were prepared which contained all the contents of the formulation except for the drug of interest. These controls were used to check for interferences from degradation products of the other two drugs and excipients. The samples were then placed in a 90°C oven for various amounts of time and analyzed to determine the amount of each drug remaining. The results are listed in Table II and some typical chromatograms are shown in Figures 2, 3, and 4. The procedures do appear to be stability indicating for the drugs and no interferences from other degradation products were seen. The actual formulation was found to be stable for at least a week at room temperature. As can be seen from the data ibuprofen in samples prepared from a methanol stock solution and exposed to acid were less stable, possibly because of ester formation, than those prepared from an acetonitrile stock solution.

System Suitability Tests

The assays for the three drugs use two different chromatographic systems. To assure the suitability of the systems used, three system suitability tests would be required (one for each drug). Each system suitability test is comprised of a resolution criterion and a precision criterion as prescribed in the literature (13-15). The precision criteria are the same for all three drugs. It was chosen as less than 2% RSD assuming an acceptance range of 95-105% of label and a 95% confidence interval (15).

The resolution criteria were chosen specifically for each of the three drugs. The resolution between the drug and its closest eluting neighbor peak(s), either a degradation product peak or another drug peak, was used as the criterion in all cases. For terfenadine the closest eluting known peaks were those for ibuprofen and MDL 9917 (a degradation product of terfenadine). The

TABLE II

ANALYSIS OF STRESSED SAMPLES OF SYNTHETIC FORMULATION

Drug	Time at 90°C	Spiked Value (mg/mL)	Assay Value (mg/mL)	% Remaining
In Water and Prepared from a Methanol Stock Solution				
Terfenadine	72 hrs	0.300	0.144	47.9
Pseudoephedrine HCl	72 hrs	0.600	0.597	99.4
Ibuprofen	72 hrs	3.000	2.485	82.8
In 0.1M HCl and Prepared from a Methanol Stock Solution				
Terfenadine	72 hrs	0.300	0.012	3.8
Pseudoephedrine HCl	72 hrs	0.600	0.501	83.4
Ibuprofen	72 hrs	3.000	0.516	17.2
In 0.1M NaOH and Prepared from a Methanol Stock Solution				
Terfenadine	72 hrs	0.300	0.298	99.3
Pseudoephedrine HCl	72 hrs	0.600	0.543	90.4
Ibuprofen	72 hrs	3.000	3.004	100.1
In Water and Prepared From an Acetonitrile Stock Solution				
Terfenadine	72 hrs	0.300	0.164	54.7
Ibuprofen	72 hrs	3.000	0.272	90.5
In 0.1M HCl and Prepared From an Acetonitrile Stock Solution				
Terfenadine	72 hrs	0.300	0.002	0.7
Ibuprofen	72 hrs	3.000	2.719	90.6
Terfenadine	16 hrs	0.300	0.212	70.6

resolution of terfenadine from both of these peaks was felt to be important so both have resolution criteria specified (Figure 5). For ibuprofen the most critical separation appeared to be between ibuprofen and pseudoephedrine so this resolution was specified. For pseudoephedrine the separation of pseudoephedrine from its oxidative degradation product was large and not a concern. Terfenadine however elutes relatively close to pseudoephedrine,

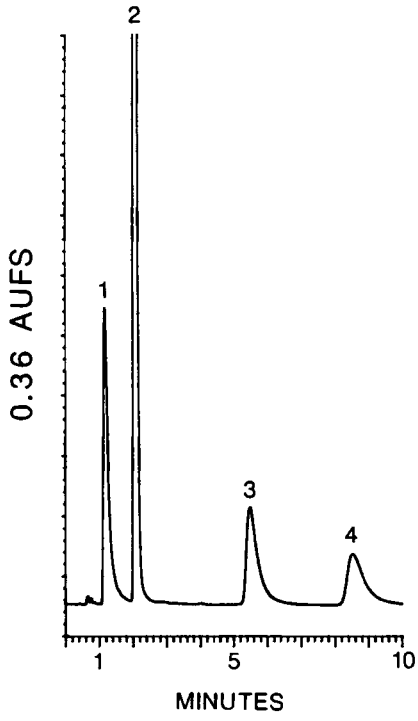


FIGURE 5. Chromatogram showing a system suitability resolution test for the terfenadine assay. For peak identity see Figure 2.

depending on the column, and therefore the resolution of terfenadine from pseudoephedrine was specified as the resolution criterion.

In summary the stability indicating assay procedures presented are accurate and precise for the quantitation of terfenadine, pseudoephedrine hydrochloride, and ibuprofen when combined in an aqueous 0.5% w/v Tween 20 and 0.5% methylcellulose animal dosing formulation.

FOOTNOTES

1. M-6000A, Waters Associates, Milford, MA 01757.
2. 710B WISP, Waters Associates.
3. Spectroflow 757, Kratos Analytical Instruments, Ramsey, NJ 07446.
4. CALS Data System, Beckman Instruments, Inc., Irvine CA 92713.
5. 3-µm Spherisorb ODS-2 (100 x 4.6 mm), Phase Separations Inc., Norwalk, CU 06850.
6. 10-µm Partisil SCX (250 x 4.6 mm), Whatman Inc., Clifton NJ 07014.
7. Model 7335, Rheodyne Inc., Cotati, CA 94928.

Acknowledgements

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