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Determination of imipramine and trimipramine by capillary electrophoresis with electrochemiluminescence detection

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ABSTRACT

The tricyclic antidepressants (TCA) imipramine (Imi) and trimipramine (Tri) were successfully analyzed by capillary electrophoresis (CE) coupling with Tris(2,2-bipyridyl) ruthenium(II)-based ($Ru(bpy)_3^{2+}$) end-column electrochemiluminescence (ECL) detection. The addition of β-CD to the running buffer was found to enable baseline separation of the two analytes and the addition of acetonitrile (ACN) as an organic additive to improve the repeatability and sensitivity of the CE method. Under the optimized separation and detection conditions (50 mM PBS (pH = 7.0) and 2 mM $Ru(bpy)_3^{2+}$ in the ECL detection cell, 20 mM Tris (pH = 2.0), 0.2 mM β-CD and 20% ACN (v/v) as running buffer), wide linear ranges of 0.1–5 μM and 0.1–5 μM were achieved, with the correlation coefficients of 0.9990 (n = 8) and 0.9980 (n = 8) for Imi and Tri, respectively. Detection limits 5 nM and 1 nM (S/N = 3) were obtained for Imi and Tri, respectively. The method was also successfully applied for the determination of Imi in pharmaceutical dosage form.

1. Introduction

Imipramine (Imi) and trimipramine (Tri) (Fig. 1) are tricyclic antidepressant (TCA) drugs, which are the most effective drugs presently available for the treatment of depression and other psychiatric disorders by blocking the reuptake of norepinephrine at nerve terminals [1,2]. The two antidepressant drugs have similar chemical structure and both of them contain a tertiary amines group. To enable their clinical applications to achieve optimum therapeutic effects and minimize side effects, analytical method with high sensitivity and free from interference is needed [3].

Many methods for the determination of Imi and Tri have been developed using techniques such as high performance liquid chromatography (HPLC) [4–8], gas chromatography (GC) [9–11], spectrophotometry [12,13], electrochemical analysis [14–18], chemometrics [19], capillary electrophoresis (CE) [20–22] and NACE [23,24]. CE has many advantages compared with other methods, such as high separation efficiency, short analysis time, good selectivity as well as low consumption. Therefore, it has been widely applied for drug analysis [25].

Ultraviolet (UV) absorbance is the most common detection method for CE, but its sensitivity is limited. Mass spectrometry (MS) method can offer high sensitivity and the information of structure of molecules, but it requires expensive apparatus and high operating cost. Laser induced fluorescence (LIF) is another sensitive detection method, but the instrument is expensive and the derivatization procedure is complex due to most of the analytes without natural fluorescence.

Electrochemiluminescence (ECL) has become an attractive and powerful detection analytical tool due to the advantages such as low equipment cost, inherent high sensitivity, good selectivity, wide linear range and simple optical setup. CE combining with ECL (CE–ECL) based on $Ru(bpy)_3^{2+}$, as an efficient and sensitive analytical technique, has been extensively applied for the determination of analytes containing tertiary amines group and their derivatives [26–30].

In the present work, CE–ECL was utilized for rapid separation and sensitive detection of Imi and Tri due to both of them containing a tertiary amines group. β-CD was added to the running buffer to obtain a baseline separation and acetonitrile (ACN) was used as organic additive in order to improve the repeatability and sensitivity. The solution in the ECL detection cell was 2 mM $Ru(bpy)_3^{2+}$ and 50 mM phosphate buffer (PBS) at pH 7.0 while the separation solution was composed of 20 mM Tris (pH = 2.0), 0.2 mM β-CD and 20% (v/v) ACN. This method was applied for the determination of Imi in pharmaceutical formulation with satisfactory results because no interferences from the tablet excipients were found.

2. Experimental

2.1. Chemicals

Tris(2,2-bipyridyl) ruthenium (II) chloride hexahydrate ($Ru(bpy)_3Cl_2 \cdot 6H_2O$) was obtained from Aldrich Chemical (Mil-

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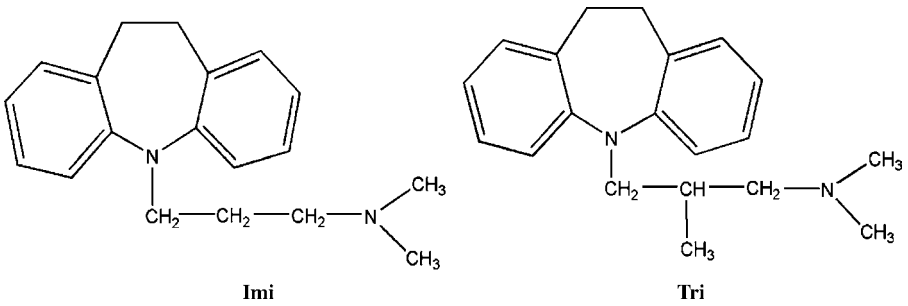

Fig. 1. Chemical structure of Imi and Tri.

waukee, WI, USA). Trimipramine maleate salt was purchased from Sigma (St. Louis, MO, USA). Imipramine hydrochloride was obtained from Merck (Darmstadt, Germany), and its drug tablets were supplied by Sine Jiu Fu Pharmaceutical Company (Shanghai, China). Before use, the tablets were ground into powder, dissolved in water, filtered and diluted to certain volume. Tris(hydroxymethyl) aminomethane (Tris) was purchased from Sigma–Aldrich, and β-CD hydrate was obtained from Lancaster. Other reagents were of analytical grade and all chemicals were used as received without further purification. Stock standard solutions of Tri, Imi, Tris and $Ru(bpy)_3^{2+}$ were stored in the refrigerator at 4 °C. Other reagents were of analytical grade and all chemicals were used as received without further purification. The 10 mM stock solutions of Imi and Tri were prepared by dissolving the standard samples in deionized water. The separation solution was prepared by dissolving β-CD and Tris with ACN–water mixtures. $Ru(bpy)_3^{2+}$ and PBS stock solutions were prepared with deionized water. They were stored in the refrigerator at 4 °C. Prior to determination, all samples and buffer solutions were filtered through 0.22 μm cellulose acetate filters (Xinya Purification Material Factory, Shanghai, China).

2.2. Apparatus

The CE–ECL system (MPI-A, Xi'an Remex Electronic Science Tech Co., Xi'an, China) included a high-voltage power, an EC potentiostat, a chemiluminescence detector and a data collection analyzer. EC detection was performed with a CH Instrument model 800 voltammetric analyzer (Austin, TX, USA).

All experiments were carried out by a conventional three-electrode system, with a 500 μm diameter Pt disk as the working electrode, a Pt wire as the counter electrode, and Ag/AgCl as the reference electrode. The electrophoretic separations were performed in a 75 μm i.d. and 30 cm length fused-silica capillary (Yongnian Optical Fiber, Hebei, China) column using a running buffer of Tris (20 mM, pH 2.0). The capillary was filled with 0.1 M NaOH overnight prior to use and rinsed with water to maximize free silanols on capillary surface. In the daily experiments, it was flushed with 0.1 M NaOH, double distilled water and separation buffer for 5 min, respectively. ECL detection was carried out in an end-column mode. The samples were injected electrokinetically for 4 s at 20 kV. The photomultiplier tube was set at 800 V to collect the ECL signals. The solution in the ECL detection cell was 2 mM $Ru(bpy)_3^{2+}$ and 50 mM PBS at pH 7.0 while the separation solution was composed of 20 mM Tris (pH = 2.0), 0.2 mM β-CD and 20% (v/v) ACN.

3. Results and discussion

3.1. Electrochemical (EC) and ECL reactions of the $Ru(bpy)_3^{2+}$/Imi and Tri system

Cyclic voltammograms (CV) and their corresponding ECL curves of 2 mM $Ru(bpy)_3^{2+}$ in 50 mM PBS (pH = 7.0) in the absence (curves a and a′) and presence of 0.3 mM Imi (curves b and b′) or 0.3 mM Tri (curves c and c′) were shown in Fig. 2. For CV measurements (Fig. 2A), with the addition of $Ru(bpy)_3^{2+}$ in buffer, the well-known reversible redox peaks appeared at about 1.1 V and 1.2 V (curve a), respectively [31]. The addition of Imi (or Tri) led to an increase in the anodic peak current but a decrease in the cathodic one (curve b or c). It can be seen that two oxidation peaks could be observed. The first one was at about 0.90 V due to the direct oxidation of Imi (or Tri). The potential of the second oxidation peak was overlapped with that of $Ru(bpy)_3^{2+}$ (1.2 V), indicating the participation of Imi or Tri as a co-reactant in $Ru(bpy)_3^{2+}$ ECL system. The possible oxidation products of Imi and Tri are shown in Fig. 3.

The corresponding ECL curves are illustrated in Fig. 2B. Adding $Ru(bpy)_3^{2+}$ to PBS, the ECL curve is typical in the potential range of 0.2–1.3 V and the highest ECL intensity was observed around 1.20 V (curve a′). The ECL intensity increased 17 and 26 times by adding Imi (curve b′) and Tri (curve c′), respectively. So the ECL method can be applied for sensitive determination of the two TCA drugs.

3.2. Performance of CE–ECL for Imi and Tri

3.2.1. Hydrodynamic voltammograms (HDVs)

As shown in Fig. 4, the effect of applied potential on ECL intensity of Imi and Tri was investigated in the range of 1.10–1.40 V. The two analytes had similar behavior of the ECL intensity vs. voltage. ECL intensity increased with the detection potential from 1.10 to 1.20 V, and then decreased. Hence, the applied potential was set at 1.20 V.

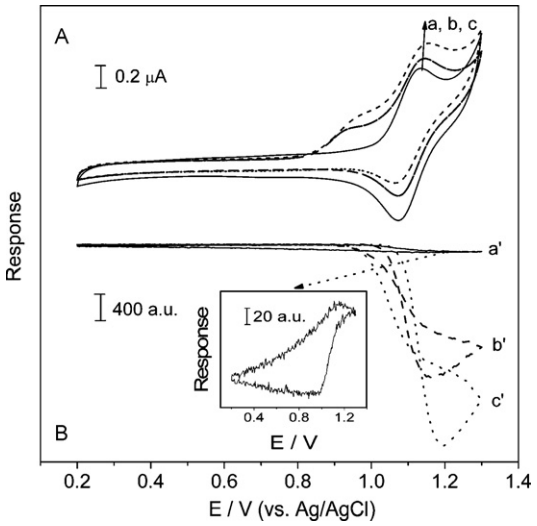

Fig. 2. Cyclic voltammograms and their corresponding ECL curves of $Ru(bpy)_3^{2+}$/Tri and Imi at Pt disc electrode. Curves a and a′, presence of 2 mM $Ru(bpy)_3^{2+}$ only; curves b and b′, adding 300 μM Imi to a; curves c and c′, adding 300 μM Tri to a. Potential scan rate, 0.1 V/s; supporting electrolyte, 50 mM PBS (pH = 7.0); PMT, 800 V.

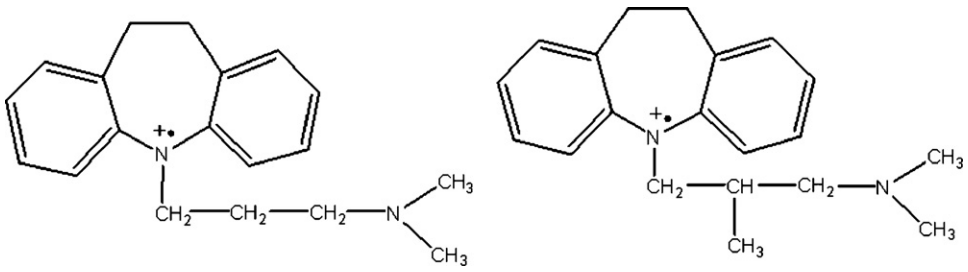

Fig. 3. Structure of oxidation products for Imi and Tri.

3.2.2. Effect of pH of PBS and Tris buffer

The pH value of buffer not only determines the charge on analytes but also has an influence on the EOF. Therefore, it has a pronounced effect on separation efficiency and resolution. In many cases, CE running buffer is the same as that in ECL detection cell in order to keep a stable and feasible environment. However, in our work, high sensitivity could not be achieved if we employed the same detection buffer and running buffer.

The influence of pH value of the detection buffer on ECL intensity was evaluated by varying the PBS pH values in the range of 6.0–9.0. The results indicated that the ECL intensity increased with pH value from 6.0 to 7.0 and then decreased with a higher pH value. The reason might be the competition of the reaction of $Ru(bpy)_3^{3+}$ with OH^- ions at higher pH values [32]. When the pH value exceeded 9.0, the noise of baseline was very large. Therefore, 50 mM PBS at pH 7.0 was used as the detection buffer in our experiment.

The effect of pH value of the running buffer on ECL intensity and resolution was investigated in 20 mM Tris buffer with different pH values (2.0, 2.5, 3.0 and 3.5) (Fig. 5). The separation resolution of the two antidepressants decreased with the pH values increase. The optimum pH value of Tris was at 2.0.

3.2.3. Addition of ACN to running buffer

Some works about the addition of organic modifiers to the aqueous buffer in CE have been reported [33–35]. One of the advantages of organic additive was to improve the selectivity. First, it can affect the acid–base properties of the analytes. Thus, the analyte with very similar pK_a values in aqueous system may be separated. Second, organic modifiers affect the electroosmotic flow via influencing viscosity, dielectric constant and conductivity of buffer [36–38]. ACN is widely used as a polar aprotic solvent. It has relatively high dielectric constant, low viscosity and low chemical reactivity. The results indicated that the influence of ACN additive on selectivity is inconspicuous. However, it could enhance the ECL intensity and improve the repeatability for determination of Imi and Tri. We found the significant degradation in ECL signal without ACN additive which is similar to that Greenway and Dolman reported [39]. The degradation in signal is caused by electrode fouling due to the oxidation product of analyte, which is insoluble in buffer. In contrast, the addition of ACN to running buffer resulted in a good repeatability. Because ACN can wash the wall of capillary and clean up the deposit on the surface of the electrode. The effect of ACN on ECL intensity of Imi and Tri was investigated with 20 mM Tris buffer solution containing different percentages of ACN (0–30%, v/v). The ECL signals of the two analytes were greatly enhanced by adding ACN to separation solution. The optimum percentage of ACN was 20%. Moreover, ACN has a very poor ECL response, which has no influence on the ECL intensity of background solution.

3.2.4. The addition of β-CD

We investigated the effect of the concentration of β-CD at 0.15, 0.20, 0.25 and 0.30 mM on the separation of Imi and Tri (Fig. 6). The typical electropherograms showed that baseline separation and high detection efficiency could be achieved when the concentration of β-CD was 0.20 mM. With the concentration of β-CD increase, the

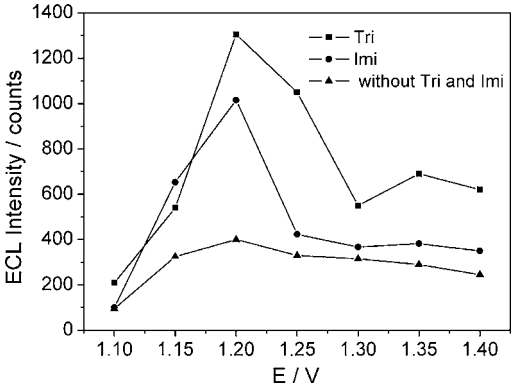

Fig. 4. Effect of detection potential on ECL intensity of 1.5 μM Imi and 0.5 μM Tri. Conditions: separation solution, 20 mM Tris (pH = 2.0); solution in the detection reservoir, 2 mM $Ru(bpy)_3^{2+}$–50 mM PBS (pH = 7.0); electrokinetic injection, 20 kV × 4 s; separation voltage, 15 kV.

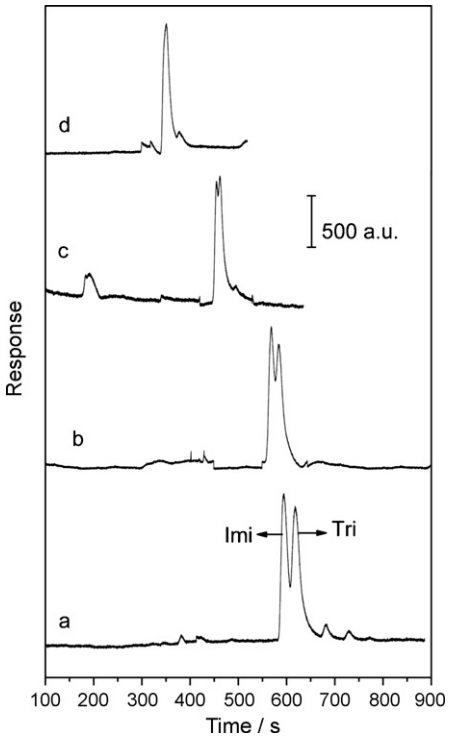

Fig. 5. Effect of the pH of Tris buffer on the separation of 1.5 μM Imi and 0.5 μM Tri. Curve a, pH = 2.0; curve b, pH = 2.5; curve c, pH = 3.0; curve d, pH = 3.5. Conditions: separation solution, 20 mM Tris with 20% (v/v) ACN; separation voltage, 10 kV. Other conditions are the same as in Fig. 2.

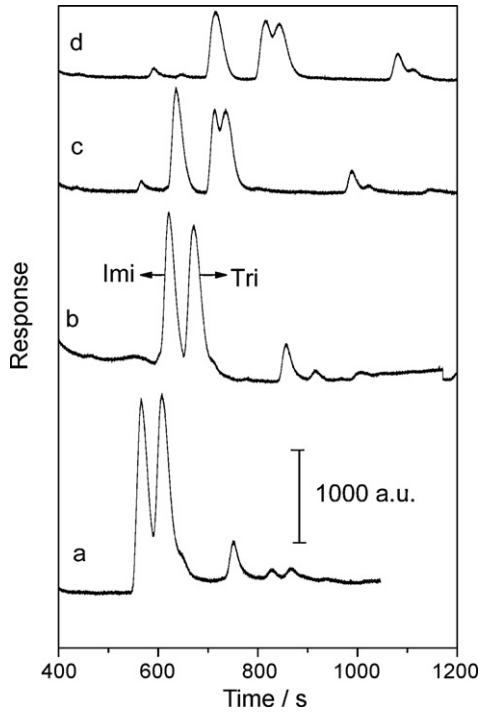

Fig. 6. Effect of the concentration of β-CD on the separation of 1.5 μM Imi and 0.5 μM Tri. The concentration of β-CD each at 0.15 (curve a), 0.20 (curve b), 0.25 (curve c), and 0.30 mM (curve d) in 20 mM Tris buffer (pH = 2.0) with 20% (v/v) ACN; separation voltage, 10 kV. Other conditions are the same as in Fig. 2.

Tri enantiomers achieved partly separation. However, the separation efficiency did not get improved with higher concentration of β-CD.

The two TCA drugs with semblable chemical structure could obtain baseline separation with β-CD additive. The mechanism is based on the inclusion of the analyte or at least its hydrophobic part into the relatively hydrophobic cavity of β-CD resulting in the difference in electrophoresis mobility between the two analytes.

3.2.5. Effect of separation voltage

The influence of the separation voltage on the ECL intensity of Imi and Tri was systematically investigated over the range of 9–18 kV. For both of them, ECL intensity increased with separation voltage from 9 to 15 kV and then decreased at a higher value. Since electroosmotic and electrophoretic velocities were directly proportional to the field strength that the use of the highest voltage possible resulted in the shortest time for separation. Based on the experiment, 15 kV was chosen to carry out the experiment.

3.3. Method evaluation

Under the optimized conditions: 50 mM PBS (pH = 7.0) and 2 mM $Ru(bpy)_3^{2+}$ in the ECL detection cell, 20 mM Tris (pH = 2.0), 0.2 mM β-CD and 20% ACN (v/v) as running buffer, electrokinetic injection 4 s at 20 kV, separation voltage at 15 kV, detection voltage at 1.20 V (vs. Ag/AgCl), the CE–ECL method was successfully applied for the separation and detection of tricyclic antidepressants Imi and Tri in 400 s (Fig. 7). As shown in Table 1, wide linear ranges of 0.1–5 μM and 0.1–5 μM, with low detection limits of 5 nM and 1 nM (S/N = 3), respectively, for Imi and Tri were achieved. The detection of Imi and Tri by CE–ECL and comparison with other methods reported are summarized in Table 2. The detection limit obtained by the CE–ECL method was lower than those by CE–UV and NACE–UV. Wide linear ranges obtained as most of the methods. Since the therapeutic ranges for Imi and Tri were 175–300 ng/ml (0.6–1.0 μM) and 150–350 ng/ml (0.4–0.9 μM). This CE–ECL method is potential to be applied in clinic studies.

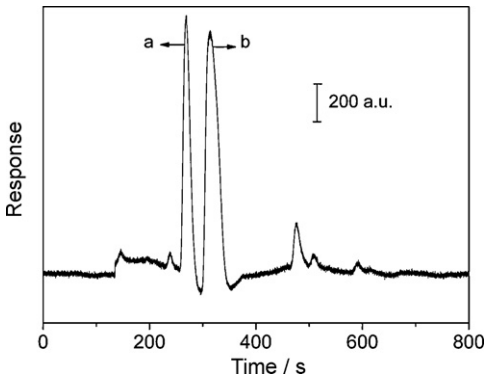

Fig. 7. Electropherogram showing the separation of 1.5 μM Imi (a) and 0.5 μM Tri (b). Running buffer: Tris (20 mM, pH = 2.0), β-CD (0.2 mM), 20% (v/v) ACN; buffer in detection reservoir: 2 mM $Ru(bpy)_3^{2+}$ with 50 mM PBS at pH 7.0; electrokinetic injection, 20 kV × 4 s; separation voltage, 15 kV; detection potential, 1.20 V; PMT, 800 V; capillary dimensions, 30 cm × 75 μm i.d.

Table 2
Detection of Imi and Tri by different methods.

Method	Analyte	Linear range (μM)	LOD (nM)	Refs.
CE–UV	Tri	62.5–250	10,000	[21]
NACE–UV	Imi	0.2–1.6	94.7	[23]
NACE–UV	Imi	0.2–3.2	34.7	[24]
CE–ECL	Tri	0.1–5	1	
Our work	Imi	0.1–5	5	

The repeatability of the method was investigated by three consecutive injections of 1.5 μM Imi and 0.5 μM Tri standard solutions with RSD values of the migration time and ECL intensity less than 1% and 3%, respectively. The inter-day variation ($n = 3$) was 1.9%, 2.1% and 3.0%, 4.1% for the migration time and ECL intensity, respectively. A good repeatability could be achieved without electrode reactivating by adding ACN to the running buffer.

3.4. Application

The validated method was applied for the commercial tablets of Imi. In the present work, the process of pretreatment is very simple (the tablets were ground into powder, dissolved in water, filtered

Table 1
Linear ranges, regression equations, detection limits and reproducibility of separation of Imi and Tri.

Analytes	Linear ranges (μM)	Regression equations $Y = BX + A$	Correlation coefficients (R)	Detection limits (nM)	Reproducibility (RSD, %)	
					Migration time	Peak intensity
Imi	0.1–5	$Y = 261X + 939$	0.9990	5	0.6[a], 1.9[b]	2.6[a], 3.0[b]
Tri	0.1–5	$Y = 317X + 1191$	0.9980	1	0.7[a], 2.1[b]	2.9[a], 4.1[b]

Y represents the ECL intensity; X represents the concentration of Imi or Tri.
[a] The intra-day RSD ($n = 3$).
[b] The inter-day RSD ($n = 3$).

Table 3
Recoveries of Imi at different spiked levels in tablet samples.

Labeled (μM)	Added (μM)	Found (μM)	Recovery (%)	RSD (%) (n = 6)
1	0	0.98	98	0.7
1	1	2.05	103	1.1

and diluted to certain volume). No interference with the analyte peak observed because most of the tablet composition is neutral. The analysis results showed in Table 3, confirmed the usefulness of this CE–ECL method in pharmaceutical study and quality control.

4. Conclusion

A simple, sensitive and rapid method was applied for the determination of tricyclic antidepressants Imi and Tri by CE coupling with ECL detection. This method is based on the reactions between aliphatic tertiary amino group in antidepressants and $Ru(bpy)_3^{2+}$. β-CD was added to the running buffer to obtain the baseline separation of the two analytes. ACN as organic additive can enhance the ECL intensity and improve the reproducibility at the same time. The limits of detection obtained for the two drugs were below the therapeutic concentration, what proves the CE–ECL method to be potential to be applied in clinical praxis.

Acknowledgements

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