

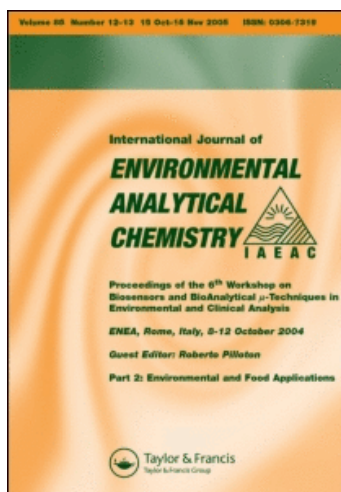
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### Dispersive liquid phase micro-extraction of aromatic amines in environmental water samples

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## Dispersive liquid phase micro-extraction of aromatic amines in environmental water samples

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A new, rapid and sensitive method for the determination of aromatic amines such as *o*-nitroaniline (NIT), alpha-naphtylamine (NAP), *o*-chloroaniline (CHL) in environmental water samples was developed with dispersive liquid phase micro-extraction coupled with high performance liquid chromatography. Preliminary experiments indicated that excellent achievements were obtained when chlorobenzene and acetonitrile were employed as the extraction solvent and dispersive solvent, respectively. Some other parameters that would have important effect on the pre-concentration of aromatic amines were investigated in detail. Under the optimal conditions, the method had excellent linear relationship between the peak area and the concentration for NIT, NAP and CHL in the concentration range of 1–50 µg L<sup>-1</sup>. The limits of detection and precisions of the proposed method were in the range of 0.1–0.7 µg L<sup>-1</sup> and 6.3–9.7%, respectively. The proposed method has been validated with two real water samples, and the results showed that excellent spiked recoveries in the range of 92.8%–111.5% were achieved. All these demonstrated that the proposed method would be very useful and applicable for the determination of such pollutants in the future.

**Keywords:** aromatic amines; dispersive liquid phase micro-extraction; high performance liquid chromatography; water samples

### 1. Introduction

In recent years, aromatic amines have been widely used in industries and they can enter the environment from the effluent, the degradation of pesticides or in other ways. The resultant environmental pollution has attracted much attention. These pollutants can be accumulated and difficult to be degraded in the environment, and they pose a serious threat to human health [1]. Dinitroaniline herbicides have been widely used in modern agriculture to control many germinating annual grass and broadleaf weeds. It has been found by some researchers that trifluralin, one of dinitroaniline herbicides, poses a potential risk to human health [2]. In addition, exposure to trifluralin for a long period

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of time would significantly increase the probability of cancer incidence [3]. Hence, it is very important to develop rapid, sensitive and simple analytical methods for aromatic amines.

At present, there have been many analytical methods and technologies used for determination of *o*-nitroaniline, *o*-chloroaniline, alpha-naphthylamine, and so on. In these methods, the often-used techniques are gas chromatography (GC) [4] and high performance liquid chromatography (HPLC) [5]. Recently, gas chromatography-mass spectrometry (GC-MS) [6–9] and electrochemical analysis [10] have been predominantly used for the analysis of aromatic amines. However, HPLC is also an important technique for the determination of aromatic amines.

In most cases, target analytes are present in the environmental samples at trace level, and the sample matrices are very complex. It was very difficult to directly analyse the pollutants with the analytical instruments. In order to gain better analytical performance and sensitivity, a sample pre-treatment procedure is necessary in the analytical process. Many sample pre-treatment technologies have been developed in recent years. Among them, solid-phase extraction (SPE) [11–16], supercritical fluid extraction [17–19], microwave-assisted extraction [20–21] and solid-phase micro-extraction (SPME) [22–27] have been introduced and have attracted much attention. Liquid phase micro-extraction as a novel sample pre-concentration technique has also been in the centre of focus in the development of analytical methods for the determination of environmental pollutants.

Assadi and co-workers developed a new technology termed dispersive liquid-liquid micro-extraction (DLLME) [28] which has been successfully applied in analysing polycyclic aromatic hydrocarbons (PAHs), triazine herbicides, organophosphorus pesticides, antioxidants, volatile phenols, chlorophenols, chlorobenzenes [28–34] and heavy metals [35–40]. Our group has developed a novel method called temperature-controlled ionic liquid dispersive liquid-phase micro-extraction and it has been successfully applied for analysing organophosphorus pesticides [41] and pyrethroid pesticides [42]. However, very few reports have been found about DLLME used for the analysis of aromatic amines [43].

The goal of this paper is to develop a rapid and sensitive determination of aromatic amines in environmental water samples with DLLME in combination with HPLC. The parameters affecting the enrichment performance were investigated and optimised in detail. The proposed method was applied to the analysis of real water samples.

## 2. Experimental

### 2.1 Reagents and chemicals

Quantities of *o*-nitroaniline, *o*-chloroaniline, alpha-naphthylamine were purchased from Beijing Chemical Reagent Factory (Beijing, China). HPLC grade acetonitrile and methanol were obtained from Guoda Chromatographic Reagent Factory (Changzhou, China). Ultrapure water was prepared in the lab using a water treatment device 'Ultra-Clear' (SG Wasseraufbereitungsanlagen, Barsbüttel, Germany) and all the other solvents were of analytical reagent grade unless stated. All the glassware used in the experiments were cleaned with deionised water, then soaked in 6 mol L<sup>-1</sup> nitric acid for 24 h and then washed with ultrapure water. A quantity of 0.5 M of sodium hydroxide and 3 M sodium hydroxide were used for adjusting the pH value of the water samples.

## 2.2 Apparatus

A high performance liquid chromatograph system, which consisted of two LC-10ATvp pumps and an SPD-10Avp, ultraviolet detector obtained from Shimadzu (Tokyo, Japan) was used for the analysis and separation. A reversed-phase ZORBAX SB-C18 column ( $150 \times 2.1$  mm ID, particle size  $5 \mu\text{m}$ ) was used for separation at ambient temperature and Chromato Solution Light Chemstation for LC system was employed to acquire and process chromatographic data. The mobile phase was methanol/water (50/50, v/v) at  $0.2 \text{ mL min}^{-1}$ , and the injection volume and detection wavelength were  $10 \mu\text{L}$  and 235 nm, respectively. Turbo Vap LV evaporator Extraction Station purchased from Zymark (USA) was used. The standard chromatogram is shown in Figure 1.

## 2.3 DLLME procedure

A volume of 10 mL ultrapure water was placed in a 10 mL glass test tube with a conical bottom, and the pH was adjusted to 12. Then the solution was spiked at the level of  $10 \mu\text{g L}^{-1}$  of standard solution. A mixture of  $500 \mu\text{L}$  acetonitrile and  $60 \mu\text{L}$  chlorobenzene was rapidly injected into the sample using a syringe, and a cloudy solution formed. Further, the solution was centrifuged for 2 min at 5000 rpm. The dispersed fine droplets of chlorobenzene were sedimented at the bottom of the conical test tube. Finally, the upper layer was removed, and the residual phase was blown to nearly dryness with mild nitrogen stream in the Evaporator Extraction Station. A quantity of  $100 \mu\text{L}$  methanol was added into the tube in order to dissolve the residual phase and  $10 \mu\text{L}$  of the final solution was injected into the HPLC system for analysis.

## 2.4 Water samples

There were two real water samples were used to validate this proposed method. They were rainwater and melted snow water samples, both collected from Henan Normal University,

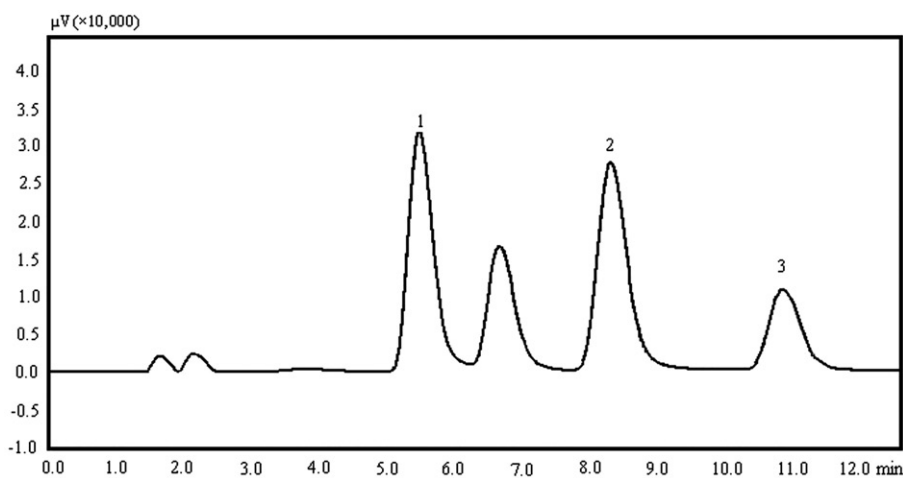


Figure 1. Standard chromatogram of the three aromatic amines. 1. *o*-nitroaniline 2.  $\alpha$ -naphthylamine 3. *o*-chloroaniline. Conditions: mobile phase, acetonitrile and ultra pure water (50/50, v/v) at flow rate of  $0.2 \text{ mL min}^{-1}$ ; concentration,  $5 \mu\text{g mL}^{-1}$  injection volume,  $10 \mu\text{L}$ ; detection wavelength, 235 nm.

Xinxiang City, Henan province. Before use, all the environmental water samples were filtered through 0.45  $\mu\text{m}$  micropore membranes and stored in brown glass bottles under low temperature, respectively.

### 3. Results and discussion

#### 3.1 Selection of extraction solvent and dispersive solvent

Usually, the type of extraction solvent and dispersive solvent are key factors in the DLLME procedure. The extraction solvent is often selected from the organic solvents, which have higher density, lower solubility in water and higher extraction capability for target compounds. Carbon tetrachloride, chlorobenzene, trichloromethane and dichloromethane are investigated as the extraction solvent and compared for extraction efficiency of the target compounds from water samples. A series of experiments were carried out using 550  $\mu\text{L}$  of acetone as disperser solvent and 60  $\mu\text{L}$  of the four organic reagents as extraction solvent, respectively. The results revealed that the best performance was obtained when chlorobenzene was used as extraction solvent and it was employed in the further experiments.

An appropriate disperser solvent can enhance enrichment performance of DLLME, but it should be miscible with extraction solvent. Here methanol, acetonitrile and acetone were investigated as the disperser solvent. A series of experiments were designed using chlorobenzene as the extraction solvent. The results showed that best performance was obtained when acetonitrile was used as disperser solvent.

#### 3.2 Effect of extraction solvent volume

In order to save extraction solvent and reduce the generation of secondary pollution, a series of experiments was designed for investigating the effect of the of extraction solvent volume. The other experimental conditions are fixed and designed by using 600  $\mu\text{L}$  of acetonitrile as dispersive solvent. The volume of extraction solvent was optimised over the range of 30  $\sim$  80  $\mu\text{L}$  and the experimental data are shown in Figure 2. From Figure 2, it was found that the best performance was obtained when 60  $\mu\text{L}$  of chlorobenzene was used in the extraction procedure.

#### 3.3 Effect of dispersive solvent volume

The volume of dispersive solvent plays an important role on the extraction performance and it was examined over the range of 300  $\sim$  800  $\mu\text{L}$ . The experimental results demonstrated that the peak areas of the three aromatic amines increased with the increase of the volume of acetonitrile in the range of 300  $\sim$  500  $\mu\text{L}$ , and then decreased slightly when the volume of acetonitrile was over 500  $\mu\text{L}$  (see Figure 3). It is because that cloudy phase did not form well at a low volume of acetonitrile and chlorobenzene could not be dispersed into infinitesimal droplets, then aromatic amines could not be transferred into the solvent extraction phase (chlorobenzene) completely. On the other hand, a much larger volume of acetonitrile enhanced the solubility of target compounds in the water sample, which also resulted in a decrease of enrichment performance. Therefore, 500  $\mu\text{L}$  acetonitrile was employed in the following experiments.

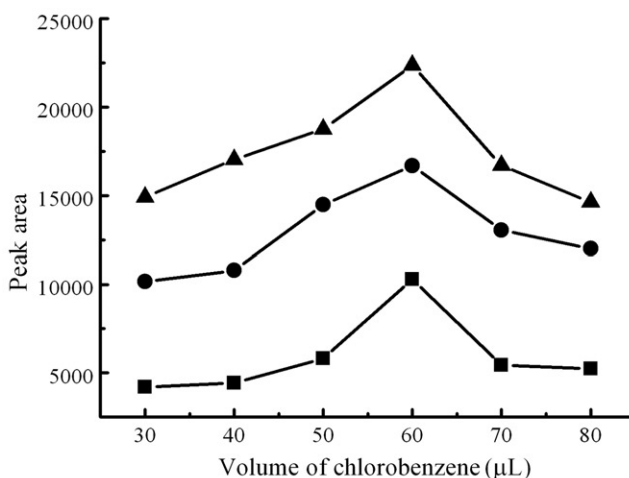


Figure 2. The effect of the volume of chlorobenzene. Conditions: sample volume, 10 mL; spiked level,  $10 \mu\text{g L}^{-1}$ ; acetonitrile volume, 600  $\mu\text{L}$ ; pH 10; extraction time, 2 min; centrifuging time, 4 min.  $\blacktriangle$ , *o*-chloroaniline;  $\bullet$ , alpha-naphthylamine;  $\blacksquare$ , *o*-nitroaniline.

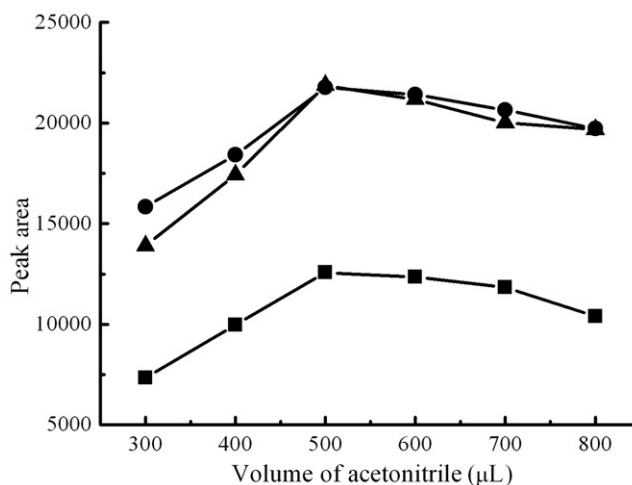


Figure 3. The effect of the volume of acetonitrile. Conditions: sample volume, 10 mL; spiked level,  $10 \mu\text{g L}^{-1}$ ; chlorobenzene volume, 60  $\mu\text{L}$ ; pH 10; extraction time, 2 min; centrifuging time, 4 min.  $\blacktriangle$ , *o*-chloroaniline;  $\bullet$ , alpha-naphthylamine;  $\blacksquare$ , *o*-nitroaniline.

### 3.4 Effect of extraction time and centrifuging time

In the DLLME procedure, extraction time and centrifugation time are two important parameters. Here extraction time was defined as the time interval between injection of the mixture of extraction solvent and dispersive solvent and the start of centrifuging. The effect of extraction time and centrifugation time were investigated over the range of 1~30 min and 1~7 min, keeping the other experimental conditions constant. The experimental results demonstrated that a short equilibrium state was reached immediately

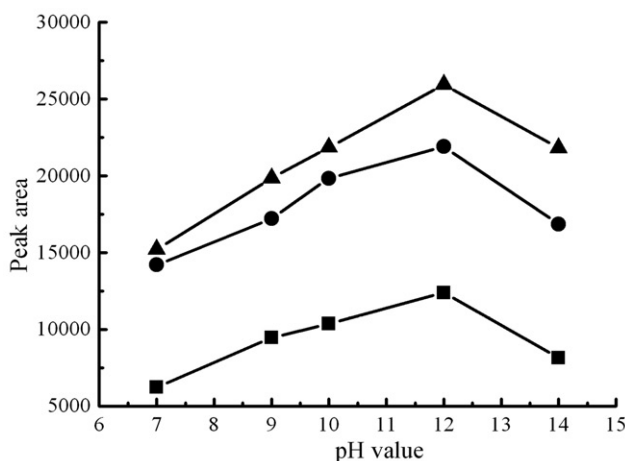


Figure 4. The effect of sample pH. Conditions: sample volume, 10 mL; spiked level,  $10 \mu\text{g L}^{-1}$ ; chlorobenzene volume, 60  $\mu\text{L}$ ; acetonitrile volume, 500  $\mu\text{L}$ ; extraction time, 2 min; centrifuging time, 4 min.  $\blacktriangle$ , *o*-nitroaniline;  $\bullet$ ,  $\alpha$ -naphthylamine;  $\blacksquare$ , *o*-chloroaniline.

and the better enrichment factor was achieved at 2 min, because the target compounds diffused into the extraction solvent quickly due to the formation of infinitely large surface area of extraction solvent droplets, which was in agreement with the report of Nagaraju and Huang [29]. A longer period would result in the decrease of the extraction efficiency owing to the fact that chlorobenzene was slowly dissolved in water with the promotion of acetonitrile. Furthermore, centrifugation time determines whether the phase separation is good or not. It was discussed in detail over a range of 1–7 min. The experimental results exhibited that the better performance was achieved at 4 min. Therefore extraction time and centrifuging time was set at 2 min and 4 min.

### 3.5 Effect of pH

Sample pH is an important parameter in the sample pre-treatment techniques. However, they are present as many states owe to the different and complex environment conditions. The  $\text{pK}_b$  of the three aromatic amines are 13.5 for NIT, 11.34 for CHL and 10.08 for NAP, which indicate that they are prone to dissociate easily in the acidic and weak alkaline environment. Thus, it can be expected the satisfied extraction efficiency can be obtained only in a strong alkaline environment. Hence sample pH would have an important effect on the enrichment of the three aromatic amines and was investigated over the range of 7–13. The results are illustrated in Figure 4. It could be seen that the extraction efficiency was not ideal over the range of 7–10 and the best performance was obtained when pH was 12; the enrichment performance then decreased with the increase of pH value. It may be that the target analytes were unstable in the acidic and weak alkaline environment. Hence, pH 12 was selected for use.

### 3.6 Analytical performance

As we are dealing with a new analytical method, some parameters such as linear range, limit of detection and precision are very important. These data were investigated in detail



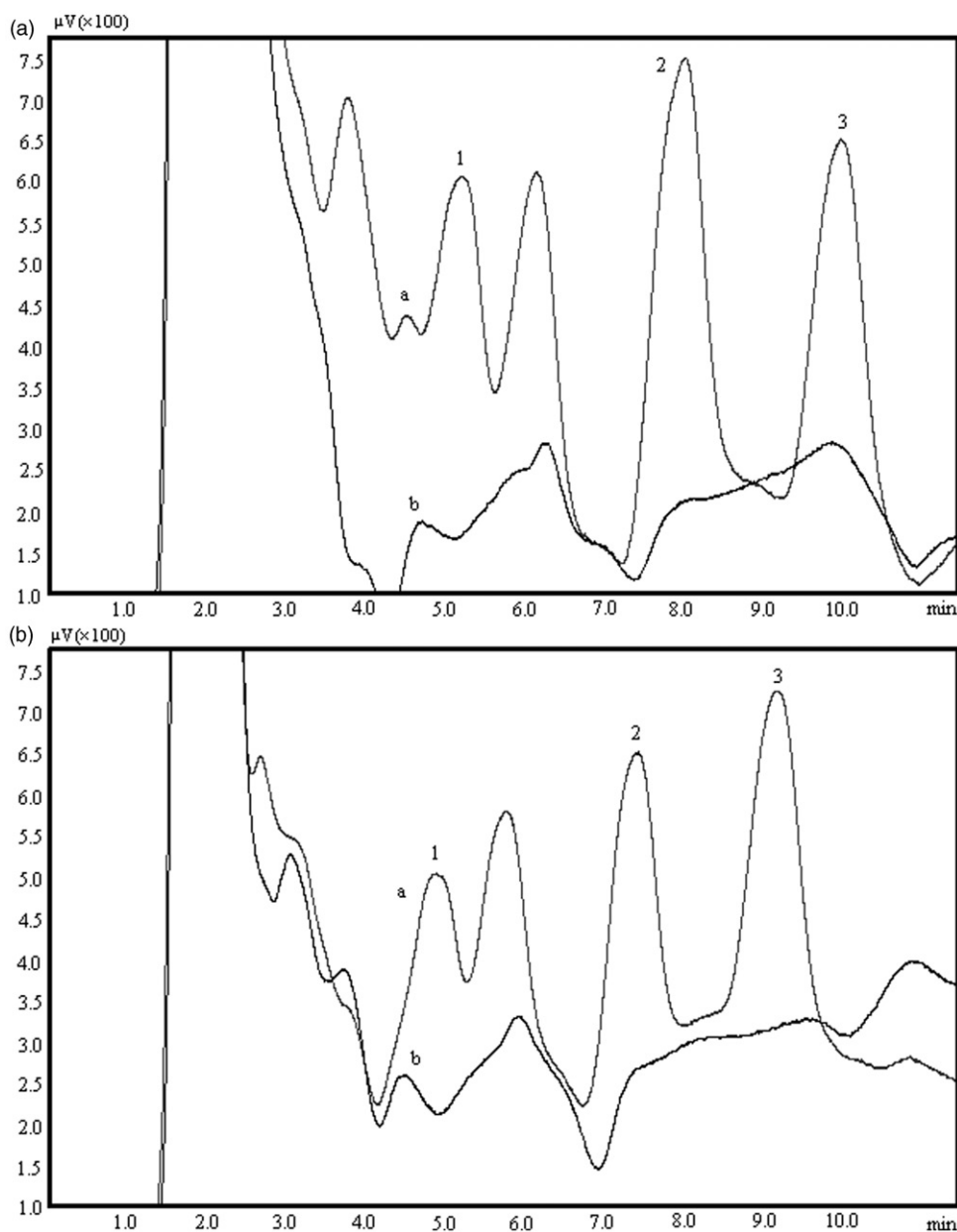


Figure 5. The chromatograms obtained from real water samples. Conditions: spiked concentration,  $5 \mu\text{g L}^{-1}$ ; sample solutions, 10 mL; chlorobenzene volume,  $60 \mu\text{L}$ ; acetonitrile volume,  $500 \mu\text{L}$ ; extraction time, 2 min; centrifuging time, 4 min; pH 12. 1, *o*-nitroaniline; 2,  $\alpha$ -naphthylamine; 3, *o*-chloroaniline. a, spiked; b, blank; A, rain water sample; B, melted snow sample.

under the optimised conditions as obtained above. The experimental results indicated that there were excellent linear relationship between peak areas and concentrations of the three aromatic amines, NIT, NAP and CHL, over the range of  $1 \sim 50 \mu\text{g L}^{-1}$  with good coefficients (0.9997, 0.9995 and 0.9992, respectively). The limits of detection (LODs)



of proposed method based on signal to noise ratio ( $S/N=3$ ) were achieved for the three aromatic amines, being 0.1, 0.2 and  $0.7\mu\text{g L}^{-1}$ , and the precisions of the established method were 9.7%, 7.2% and 6.3% (RSD,  $n=5$ ) for the three aromatic amines, respectively.

### 3.7 Real water samples analysis

In order to validate the applicability of the proposed method, two real world samples such as rainwater and melted snow water were used for analysis. The results showed that no target aromatic amines were found in the samples. In order to assess matrices effects, the two real water samples were spiked with standard solution at the concentration of  $5\mu\text{g L}^{-1}$ , respectively. The spiked recoveries of target aromatic amines in rainwater and melted snow water samples were 96.6%, 111.5%, 96.5% and 99.3%, 92.8%, 102.2% for NIT, NAP and CHL, respectively. And the LODs of the three aromatic amines in rainwater and melted snow water samples were 1.04, 0.79,  $0.75\mu\text{g L}^{-1}$  and 1.22, 0.91,  $0.83\mu\text{g L}^{-1}$ , respectively. The typical chromatogram is shown in Figure 5.

## 4. Conclusion

Aromatic amines have been proved to have a potential risk for human health, and have attracted much attention. To develop novel, sensitive, simple and low cost analytical methods for analysing them is therefore of great importance. This paper developed a simple, rapid, easy to operate, low cost and sensitive method for the analysis of aromatic amines based on a DLLME in combination with HPLC. The low LODs and satisfied spiked recoveries obtained from the real water samples demonstrated the advantage of the proposed method. All these data indicated that the developed method would be a competitive alternative in the routine analysis of aromatic amines in the environmental monitoring.

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