Ultrasound Assisted Surfactant Enhanced Emulsification Microextraction and Spectrofluorimetry for Determination of Oxadiazon in Agricultural Water Samples

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ABSTRACT: *In this work, an environmentally friendly sample pre-treatment method, Ultrasound Assisted Surfactant Enhanced Emulsification Microextraction (UASEME) followed with spectrofluorimetry was performed for determination of oxadiazon residues in water samples. After the determination of the most suitable extraction solvent and its volume, other parameters such as type and concentration of surfactant, pH, extraction time and ionic strength were optimized. Under the best conditions for extraction recovery, 50µL of chlorobenzene was chosen as extraction solvent and Tween 20 in concentration of 0.06 mmol/L as emulsifier and 2 min was the required time for quantitative analysis, without ionic strength and pH adjustment. Limit of detection and relative standard deviation were calculated as 0.05µg/L and 3.2% respectively. The procedure was applied successfully for assessing matrix effect in real water samples with relative recovery 96-104% with the precision in the range of 2.9-3.4%. The results demonstrate that UASEME procedure as reported is reliable, rapid and easy to use for analysis of oxadiazon in water samples.*

KEY WORDS: *Ultrasound assisted surfactant enhanced emulsification microextraction , Oxadiazon, Spectrofluorimetry*

INTRODUCTION

Oxadiazon,5-tert-butyl-3-(2,4-dichloro-5-isopropoxyphenyl)-l,3,4-oxadiazol-2(3H)-one is an effective herbicide for control of obnoxious grasses and broad leaf weeds in a wide variety of crops, e. g.,citrus fruit, vines, cotton, rice, soya beans and onions [1]. The relatively low toxicity by ingestion and by dermal route of this herbicide is one of the reasons for its widespread use [2]. Oxadiazon is stable inneutral or acid media but unstable in alkaline media, DT 50 (half-life) 38 d (pH 9, 25 °C). Maximum concentration of oxadiazon allowed is 0.01 mg/kg. Different analysis methods such as Gas Chromatography (GC) with different detection systems, Flame Ionization Detector (FID) [3], Electron Capture Detector (ECD) [4], Mass Spectrometry (MS) [5], High Performance Liquid Chromatography (HPLC) [6], have been reported for the determination of oxadiazon in different matrixes. According to the low concentration of herbicides and pesticides in different natural samples, sample

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preparation has an essential role to obtain accurate and sensitive results. The most commonly sample preparation procedures for the analysis of oxadiazon are Liquid-Liquid Extraction (LLE), Solid Phase Extraction (SPE), Head Space-Solid Phase Micro Extraction (HS-SPME) in combination to HPLC and GC. Determination of oxadiazon was performed in different matrixes such as water, soils, grapes and wine [7-9]. In 2006, *Assadi et al.* has developed an interesting mode of microextraction named, Dispersive Liquid-Liquid Micro Extraction (DLLME), which showed many advantages such as rapidity, low cost, simplicity, high enrichment factor for determination of wide range of compounds [10-16]. In this microextraction mode, extraction solvent is dispersed in sample solution by the assistance of a water miscible organic solvent. This solvent which is named dispersive solvent could decrease the partition coefficient of analyte to the extraction phase, which is known as major defect of DLLME. Ultrasound Assisted Emulsification MicroExtraction (UAEME) was used for the first time by *Garcia-Jares et al.* for the determination of synthetic musk fragrances, phthalate esters and lindane in environmental waters [17]. In this microextarction procedure, a microvolume of water immiscible solvent is emulsified in sample solution by the assistance of ultrasound energy. By formation of tiny droplets of organic solvent the target analytes are extracted to the extraction solvent and after centrifuging the sedimented phase are determined by analysis methods. So there is no need to use a polar solvent such as methanol or acetonitrile to disperse the extraction solvent into the sample solution. UAEME has been also used for the determination of polybrominated diphenyl ethers, polycyclic aromatic hydrocarbons, phenolic preservatives, polychlorinated biphenyls organochlorine and organophosphorus pesticides in water samples [18-22]. The low mass transfer efficiency of analyte between the two immiscible phases in this extraction mode may cause the extraction time long [23-27]. Surfactants have been widely used in separation sciences such as cloud point extraction. Recently they have been used as surface active agents in order to enhance the extraction efficiency in USAEME. They could accelerate the dispersion of extraction solvent in aqueous sample solution and so speed up the mass transfer to organic phase. This novel microextraction system was named Ultrasound-Assisted Surfactant Enhanced Emulsification Microextraction (UA,SEME) [28], could decrease the extraction time lower than 5 minutes. In aqueous solutions and at Critical Micelle Concentration (CMC), surfactants begin to form aggregates that are in dynamic equilibrium with the monomers in the bulk aqueous solution. An important property of micelles is to enhance the solubility of hydrophobic substances in aqueous solutions [29, 30]. So the analysis by this microextraction mode is performed in the concentration lower than CMC [31].

EXPERIMENTAL SECTION

Reagents and materials

All chemicals used in this research were of analytical-reagent grade. Chlorobenzene (C_6H_5Cl), chloroform ($CHCl_3$), carbon tetrachloride (CCl_4), tetra chloride ethylene (C_2Cl_4), sodium hydroxide (NaOH) and sodium chloride (NaCl) were obtained from Merck chemical company (Darmstadt, Germany). Sodium dodecyl sulfate, SDS ($C_{12}H_{25}OSO_3Na$), Triton X-100, Tween 20, Tween 80 and Triton X-100 were purchased from Sigma-Aldrich. A stock standard solution containing 1000 mg/L of oxadiazon was prepared in methanol and was stored in the dark at 5ºC. Other working solutions in lower concentrations were prepared daily immediately prior to analysis. Natural water samples were collected from the agricultural fields in the north of Iran.

Equipments

Spectrofluorimetric analyses were made using a Perkin Elmer LS 50 spectrofluorimeter equipped with xenon discharge lamp. The micro fluorimetry cell (model: Q-03401, www.at.fishersci.com) was used for transportation of extracted phase to the analysis instrument. Excitation and emission slits of instrument both were adjusted to 10 nm. Ultrasonic cleaning system with voltage line 230V, frequency of 50-60 HZ, and centrifuge from Hettich (Tuttlingen, Germany, www.hettichlab.com) was used for centrifuging. The pH-meter model 692 (Herisau, Switzerland,) supplied with a glass combined electrode and universal pH indicator (pH 0-14) from Merck (Darmstadt, Germany) were used for pH measurements. A 100 µL syringe was purchased from Hamilton (USA) for injection of organic phase drops in the sample solution and also measuring the volume of sedimented phase. All 10 mL screw cap glass test tubes

with conic bottom (as extraction vessel), were remained in nitric acid (1mol/L) for 24 hours and maintained at 250 °C, for cleaning in order to good sedimentation of organic solvent droplets in the centrifuging step.

Recommended UASEME procedure

Aliquots of 5.00 mL sample solution containing 30 µg/L of oxadiazon was placed in a 10 mL screw cap glass tube with conical bottom. The phosphate buffer was used for adjustment of pH=5 in sample solutions. 20 µL of 1.0 $\times 10^{-2}$ mol/L Tween20 as emulsifier were added into the sample solution (the concentration of Tween20 in sample solution is 4×10^{-5} mol/L), and 50 µL of chlorobenzene (extraction solvent) were added into the sample solution. The tube was immersed into an ultrasonic water bath and dispersion of very fine droplets of chlorobenzene caused high turbidity and cloudy state of aqueous phase. The procedure was performed for 5 minutes at 25°C. For disrupting of this cloudy solution, centrifugation at 5000 rpm for 4 minutes was performed and the organic phase was sedimented at the bottom of the conical tube. The sedimented organic phase (48±0.5µL) was completely removed using a 100 µL Hamilton syringe and transferred to the micro fluorometer cell and 100 µL of ethanol was added prior to spectrofluorimeteric determination.

RESULTS AND DISCUSSION

In this study, the applicability of UASEME with spectrofluorimetery was explored for the extraction and determination of oxadiazon (5-*tert*-butyl-3-(2,4-dichloro-5-isopropoxyphenyl) -1,3,4- oxadiazol -2(3H)-one,) (Fig. 1) in water for the first time. The variables affecting the extraction recovery such as volume and type of extraction solvent and surfactant, ionic strength, pH, time were investigated. All the experiments were performed triplicate and the means of the results were used for optimization. The intensity of fluorescence which is related to the number of moles of extracted analyte was used to evaluate the extraction efficiency. The important point in use of spectrofluorimetry in combination with microextraction process is that the solvent should not have fluorescence signal in the wave length which the analyte shows fluorescence signal. Fig. 2(a) shows the oxadiazon fluorescence spectrum in the wavelength region from 305 to 505 nm and (b) is the spectrum of

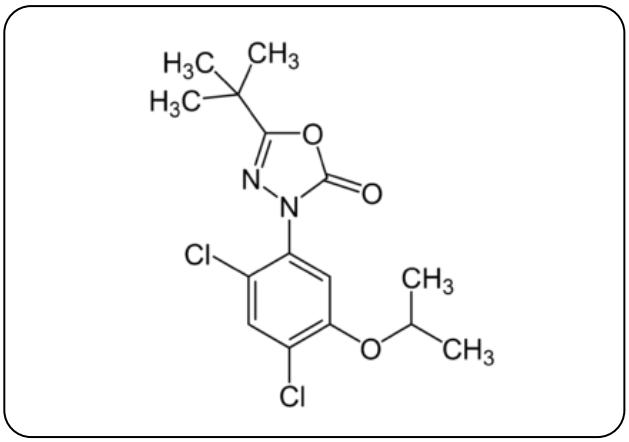


Fig. 1: Chemical structure of the oxadiazon.

the solvent in the same region. So it can be seen that in the selected wavelength region, extraction solvent has no interference with the extracted analyte signal. Therefore, the fluorescence intensity is measured at 348.5±5 nm, with the excitation wavelength at 305±5 nm.

Selection of extraction solvent

Selection of a suitable extraction solvent is critical to achieve an efficient UASEME procedure. The desired characteristics for appropriate extraction solvent are low water solubility, high extraction capacity for the target analyte, and form a stable emulsion system in the presence of surfactant under ultrasound energy. Based on these considerations four solvents, $CHCl_3$, CCl_4, C_2Cl_4, C_6H_5Cl, CH_2Cl_2 were contemplated to be appropriate in this work. Preliminary experiments were performed by using 100 µL of each solvent. The stable emulsion solution was obtained by each of these solvents except CH_2Cl_2 which showed high solubility in water. The results obtained by other extraction solvents in the presence of Tween20 as emulsifier are reported in Fig. 3. As can be seen among the tested solvents C_6H_5Cl acted most effective and had the less interference with analyte profile. So in further experiments C_6H_5Cl was used as extraction solvent.

Effect of extraction solvent volume

Different volumes of C_6H_5Cl ranging from 30-80 µL were examined with the same UASEME procedures. The results are offered in Fig. 4. The results revealed that when the volume of the extraction solvent was increased, the recovery increased until 50 µL then remained

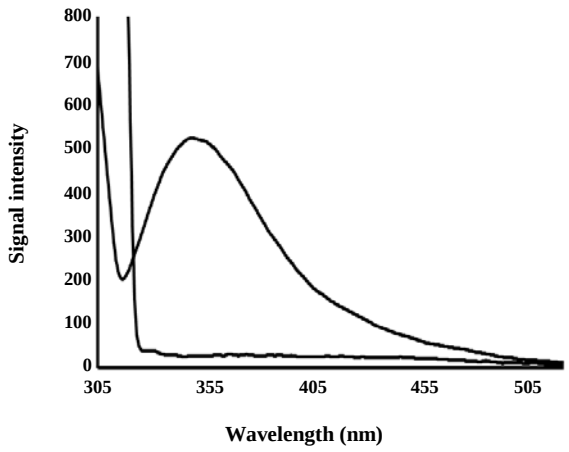


Fig. 2: Fluorescence emission profile of oxadiazon. (a) profile of extraction solvent (chlorobenzene) and Tween 20; (b) profile of extracted analyte in chlorobenzene. Experiment conditions: sample volume: 5mL; extraction solvent volume: 100 µL; extraction time 2 min; no salt addition; concentration of analyte: 30 ng/mL; concentration of Tween 20: 0.06 mmol/L.

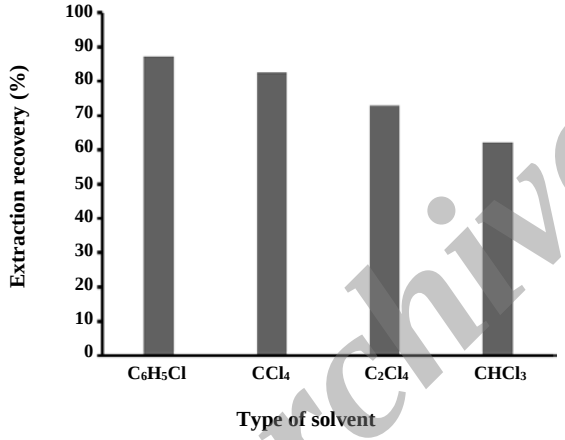


Fig. 3: Extraction solvent type and their extraction efficiency. Experiment conditions: sample volume: 5mL; extraction solvent volume: 100 µL; extraction time 2 min; no salt addition; concentration of analyte: 30 ng/mL; concentration of Tween20: 0.06mmol/L.

constant up to 70 µL. At higher volumes up to 80 and 90 µL noticeable decline appeared which might be because the emulsification phenomenon was not stable in large volume of solvent and on the other hand the presence of chlor in extraction solvent caused the quenching effect in fluorescence signal. So 50 µL was the optimum volume for extraction solvent.

Effect of the type and concentration of surfactant

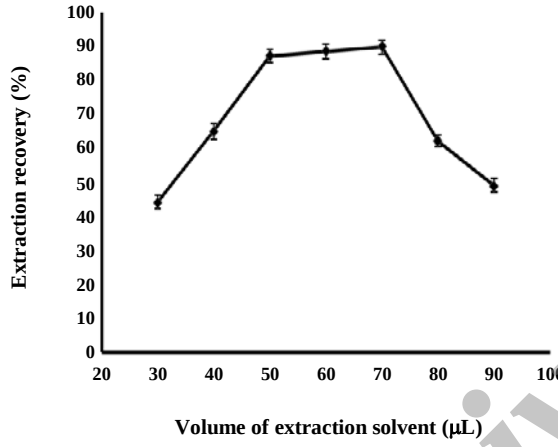
In order to enhance the extraction efficiency surfactant was served as an emulsifier which accelerates the formation of the fine droplets of the water immiscible extraction solvent into the aqueous solution under ultrasound radiation. By this way extraction time decreased and the speed of mass transfer increased impressively. The effect of different surfactants (SDS, Triton X-100, Tween20, Tween80) was investigated. The properties of selected surfactants are reported in Table 1. The effect of different surfactants in extraction process depend on the Hydrophile-Lipophile Balance values (HLBs) the surfactants, hydrophobicity and analyte polarity. When the HLB values of the surfactants range from 8-18 it can be used as emulsifier. The HLB value of these surfactants are 40, 13.4,16.7,15 respectively. Since the HLB value of SDS is out of this range, the extraction efficiency by using this surfactant was not acceptable. Triton X-100 and Tweens which are polyoxyethylene nonionic surfactants with good hydrophobicity, showed better response specially by using Tween20 (Fig. 5). So Tween20 was used as emulsifier in subsequent studies. Surfactant concentration is another important parameter for obtaining satisfactory extraction effect. Different concentrations of Tween20 (0.01-0.08 mmol/L) were examined. The results revealed that by increasing the concentration of surfactant, the extraction efficiency increased. But when the concentration of Tween20 in the sample solution was higher than critical micellar concentration, (CMC, 0.06 mmol/L) the efficiency decreased. One of the most important problems happens by increasing the concentration of surfactant is higher solubility of analyte in water and foam formation which makes separation difficult. So 0.05 mmol/L was used as appropriate concentration of Tween20 in extraction process.

Effect of ionic strength

The influence of the ionic strength on the performance of UASEME was investigated by using various concentrations of NaCl (0-10% W/V) in sample solution. The results (Fig. 6), state that increasing ionic strength had no special impact on extraction efficiency but the volume of deposited phase increased due to the reduction in solubility of organic solvent in water. Overall, salt addition decreases the solubility of analyte and promotes

Table 1: Selected optimum conditions for UASEME-Spectrofluorimetry.

Feature	Value
Microextraction solvent (Chlorobenzene)	80 µL
Sample volume	5 mL
Salt addition	No effect
Sample pH	5
Extraction time (min)	10
Extraction temperature	25±3 °C
Excitation wavelength (nm)	315±5
Emission wavelength (nm)	350±5
Slit width	7



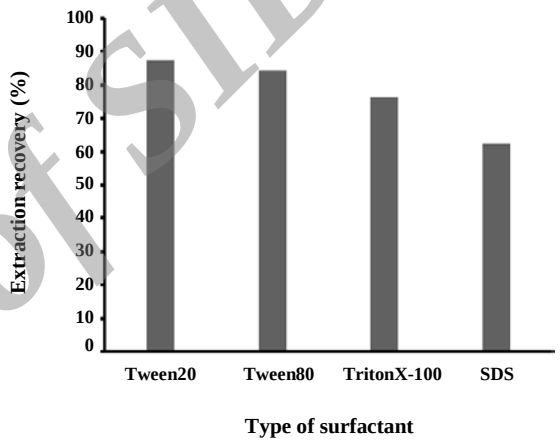


Fig. 4: Correlation between the added extraction phase (chlorobenzen) volume and extraction efficiency. Experiment conditions: sample volume: 5mL; extraction time: 2 min; no salt addition; concentration of analyte: 30 ng/mL; concentration of Tween20: 0.06mmol/L.

Fig. 5: Selection of surfactant. Experiment conditions: sample volume: 5mL; extraction solvent (chlorobenzen) volume: 50 µL; surfactants: Tween20, Tween80, SDS, Triton X-100. Extraction time 2 min; no salt addition; concentration of analyte: 30 ng/mL.

mass transfer and extraction efficiency but on the other hand presence of salt in sample solution increases density and viscosity of the solution which can prevent formation of fine droplets of organic phase. Finally salt addition was not considered in this study.

Effect of sample pH

In order to establishment of an efficient UASEME procedure, pH of aqueous solution should be adjusted. Different initial sample solutions in different pH (2-12), were prepared and the effect of pH was investigated. According to the results (Fig. 7) obtained, there were no differences in response when acidic, neutral or low basic pH values were used which is agreeable as reported before [32]. But in highly alkaline solution the intensity decreased which may be in the result of oxadiazon hydrolysis. As the initial aqueous sample solution the pH was measured 6, no further pH adjustment was performed in subsequent experiments.

Effect of Ultrasound assisted surfactant enhanced emulsification microextraction time

Time in this kind of extraction is described as the time interval between the addition of extraction solvent and

Table 2: Properties of different surfactants.

surfactant	TritonX-100	Tween20	Tween80	SDS
CMC (mmol/L)	0.2-0.9	0.06	0.012	7-10
HLB	13.5	16.7	15	40
Cloud Point (ºC)	65	76	65	>100

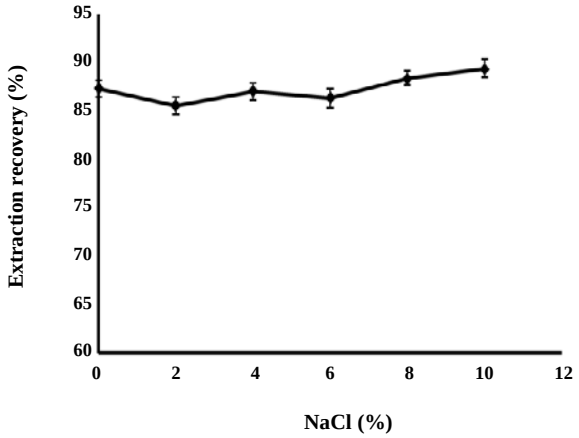


Fig. 6: Ionic strength effect on the extraction efficiency. Experiment conditions: sample volume: 5mL; extraction solvent volume: 50 µL; extraction time 2 min; no salt addition; concentration of analyte: 30 ng/mL; concentration of Tween20; 0.06mmol/L.

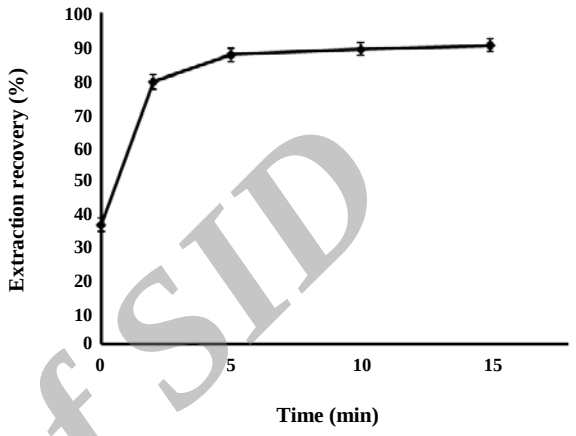


Fig. 7: Effect of pH on the extraction efficiency of oxadiazon. Experiment conditions: sample volume 5mL; extraction solvent (chlorobenzen) volume: 50 µL; extraction time 2 min; no salt addition; concentration of analyte: 30 ng/mL; concentration of Tween20: 0.06mmol/L.

the end of sonication prior to centrifugation onset. Time can affect emulsification and mass transfer process. In the present study extraction time was investigated in the range from 0-15 minutes. Fig. 8 shows the extraction recovery of extracted analyte versus extraction time. As can be seen, the recovery increased up to 2 minutes, and then remained almost constant. Therefore 2 minutes of sonication time was chosen for the further experiments.

Analytical figures of merit

Under optimum conditions which are reported in Table 2, the analytical performance of proposed method was validated. Calibration graphs were constructed using solutions of oxadiazon of known concentrations and the linearity of the method was obtained over the range of 0.1-60 µg/L with determination coefficient of 0.9979. Limit Of Detection (LOD), was calculated as $3S_b/m$ (S_b, standard deviation of the blank signals and m, slope of calibration curve after preconcentration). Precision, expressed as Relative Standard Deviation (RSD), was evaluated as 3.2% in terms of repeatability based on the peak intensity for 5 replicates and preconcentration factor was 104 for proposed method.

Analysis of the real samples

To evaluate the capability of the method, the procedure was performed to the analysis of oxadiazon in ground water and agricultural water. Samples were collected from garden earth and agricultural champ, in north of Iran. The results showed that the analysed samples were free of oxadiazon and for assessing matrix effect, the water samples were spiked with the analyte at different levels and the method was applied as reported. Table 3 shows the results. It is revealed that matrix has no special effect on method efficiency.

Table 3: Relative recoveries obtained in the determination of oxadiazon in water samples by UASEME-Spectrofluorimetry.

Real sample	Spiked (ng/mL)	Found (ng/mL)	RR[a]%	RSD (%), n=3
Agricultual water (I)	0	0		
	5	5.2	104	3.2
	8	8.1	101.2	3.4
Agricultual water (II)	0	0		
	5	4.8	96	2.9
	10	10.2	102	3.2

[a]*RR: Relative Recovery*

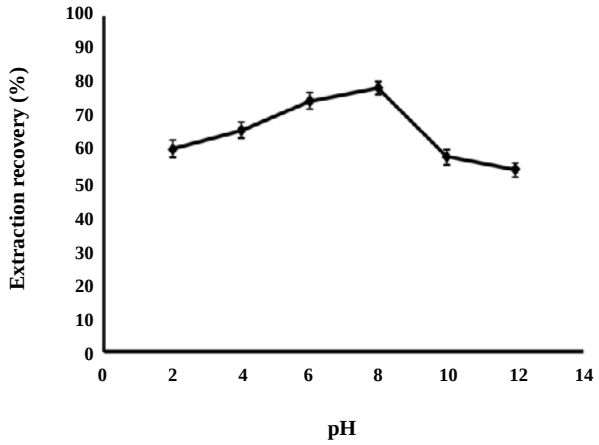


Fig. 8: Effect of time on the extraction efficiency of oxadiazon. Experiment conditions: sample volume 5mL; extraction solvent (chlorobenzen) volume: 50 µL; extraction time 2 min; no salt addition; concentration of analyte: 30 ng/mL; concentration of Tween20; 0.06mmol/L.

CONCLUSIONS

In this paper, UASEME coupled with spectrofluorimetry was developed for determination of oxadiazon in water samples. Here application of nonionic surfactant as emulsifier has shortened the time of analysis. The method provided low detection limit, appropriate repeatability, good extraction recoveries, low organic solvent consuming which is expected in sample preparation techniques. So this inexpensive and environmentally friendly method can be used with acceptable results.

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