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Original Research Article

A rapid and sensitive method for the determination of dibutyl phthalate in wine by flow-injection chemiluminescence analysis

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ABSTRACT

A sensitive method for the determination of picogram level dibutyl phthalate (DBP) in wine by flow-injection chemiluminescence (FI–CL) analysis is presented for the first time, which was based on the quenching effect of DBP on the luminol–myoglobin (Mb) CL system. The decrement of CL intensity was linearly proportional to the logarithm of DBP concentration in the range of 0.1–100 pg mL^{-1} with the detection limit of 0.03 pg mL^{-1} (3σ). At a flow rate of 2.0 mL min^{-1}, a complete determination of DBP including sampling and washing could be accomplished in 0.5 min, giving the maximum sample throughput of 120 h^{-1}. The proposed method was successfully applied to the determination of DBP in wine, human serum and urine samples with the relative standard deviations (RSDs) of less than 3.0% ($n = 5$). The molecule docking results showed that DBP interacted with the amino acid residues near the heme moiety of Mb. The possible CL mechanism of luminol–Mb–DBP reaction should be that the binding of Mb with DBP forming a 1:1 complex (binding constant $K = 1.55 \times 10^4$ L mol^{-1}) led to the conformational change of Mb and resulted in the quenching of CL intensity.

1. Introduction

Dibutyl phthalate (1,2-benzenedicarboxylic acid dibutyl ester, DBP, MW 278) is a phthalate ester as shown in Fig. 1. DBP is extensively used in industrial products such as plastic polyvinyl chloride (PVC) piping, which can increase the flexibility of high molecular polymers (Zhu et al., 2012). DBP can easily migrate from plastic materials to the environment and even to substances in contact with it, such as palm oil in food and drink (The Central News Agency, 2011), plastic wrapped food (Hubinger and Havery, 2006), drinking water (Bach et al., 2012), pharmaceuticals (Gregory and Mark, 2006) and personal care products (e.g., perfumes, lotions and cosmetics) (Koniecki et al., 2011). Research has shown that DBP can interfere with normal hormone levels and influence the most basic regulatory physiological functions of the human body (He et al., 2010; Heng et al., 2012; Park et al., 2012; Song et al., 2012), which may result in tumors, cancers, deformity, mutation and kidney diseases (Cirillo et al., 2011; Struve et al., 2009; Weuve et al., 2010). In the European Union and the United States of America, the use of DBP has been restricted in children's toys and childcare articles (EU directive 2005/84/EC) (Official Journal of the European Union, 2005). In 2012, DBP was detected in JiuGui Wine at the level of 1.04 mg kg^{-1} (Administration of Quality and Technology Supervision of Hunan Province, 2012), which is 2.47 times higher than the allowed standard of 0.3 mg kg^{-1} (Ministry of Health of the People's Republic of China, 2011); this promptly resulted in much attention being paid to public food safety issues. Due to the increasing concern about the health implications and widespread existence of DBP in various matrices, it is of great significance to develop a reliable analytical method for detection and quantification of DBP at very low levels in food, biological and environmental samples.

Many analytical methods have been utilized for the determination of DBP, including gas chromatography–mass spectrometry (GC–MS) (Del Carlo et al., 2008; Jiao et al., 2012; López-Nogueroles et al., 2013; Wu et al., 2012), high performance liquid chromatography–MS (HPLC–MS) (Jen and Liu, 2006; Li et al., 2011; Wang et al., 2011), fluorescence (FL) (Zhang et al., 2006), enzyme-linked immunoassay (ELISA) (Wei et al., 2011), and chemiluminescence (CL) (Qiu et al., 2013). CL coupled with flow-injection analysis (FIA) has attracted increasing attention in various fields owing to its high sensitivity, low limit of detection (LOD), wide linear dynamic range, rapid measurements, less reagent consumption as well as simple instrumentation (Wang et al., 2009; Llamas et al., 2011; Zhang et al., 2011).

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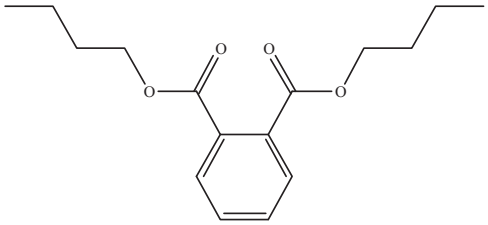

Fig. 1. Chemical structure of DBP.

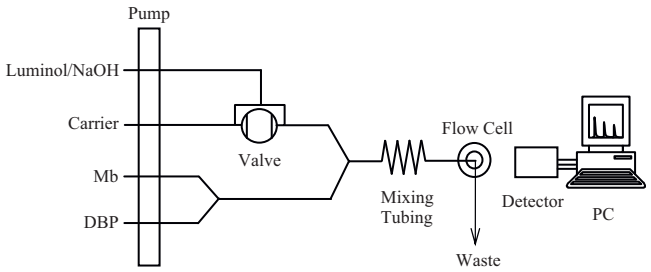

Fig. 2. Schematic diagram of the FI–CL system for the determination of DBP. Luminol: 2.5×10^{-5} mol L^{-1}; Mb: 5.0×10^{-8} mol L^{-1}; NaOH: 0.025 mol L^{-1}; flow rate: 2.0 mL min^{-1}; and high voltage: −700 V.

It has been reported that myoglobin (Mb) could accelerate the electron transfer rate of excited 3-aminophthalate leading to enhancement of luminol CL intensity (Song et al., 2004). In this paper, it was found that DBP could quench the CL intensity of the luminol–Mb system, and the decrement of CL intensity was linearly proportional to the logarithm of DBP concentration in the range of 0.1–100 pg mL^{-1} with the detection limit of 0.03 pg mL^{-1}. The linear equation was $\Delta I = 4.18 \ln C_{DBP} + 16.70$, $R = 0.9988$, with the relative standard deviations (RSDs) of less than 3.5% ($n = 7$). The simple and rapid method was successfully applied to the determination of DBP in wine, human serum and urine. The binding constant ($K = 1.55 \times 10^4$ L mol^{-1}) and the number of binding sites ($n = 0.86$) of Mb with DBP were obtained by the FI–CL model (Wang and Song, 2010), which indicated that a 1:1 Mb–DBP complex was formed online. The molecule docking results showed the binding mode and specific binding sites of DBP to Mb revealing that DBP interacted with the amino acid residues near the heme moiety of Mb.

2. Materials and methods

2.1. Apparatus

A peristaltic pump of the IFAS-E Luminescence Analyzer (Xi'an Remax Analysis Instrument Co. Ltd., Xi'an, China) was used to deliver all streams. PTFE tubing (1.0 mm i.d.) was used throughout the manifold for carrying the CL reagents. A six-way valve with a loop of 100 μL was used for sampling. The flow cell was made by coiling 15 cm of colorless glass tube (1.0 mm i.d.) into a spiral-shape disk (2.0 mm i.d.) and placed close to the photomultiplier tube (PMT). The CL signal produced in the flow cell was detected without wavelength discrimination, and the PMT output was recorded by PC with an IFAS-A client system (Remax, Xi'an, China). A UV-vis spectrophotometer (Shimadzu, UV-2500PC, Japan) was used.

2.2. Reagents

All chemicals used were of analytical reagent grade. Water purified in a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout. Standard solution of DBP was supplied by the Sinopharm Chemical Reagent Co. Ltd, Xi'an, China. The DBP (Sinopharm Chemical Reagent Co. Ltd, Xi'an, China) stock solution (1.0 mg mL^{-1}) was prepared by ethanol. Luminol (2.5×10^{-2} mol L^{-1}) was used as supplied to prepare stock standard solution by dissolving 0.44 g luminol (Fluka, Biochemika, Switzerland) in 0.1 mol L^{-1} NaOH solution in a 100 mL calibrated flask; Horse heart Mb (Sigma–Aldrich, St. Louis, MO, USA) was purchased from local market and used as received without further purification. Mb stock solution (1.0×10^{-5} mol L^{-1}) was prepared in a 25 mL calibrated flask.

2.3. Procedures

The schematic profile of equipment in the flow system was described in Fig. 2. The flow system consisted of four lines of luminol/NaOH, carrier solution, Mb, and DBP at a flow rate of 2.0 mL min^{-1}. Purified water was first employed to wash the entire flow system until a stable baseline had been recorded. Then luminol standard solution (100 μL) was injected into the flow line by the six-way valve, thereafter, merged with the premixed homogeneous stream of Mb and DBP. The entire mixed solution was then delivered to the CL cell in an alkaline medium, with the produced CL signal detected by the PMT at a high voltage of −700 V. The concentration of DBP could be quantified by the accompanied decrement of CL intensity, $\Delta I = I_0 - I_s$, where I_s and I_0 were the CL signal in the presence and in the absence of DBP solution, respectively.

2.4. Sample preparation

2.4.1. Wine samples preparation

The samples of XiFeng Wine (batch 20091214401FJ001, 46° and 20111026601HV46, 50°) were purchased from local market. According to the literature (Standard of the People's Republic of China, 2008, GB/T 21911−2008), the wine sample (5.0 mL) was measured exactly and placed into test tube, and then put into boiling water bath to evaporate the alcohol. The obtained solution was stood for cooling and was added to *n*-hexane by shaking. Then the upper organic phases was extracted and evaporated just to dryness in a gentle water bath, and the residue was dissolved with 5.0 mL ethanol. Suitable aliquot samples from this solution were taken for determination.

2.4.2. Treatment of human serum and urine samples

The serum samples supplied by the Hospital of Northwest University and the urine samples collected from three volunteers were spiked before determination. To prepare the spiked samples, known quantities of standard solution of DBP were spiked into 1.0 mL of serum and 5.0 mL of urine. After homogenization and dilution with the factor of suitable fold for serum and urine samples, the samples were processed according to the proposed procedure.

3. Results and discussion

3.1. Effects of luminol, NaOH, and Mb concentrations

The effects of luminol and NaOH concentrations on the CL intensity were investigated over the ranges of 1.0×10^{-7}–1.0×10^{-4} mol L^{-1} and 1.0×10^{-2}–0.5 mol L^{-1}, respectively. The optimal CL intensity could be obtained when using a concentration of 2.5×10^{-5} mol L^{-1} luminol. With regard to the concentration of NaOH, 2.5×10^{-2} mol L^{-1} NaOH gave the maximum CL intensity.

The effect of the Mb concentration was tested over the range from 5.0×10^{-10}–5.0×10^{-7} mol L^{-1}. The CL intensity increased when the Mb concentration was increased up to 5.0×10^{-8} mol L^{-1}, but decreased at a higher concentration.

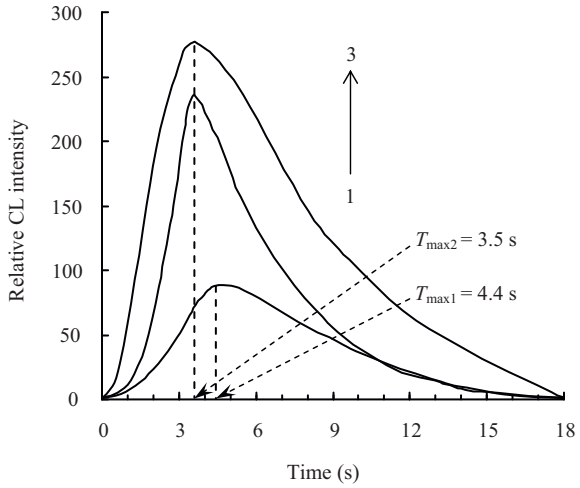

Fig. 3. CL intensity–time profile of different systems. Curve 1: 2.5×10^{-5} mol L^{-1} luminol; curves 2: luminol–Mb with 5.0 pg mL^{-1} DBP system; curve 3: 2.5×10^{-5} mol L^{-1} luminol with 5.0×10^{-8} mol L^{-1} Mb.

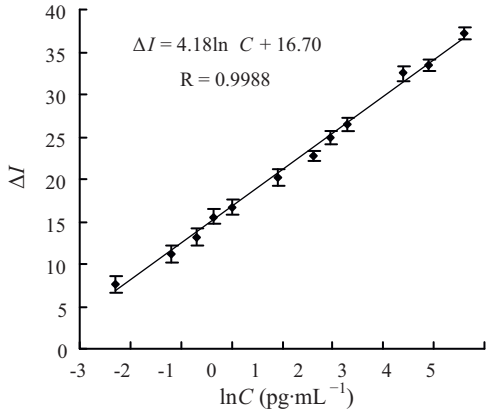

Fig. 4. Plot of ΔI vs. lnC. Linear range of DBP concentration: 0.1–100 pg mL^{-1}.

Therefore, 5.0×10^{-8} mol L^{-1} Mb was used in all subsequent experiments.

3.2. Effect of flow rate and the length of mixing tubing

The effect of the mixing tube length on CL intensity was tested from 5.0 to 20.0 cm. It could be observed that the CL intensity was much stronger using 15.0 cm mixing tube than that of other mixing tube in the presence of 1.0 pg mL^{-1} DBP. Thus, 15.0 cm mixing tube was selected. The influence of flow rate on determination was examined by investigating the signal-to-noise ratio (S/N) under different flow rate, and the flow rate of 2.0 mL min^{-1} offering highest S/N ratio was then chosen as suitable condition considering analytical precision.

3.3. The relative CL intensity–time profile

The relative CL intensity–time profile was shown in Fig. 3. It can be seen that the maximum CL intensity (I_{max}) of luminol (curve 1) was 83 at the time (T_{max}) of 4.4 s; I_{max} of luminol–Mb system (curve 3) was increased from 83 to 272, and T_{max} was shortened from 4.4 to 3.5 s compared with luminol system; while in the presence of DBP (5.0 pg mL^{-1}), the I_{max} of luminol–Mb–DBP system (curve 2) was decreased from 272 to 244 with the same T_{max} at 3.5 s as luminol–Mb system.

3.4. Operational stability and analytical performance of the FI–CL system for DBP determination

The operational stability of the FI–CL system was tested by injecting 100 μL luminol solution into the flow system which further merged with the mixed solution of DBP (5.0 and 50 pg mL^{-1}, respectively) and Mb. Then, the relative CL intensity ($\Delta I = I_0 - I$) was recorded to test the stability of the system. The experiment lasted for 5 days with the FI–CL system regularly used over 8 h per day, and the stability and precisions of FI–CL system for DBP determination was shown in Table 1. It was found that ΔI kept stable under the fluctuation of I_0 and RSDs were less than 3.0%, which showed that the system exerted very good stability.

Under the optimum conditions described, the decreased CL intensity was proportional to the logarithm of DBP concentration over the range from 0.1 to 100 pg mL^{-1}, and the LOD was 0.03 pg mL^{-1} (3σ). The linear equation was $\Delta I = 4.18 \ln C_{DBP} + 16.70$, $R = 0.9988$ ($n = 7$). The calibration graph of DBP determination was shown in Fig. 4.

3.5. Interference studies

Considering the effect of the matrix for the determination of DBP in wine, human urine and serum, the interference of foreign substances was tested. Presuming interference at 5% level, tolerable concentrations of interference with respect to 10 pg mL^{-1} DBP were less than 50 μg mL^{-1} ethyl acetate, ethyl lactate; 10 μg mL^{-1} isobutyl alcohol, glucose, lactose, borate, lactic acid, malic acid, fructose, Ac^- and SO_4^{2-}; 5.0 μg mL^{-1} ethanol, methanol, isoamyl alcohol, ethyl hexanoate, tartrate, sucrose, citrate acid, salicylic acid, acetaldehyde, lactic acid, heptanoic acid, hexanoic acid, Ca^{2+}, Ba^{2+} and Mg^{2+}; 500 ng mL^{-1} dioctylphthalate; 300 ng mL^{-1} dimethyl phthalate and di-isononyl phthalate; 50 ng mL^{-1} ascorbic acid and uric acid; 10 ng mL^{-1} Cu^{2+}, Zn^{2+} and Fe^{3+}. Compounds abundant in human urine and serum such as salt, lipid and proteins caused no obvious interference for the determination of DBP.

4. Application

4.1. Determination of DBP in wine sample

The samples of XiFeng wine were purchased from the local market and prepared as described under Sample preparation. The contents of DBP in the samples were quantified according to the standard addition method, and the results are listed in Table 2. The

Table 1
The stability and precisions of FI–CL system for DBP determination[a].

Time (day)	I_{CL} (blank)	RSD (%)	I_{CL}(5.0 pg mL^{-1})	RSD (%)	I_{CL}(50 pg mL^{-1})	RSD (%)
1st	277	2.0	254	2.6	243	2.5
2nd	272	1.9	250	1.8	242	2.4
3rd	274	1.7	251	2.2	248	2.6
4th	276	1.9	252	2.0	245	2.3
5th	275	1.6	255	2.7	247	2.7

[a] Each result is the average of 7 separate determinations.

Table 2
Results of determination of DBP in wine[a].

Sample No.	Added (pg mL^{-1})	Found (pg mL^{-1})	RSD (%)	Recovery (%)	Content (μg mL^{-1})	
					Proposed method	GC–MS
1-1	–	9.6	2.0	103.3	0.10 ± 0.01	0.10 ± 0.05
	3.0	12.7	1.9			
1-2	–	9.9	2.1	96.0	0.09 ± 0.01	
	5.0	14.7	1.5			
1-3	–	9.9	1.1	99.0	0.09 ± 0.01	
	10.0	19.8	0.7			
1-4	–	9.6	1.3	101.0	0.09 ± 0.01	
	30.0	39.9	1.1			
1-5	–	10.8	1.4	99.4	0.10 ± 0.01	
	50.0	60.5	1.0			
2-1	–	10.7	2.9	103.3	0.22 ± 0.01	0.21 ± 0.06
	3.0	13.8	2.5			
2-2	–	10.5	0.9	101.3	0.21 ± 0.02	
	5.0	15.5	0.8			
2-3	–	9.8	1.4	101.0	0.20 ± 0.02	
	10.0	19.9	1.1			
2-4	–	10.1	1.5	100.8	0.21 ± 0.01	
	30.0	41.1	1.3			
2-5	–	10.0	1.0	102.1	0.20 ± 0.02	
	50.0	61.0	0.7			

[a] The average of 5 determinations.

Table 3
Results of determination of DBP in human serum and urine[a].

Sample No.[b]	Added (pg mL^{-1})	Found (pg mL^{-1})	RSD (%)	Recovery (%)	Content (ng mL^{-1})	
					Proposed method	Spiked
1-1	–	0.5	1.4	101.1	5.1	5.0
	0.3	0.8	0.6			
1-2	–	0.5	2.9	98.0	4.9	5.0
	0.5	1.0	2.6			
1-3	–	0.5	2.8	98.6	4.9	5.0
	0.7	1.2	1.2			
2-1	–	30.1	2.3	101.0	30.1	30.0
	10.0	40.2	2.1			
2-2	–	30.2	1.8	99.3	29.9	30.0
	30.0	60.0	1.1			
2-3	–	29.6	1.4	101.2	29.7	30.0
	50.0	80.2	1.1			

[a] The average of 5 determinations.
[b] No. 1-1 to 1-3 human serum, No. 2-1 to 2-3 human urine.

recoveries ranged from 96.0 to 103.3%, with RSDs of <3.0%. The same samples were also determined by GC–MS (Agilent, GC/MS-5975C, USA), which were performed by Xi'an Products Quality Supervision and Inspection Institute (Standard of the People's Republic of China, 2008, GB/T 21911–2008). It was found that the results by proposed CL method were well agreed with the GC–MS.

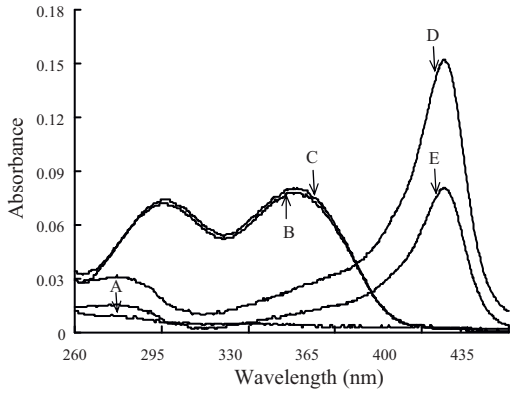

Fig. 5. UV absorption spectra profile of different reaction types. (A) DBP; (B) luminol; (C) luminol–DBP; (D) Mb; and (E) Mb–DBP. The concentrations of DBP, luminol and Mb were 1.0×10^{-6} mol L^{-1}, 1.0×10^{-5} mol L^{-1} and 1.0×10^{-6} mol L^{-1}, respectively.

4.2. Determination of DBP in spiked human serum and urine samples

The determination results of DBP in spiked human serum and urine samples are given in Table 3. The method was verified by determination of recoveries which changed from 98.0 to 101.1% for human serum, and from 99.3 to 101.2% for urine. It was obvious that the proposed method was very sensitive for the determination of DBP in biological fluids.

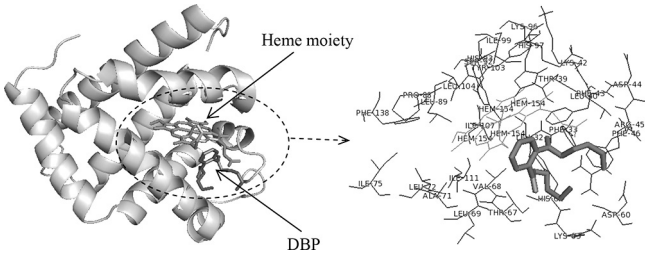

Fig. 6. The binding mode and specific binding site of DBP to Mb. The results were obtained by AutoDock 4.2 (The Scripps Research Institute).

Table 4
Compared different methods for determination of DBP.

Methods	Sample	Liner range ($\mu g\,mL^{-1}$)	LOD ($\mu g\,mL^{-1}$)	RSD (%)	Refs
GC–MS	Wine	1.8×10^{-2}–5.0	1.8×10^{-2}	16.0	Del Carlo et al. (2008)
	Water	5.0×10^{-2}–10	1.0×10^{-2}	10.0	Jiao et al. (2012)
	Perfume	0.2–1.0	5.4×10^{-2}	6.0	López-Nogueroles et al. (2013)
HPLC–MS	Food contacted plastic	1.0×10^{-2}–0.75	4.0×10^{-4}	4.0	Jen and Liu (2006)
	Milk	5.0×10^{-2}–40	2.5×10^{-2}	8.5	Li et al. (2011)
FL	Water	0.1–30	2.0×10^{-2}	10.0	Zhang et al. (2006)
CL sensor	Drink sample	1.1×10^{-2}–5.8	5.8×10^{-4}	4.0	Qiu et al. (2013)
The proposed CL	Wine	1.0×10^{-7}–1.0×10^{-4}	1.0×10^{-8}	3.0	This work

4.3. Possible CL mechanism

The possible reaction mechanism of Mb–DBP was investigated using the UV–vis, CL and molecule docking methods. As shown in Fig. 5, it was found that $A_{max,\ 409\ nm}$ of Mb was decreased from 0.1520 to 0.0810 in the presence of DBP, which suggested that Mb should interact with DBP. By the FI–CL model for protein–small molecule interaction (Wang and Song, 2010), the binding constant ($K = 1.55\times10^4\ L\ mol^{-1}$) and the number of binding sites ($n = 0.86$) of Mb with DBP were obtained, which indicated that a 1:1 Mb–DBP complex was formed online. The molecule docking results in Fig. 6 shows the binding mode and specific binding sites of DBP to Mb revealing that DBP could interact with the amino acid residues near the heme moiety of Mb.

The possible reaction mechanism of luminol–Mb–DBP should be explained as follows: (1) Mb could accelerate the electrons transferring rate of excited 3-aminophthalate, which led to the CL enhancement of luminol (Song et al., 2004); (2) DBP might interact with Mb forming a 1:1 Mb–DBP complex online, which caused the conformational change of Mb and resulted in the quenching of CL intensity from the luminol–Mb system.

Comparison with reported methods for the determination of DBP is summarized in Table 4.

5. Conclusion

A sensitive method for the determination of picogram level DBP in wine was developed using FI–CL. It is clear that the proposed CL method offers a wide linear calibration range, high sensitivity and reduced reagent consumption for the determination of DBP. Since no background signal or stray light effect exists in CL detection, and it is probable that emission spectral bands rely in the dispersion line at the low concentration, the proposed CL method exhibits relatively low LOD for the determination of picogram level DBP. Satisfactory performance of the quantification of DBP in wine and biological fluids has demonstrated that the method is practical and suitable not only for quality control analysis but also for the analysis of complex biological samples.

Acknowledgments

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