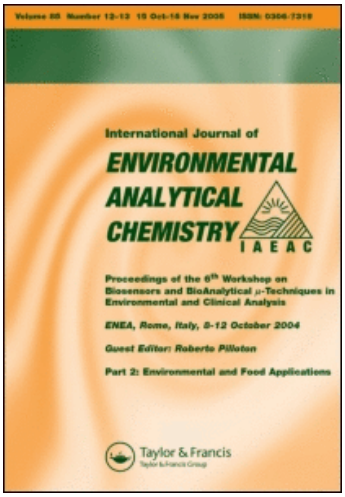

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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Online publication date: 17 September 2010

To cite this Article Topuz, Sibel and Alpertunga, Buket(2003) 'Determination of Triazines and *N*-Methylcarbamate Pesticides in Water by High-Performance Liquid Chromatography-Diode Array Detection', International Journal of Environmental Analytical Chemistry, 83: 9, 787 — 795
To link to this Article: DOI: 10.1080/0306731031000104731
URL: http://dx.doi.org/10.1080/0306731031000104731

Intern. J. Environ. Anal. Chem.
Vol. 83, No. 9, pp. 787–795, September 2003

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DETERMINATION OF TRIAZINES AND *N*-METHYLCARBAMATE PESTICIDES IN WATER BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-DIODE ARRAY DETECTION

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(Received 16 August 2002; in final form 14 February 2003)

A rapid and selective method for the simultaneous determination of triazine herbicides (atrazine, its degradation product desethylatrazine, simazine, prometryn, terbutryn) and *N*-methylcarbamate insecticides (propoxur, carbaryl and methiocarb) in surface water has been developed. A 0.5 L of the water sample was preconcentrated by passage through a 1 g C_{18} solid-phase extraction cartridge. The retained compounds were eluted with 5 mL of methanol from the cartridge. The pesticides were separated and quantified by reversed-phase high-performance liquid chromatography with UV diode-array detection. Analytical separation was performed using a concave gradient elution with acetonitrile and water on a C_{18} column. Prometryn and terbutryn were determined at 240 nm; propoxur, methiocarb at 204 nm and the others at 220 nm. Recoveries varied from 85 to 102% over concentrations at 0.025 and 0.2 $\mu g\,L^{-1}$. The limits of detection for the compounds investigated are in the range of 0.005–0.012 $\mu g\,L^{-1}$.

Keywords: High-performance liquid chromatography; Diode-array detection; Water analysis; Pesticides; *N*-methylcarbamates; Triazines

INTRODUCTION

Pesticide contamination has become a world environmental concern because many pesticides have been used extensively for a number of years, and some of them and their degradation products are now found in surface and ground waters. Triazine herbicides comprise an important class of herbicides used for pre- and post-emergence weed control. They and their degradation products are very toxic and highly resistant and survive many years in the soil and water [1,2]. Atrazine has been classified as a possible human carcinogen and has been banned in certain countries [3]. *N*-methylcarbamate (NMC) insecticides are another important class of pesticides. Since their introduction in the 1950s they have been used worldwide on a large number of crops. The analysis of water samples for NMCs has attracted increase attention in recent years because,

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ISSN 0306-7319 print: ISSN 1029-0397 online © 2003 Taylor & Francis Ltd
DOI: 10.1080/0306731031000104731

even though the half-lives of most of the carbamates in natural waters are not very long, their residues are persistent enough to be found in the water environment [4–6]. To prevent water pollution by these pesticides, precise information on their concentration levels is necessary.

The Council of the European Community (EC) limit the concentration of individual pesticides and toxic transformation products in drinking water to $0.1\,\mu g\,L^{-1}$ and the total concentration to $0.5\,\mu g\,L^{-1}$; in surface water, these limits are $1–3\,\mu g\,L^{-1}$ [7]. These rigorous standards for water quality require the availability of suitable analytical methods with high sensitivity and selectivity.

High-performance liquid chromatography (HPLC) [5,8–25] and gas chromatography (GC) [5,18,20,22,26–29] are the most used techniques for monitoring of pesticides in water. HPLC is favored over GC for acidic pesticides, with medium and high polarities, low volatilities and thermal instabilities. The use of diode-array detection (DAD) in HPLC presents multiwavelength detection and spectral comparison [8,15,21,30–35]. Obviously, this technique is much easier to handle than HPLC coupled to mass spectrometry and can be economically used in analyzing samples in routine methods.

Solid-phase extraction (SPE) [36–41] is very attractive choice for the trace enrichment of samples prior to instrumental analysis owing to its many advantages over conventional liquid–liquid extractions (LLE), such as decreased use of hazardous solvents, extractions that are not hindered by the formation of emulsions, high extraction efficiency, variety of adsorbents and convenience in automation [42–46]. The most popular SPE adsorbent for pesticides in water is octadecyl (C_{18}) bonded porous silica [3,26,47–50].

In this study, a rapid and selective HPLC method for the simultaneous determination of triazine herbicides atrazine, its degradation product desethylatrazine (DEA), simazine, prometryn, terbutryn and NMC insecticides propoxur, carbaryl and methiocarb in surface water has been developed. DAD was used, in order to ensure the selectivity of the method, together with C_{18} SPE. The analytical method was applied to the monitoring of the seven pesticides and a degradation product in surface water samples.

EXPERIMENTAL

Chemicals and Materials

All pesticide standards were of 98–99% purity. Simazine, atrazine and prometryn were purchased from Labor Dr. Ehrenstorfer (Augsburg, Germany); DEA, propoxur, carbaryl, methiocarb and terbutryn were purchased from Riedel-de Haën (Seelze, Germany). HPLC grade methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). Ultra-pure water was prepared by ultra-filtration with a Milli-Q water purification system from Millipore (Bedford, MA, USA).

C_{18} SPE cartridges (Discovery DSC 18–1 g/6 mL; Supelco, USA) were used in the preconcentration step. Mobile phase and sample filtration were obtained using a 0.2 μm membrane filter (Phenomenex, CA, USA).

Solutions

Stock standard solutions of $500\,\mu g\,mL^{-1}$ of each compound were prepared in methanol. Working standard solutions of all pesticides, at concentrations range of

0.05–0.4 ng μL^{-1}, were obtained by dilution with acetonitrile–water (30 : 70, v/v). Standard solutions were stored at 4°C.

Apparatus

Liquid chromatographic analyses were performed with a Thermo Separation Products liquid chromatograph (Model Spectra System®, TSP, CA, USA), equipped with HPLC pump (Spectra Series pump P4000), vacuum degasser for liquid chromatography (solvent degasser SCM 1000), rheodyne injection valve (injection volume: 50 µL), photodiode-array detector (UV 6000LP). System parameters were controlled with system controller (SN 4000) and chromatographic data were collected and recorded using the PC 1000 system software. The separation was carried out using a C_{18}, 5 µm Luna column (4.6 × 250 mm, Phenomenex, CA, USA) fitted with guard column (4 mm L × 3 mm ID, Phenomenex, CA, USA) packed with same material.

Chromatographic Conditions

The chromatographic separation was carried out using a concave gradient profile of acetonitrile and water, going from 30% of acetonitrile to 70% in 45 min this condition was held for 5 min and then back to the initial conditions in 10 min. The flow rate of the mobile phase was 1 mL min^{-1} and column temperature was ambient.

For multiwavelength monitoring, the DAD was set at 204, 220 and 240 nm with a bandwidth of 4 nm. Absorbance spectra were recorded in the 200–360 nm range.

Extraction Procedure

Water samples (500 mL) were pumped through C_{18} cartridges conditioned with 10 mL methanol and 10 mL Milli-Q water at 8–10 mL min^{-1} flow rate. After adsorption of the pesticides, the cartridges were washed 5 mL Milli-Q water, dried for 15 min under vacuum and desorption was carried out with 5 mL methanol. The eluent was evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was dissolved in 0.25 mL of mobile phase for HPLC injection.

Water Samples

The proposed analytical scheme was used for the analysis of 40 surface water samples collected from the streams and lakes that nourish the six water reservoirs of the city of Istanbul. On the European side, the Terkos Lake, the Büyükcekmece Lake, the Sazlýdere Dam and the Alibeyköy Dam; on the Anatolian side, the Ömerli-Darlık Dam and Elmalı Dam. Samples were collected in 1 L glass bottles. They were brought to the laboratory the same day of sampling and were stored at 4°C in the dark until SPE, which was carried out in four days or less after sampling. All samples were filtered through 0.2 µm membrane filters before the preconcentration step, to eliminate particulate matter.

RESULTS AND DISCUSSION

In order to find the optimum conditions for the separation of the target pesticides, a concave gradient elution was chosen. This afforded good resolution in a reasonable

time. Acetonitrile was preferred to methanol as organic modifier due to its lower operating pressure and UV absorbance in the range work. The use of DAD enables confirmation of the results through spectral comparison and also offers the possibility of avoiding matrix interferences by choosing different wavelengths. The pesticides studied are highly absorbing substances in the UV region of the spectrum, with absorption maximum at 204 nm for propoxur and methiocarb, 220 nm DEA, simazine, atrazine and carbaryl. Although at 220 nm prometryn and terbutryn have also maximum absorbance values, their determination at 240 nm is more accurate because the background of matrix is higher at 220 nm than at 240 nm.

SPE with C_{18} cartridges was used in order to achieve suitable sensitivity. Since the analytes had different polarities the optimum elution conditions for maximum recoveries of the pesticides were investigated. The first parameter studied was the extraction solvent for the elution of the pesticides. Different volumes of acetonitrile and methanol or mixtures of these solvents were used. In each case, the cartridge was conditioned with the solvent or mixture used for the elution. The recovery values obtained are shown in Table I. The best recoveries were obtained with 5 mL of methanol. In the case of acetonitrile, the recoveries were lower than 50% for prometryn and terbutryn, although a good baseline and less interfering peaks were obtained. Two different sample volumes (500 and 1000 mL) preconcentrated through the cartridge to test differences in recoveries (Table II). A 500 mL volume was chosen as the optimum volume.

The procedure is illustrated in Figs 1 and 2, which show the chromatograms of a Milli-Q and a surface water sample blank and spiked with pesticides at the 0.1 $\mu g\,L^{-1}$ level, respectively. The performance of the method was tested for Milli-Q and surface

TABLE I Efficiency of eluent on the recoveries (%) of the pesticides (0.1 $\mu g\,L^{-1}$)

Compound	*Eluent*			
	Acetonitrile 10 mL	*Methanol* 10 mL	*Methanol* 5 mL	*Acetonitrile–methanol* (1 : 1) 5 mL
DEA	75.2	95.3	96.6	101.2
Simazine	90.8	81.1	98.1	94.5
Propoxur	81.2	92.4	97.2	89.6
Atrazine	94.3	82.7	95.1	97.1
Carbaryl	96.1	90.7	94.6	94.4
Methiocarb	93.8	83.4	98.6	85.1
Prometryn	42.6	98.9	96.2	56.4
Terbutryn	47.4	91.5	97.7	53.6

TABLE II Recoveries (%) obtained after SPE with C_{18} cartridges of 500 and 1000 mL of Milli-Q water spiked with 0.1 $\mu g\,L^{-1}$ of each pesticide

Compound	*Sample volume*	
	500 mL	1000 mL
DEA	97.2	81.6
Simazine	96.6	76.6
Propoxur	95.3	72.6
Atrazine	96.2	80.1
Carbaryl	96.2	78.8
Methiocarb	99.4	89.2
Prometryn	96.8	91.4
Terbutryn	98.2	90.6

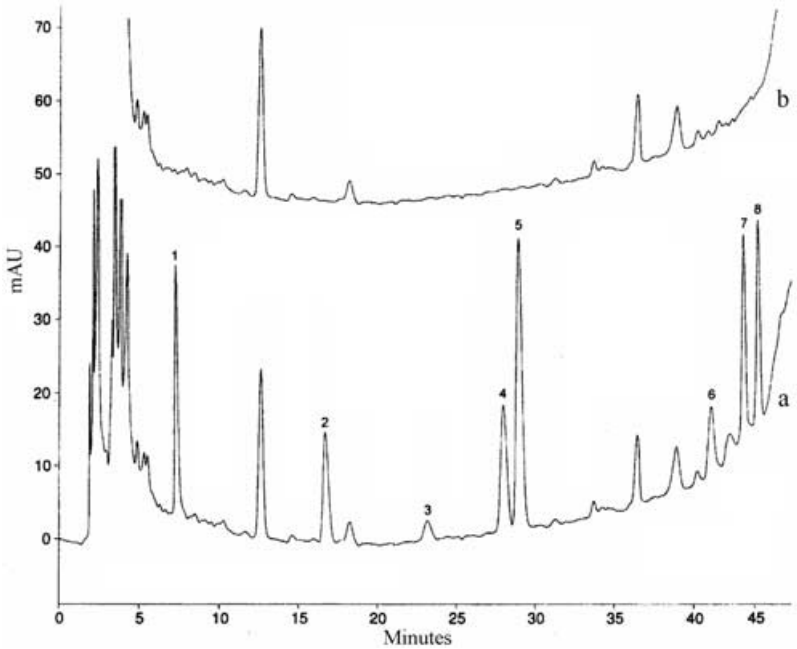

FIGURE 1 LC-DAD chromatograms of (a) a Milli-Q water sample spiked at 0.1 μg L^{-1} with pesticides registered at 220 nm. Peaks: 1 = DEA; 2 = simazine; 3 = propoxur; 4 = atrazine; 5 = carbaryl; 6 = methiocarb; 7 = prometryn; 8 = terbutryn; (b) a Milli-Q water sample blank.

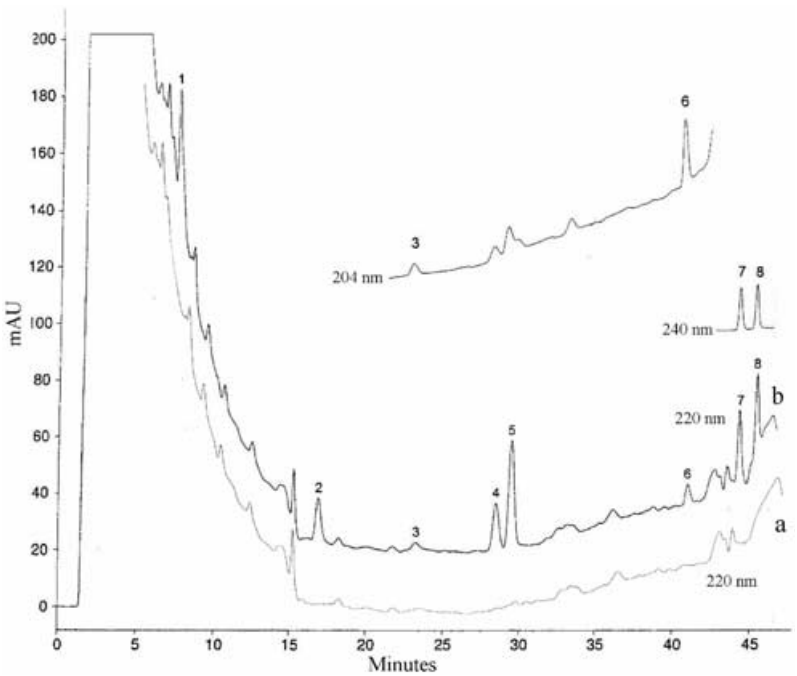

FIGURE 2 LC-DAD chromatograms of (a) a surface water sample blank; (b) a surface water sample spiked at 0.1 μg L^{-1} with pesticides registered at 204, 220, 240 nm.

water samples. As no peaks corresponding to any pesticide studied were present, this water was used as the blank for the recovery studies. Recoveries obtained for Milli-Q and a surface water sample spiked at two pesticide levels are shown in Table III. As can be seen, similar recoveries were obtained either using Milli-Q and surface water samples at 0.025 and 0.2 $\mu g\,L^{-1}$ levels of spiking. Only DEA has a higher error, due to a coeluting peak from the matrix at that interval (Fig. 2). Therefore background subtraction (as shown in Fig. 3) would prove to be valuable asset in the determination of DEA in surface water sample [34,51]. After background subtraction, the mean recovery and RSD of DEA were 93 and 5%, respectively. Linearity was observed for all of the pesticides studied in the range of 0.025–0.2 $\mu g\,L^{-1}$ in Milli-Q water and the correlation coefficients were usually higher than 0.9987. The linearity

TABLE III Mean recoveries (%) and relative standard deviations (RSD) of pesticides in Milli-Q and surface waters ($n = 4$)

Compound	*Recovery*[a] *%* (RSD)			
	Milli-Q water		*Surface water*	
	0.025 $\mu g\,L^{-1}$	0.2 $\mu g\,L^{-1}$	0.025 $\mu g\,L^{-1}$	0.2 $\mu g\,L^{-1}$
DEA	99 (2)	99 (2)	122 (12)[b]	102 (4)
Simazine	96 (2)	100 (1)	87 (3)	101 (6)
Propoxur	95 (1)	98 (1)	85 (5)	97 (5)
Atrazine	99 (3)	102 (6)	91(4)	93 (6)
Carbaryl	100 (4)	95 (1)	85 (4)	89 (7)
Methiocarb	101 (4)	99 (2)	93 (5)	96 (7)
Prometryn	100 (5)	98 (3)	88 (6)	95 (6)
Terbutryn	102 (6)	99 (2)	91 (4)	86 (3)

[a]Without any background subtraction; [b]High recovery values due to interferences in the chromatograms (see Fig. 2).

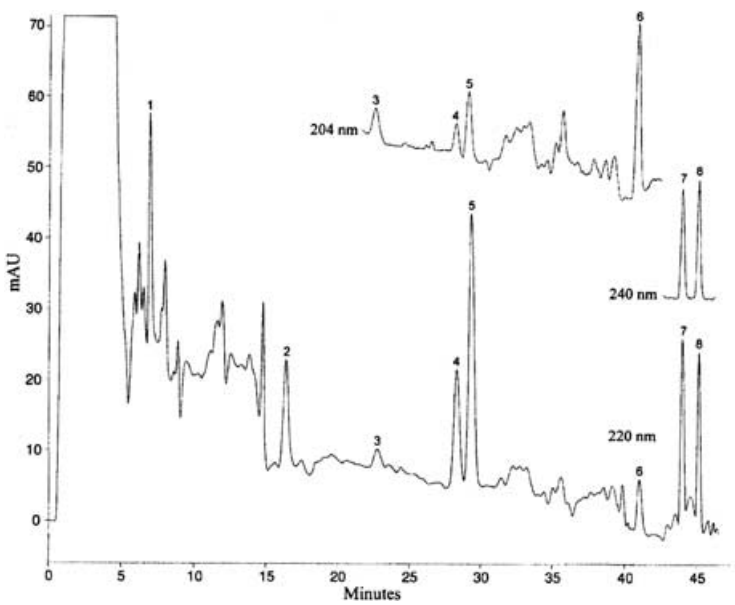

FIGURE 3 LC-DAD chromatograms of a surface water sample spiked at 0.1 $\mu g\,L^{-1}$ with pesticides after subtracting surface water sample blank as a background.

values were also checked for surface water and the corresponding results were similar as reported for Milli-Q water. The limits of detection (LODs) for the pesticides investigated are in the range of 0.005–0.012 $\mu g/L^{-1}$ for Milli-Q water (Table IV) and similar values were obtained for surface water (without any background subtraction). The use of DAD allows the identification of DEA at the level of 0.008 $\mu g\,L^{-1}$ although an interference due to the surface water sample.

The described method was applied to the monitoring of the selected pesticides in 40 surface water samples, collected from the streams and lakes that nourish the six water reservoirs of the city of Istanbul.

TABLE IV Limits of detection (LODs) and detection wavelength of each pesticide (in the range of 0.025–0.2 $\mu g\,L^{-1}$) in Milli-Q water ($n = 4$)

Compound	*LODs*[a] ($ng\,L^{-1}$)	*Detection wavelength* (nm)
DEA	8	220
Simazine	8	220
Propoxur	12	204
Atrazine	8	220
Carbaryl	5	220
Methiocarb	6	204
Prometryn	8	240
Terbutryn	8	240

[a]LODs (twice the noise).

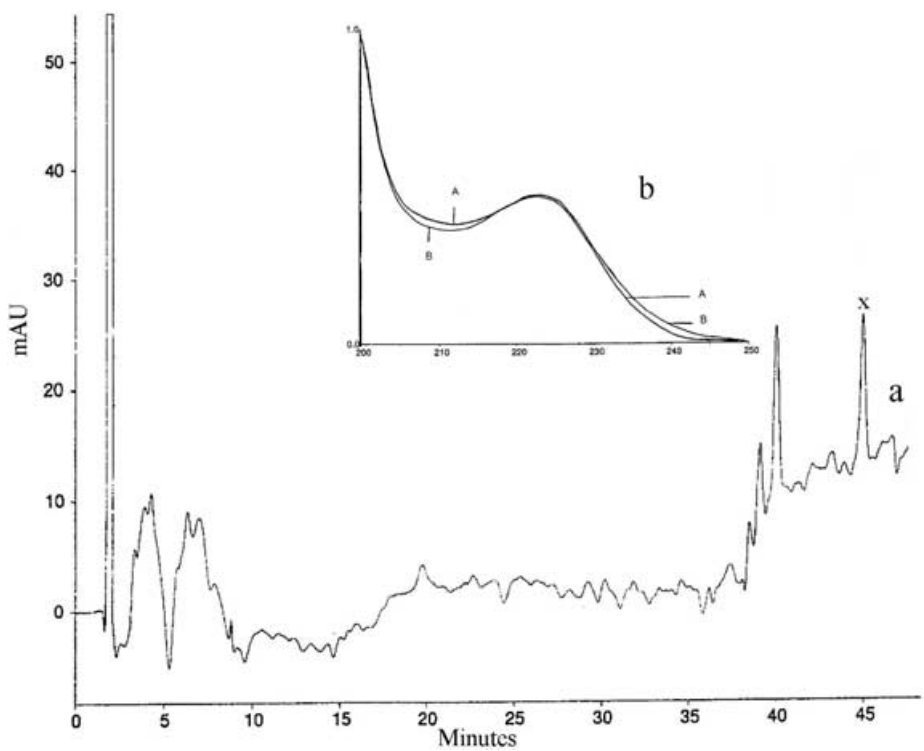

FIGURE 4 (a) LC-DAD chromatogram of the sample containing terbutryn (0.074 $\mu g\,L^{-1}$) registered at 240 nm; (b) comparison of standard spectra and unknown peak spectra for terbutryn. 'A' refers to the peak of the unknown compound and 'B' to the standard.

The identification of pesticides was accomplished on the basis of the retention times and by comparison between the UV spectrum of the reference compound in the library and the UV spectrum of the detected peak in the sample. A match equal to or higher than 990 was fixed to confirm identification between both spectra for all the pesticides determined. Quantitation of the pesticides in the sample extracts was performed by use of external standard calibration curves. Terbutryn was the only pesticide, detected in three water samples at the concentrations of 0.025; 0.027; $0.074\,\mu g\,L^{-1}$, under the limits of $0.1\,\mu g\,L^{-1}$ fixed by EU (Fig. 4).

CONCLUSIONS

In this work, a very sensitive, reliable and rapid procedure based on SPE-RPLC and DAD has been developed which allows the simultaneous determination of seven triazine and NMC pesticides which selected owing to their frequency of use and a degradation product in surface waters. The use of DAD enables confirmation of the results through spectral comparison and also offers the possibility of avoiding matrix interferences by choosing different wavelength. Calibration of the method using surface water spiked with selected pesticides revealed good linearity within the 0.025–$0.2\,\mu g\,L^{-1}$ range, with limits of detection around 0.005–$0.012\,\mu g\,L^{-1}$ when 500 mL of water was preconcentrated. The procedure has been successfully applied in a program for monitoring of surface water carried out by our laboratory. Terbutryn was found in three water samples at the concentrations of 0.025; 0.027; and $0.074\,\mu g\,L^{-1}$ under the limits of $0.1\,\mu g\,L^{-1}$ fixed by EU.

Acknowledgment

The authors would like to thank the Research Fund of the University of Istanbul for supports of this work (Project number: T-733/280699).

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