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Screening methods for asulam, oxine-copper and thiram in water by high-performance liquid chromatography after enrichment with a minicolumn

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ABSTRACT

A syringe-type minicolumn containing 40 μl of C_{18} bonded silica was used for pretreatment of the samples in the determination of methyl-N-(aminophenylsulphonyl)carbamate (asulam), bis(8-quinolinolato)copper (oxine-Cu) and bis(dimethylthiocarbamoyl)disulphide (thiram) in ground, tap and river water by reversed-phase high-performance liquid chromatography (HPLC). The low levels of pesticides were adsorbed on the minicolumn, which was then connected to the injector of the HPLC system, and directly injected into the loop by eluting with a small amount of eluent. Average recoveries of asulam, oxine-Cu and thiram in water samples at concentrations of 5 μg/l were 91.5, 77.1 and 87.3%, respectively. The detection limits of asulam from 300 μl of water sample and of oxine-Cu and thiram from 1 ml were 0.2, 1.0 and 0.5 μg/l, respectively. It took only about 10 min to obtain a chromatogram on HPLC, including sample pretreatment. The syringe-type minicolumn was useful to eliminate compounds coexisting in water. This small-scale procedure enabled the sample volume required and the amount of organic solvent for elution to be reduced.

INTRODUCTION

High-performance liquid chromatographic (HPLC) determination of organics in water has generally been performed by injecting an aliquot of the extract prepared by solid-phase or liquid–liquid extraction [1–3] and by on-line pre-column sample enrichment using a column-switching valve system [4–6]. The former off-line method requires more than 100 ml of water sample to determine pesticides at the level of a few micrograms per litre. It takes time for sample preparation and requires extraction and concentration steps before analysis by HPLC. Moreover, a large volume of organic solvent waste is produced during liquid–liquid partition. As environmental pollution by chemicals including organic solvents is an object of public concern, it is preferable to be able to reduce the amount of organic solvent used in laboratories when measuring the level of a contaminant. The latter on-line method has some merits, for example it is a highly sensitive and simple technique. However, an enrichment column is usually used repeatedly for many water samples, and cross-contamination becomes a problem, especially in the case of trace levels in water, unless extensive washing between samples is carried out.

The object of this work was to develop a rapid and highly sensitive HPLC method to detect pesticides at microgram per litre levels in a small volume of water sample.

A disposable syringe-type minicolumn packed with silica gel, Extrashot-Silica, developed by Homma *et al.* [7] permits direct sample injection into an HPLC system without tedious sample preparation, and an improved version has been applied to the determination of theophylline and methylpyrazines in biological fluids such as plasma and urine [8,9].

We investigated analytical methods using a syringe-type minicolumn packed with C_{18} bonded sil-

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ica to detect methyl-N-(4-aminophenylsulphonyl)-carbamate (asulam), bis(8-quinolinolato)copper (oxine-Cu) and bis(dimethylthiocarbamoyl)disulphide (thiram). These pesticides are scattered copiously on golf courses in Japan to keep them green. Regulation levels of these pesticides were established recently in Japan (1990).

EXPERIMENTAL

Reagents and chemicals

Asulam, oxine-Cu and thiram were purchased from GL Science (Tokyo, Japan). Acetonitrile and methanol were of HPLC grade, and the other compounds obtained from Wako (Osaka, Japan) were of analytical grade. Stock solutions were prepared at 100 mg/l and stored at 4°C with acetonitrile as the solvent for asulam and thiram, and methanol for oxine-Cu.

Apparatus

A syringe-type minicolumn, Extrashot-ODS, was obtained from Kusano Kagakukikai (Tokyo, Japan). The minicolumn [8] had a 51-mm-long needle made of stainless-steel and the column portion (27 mm × 7 mm I.D.) was prepacked with 40 μl of C_{18} bonded silica (particle size 70 μm) in an internal tube made of PTFE.

The HPLC system consisted of a Model 880-PU pump (Jasco, Tokyo, Japan) a Model 870 ultraviolet detector (Jasco), a Model 7125 injector (Rheodyne, Cotati, CA, USA) with a 100-μl loop and a Model C-R4A chromatographic integrator (Shimadzu, Kyoto, Japan). An Ultrasphere-octadecylsilane (ODS) reversed-phase column (250 mm × 4.6 mm I.D., Beckman, San Ramon, CA, USA) was used to analyse asulam and thiram, and ODS-Cu (150 mm × 4.6 mm I.D., GL Science) was used for oxine-Cu.

Chromatographic conditions of HPLC

Asulam and thiram were monitored by ultraviolet detection at 270 nm, and oxine-Cu at 240 nm.

The mobile phases for asulam, thiram and oxine-Cu were acetonitrile–50 m*M* potassium phosphate buffer, pH 3.2 (30:70, v/v), acetonitrile–water (60:40, v/v) [10] and acetonitrile–100 m*M* potassium phosphate buffer pH 3.0, (10:90, v/v), respectively, as recommended by the manufacturer of the ODS-Cu column. The flow-rate of the mobile phase was 1 ml/min.

Analytical procedures

Sample pretreatment. A 10-ml aliquot of water sample was dispensed into a 13-ml glass-stoppered test tube. When the water sample contained free available chlorine, HOCl and OCl^-, they were removed by sodium sulphite. Water samples were acidified with 20 μl of 35% hydrochloric acid for asulam, and with 10 μl of phosphoric acid for thiram.

Conditioning of syringe-type minicolumn. The minicolumn, Extrashot-ODS, was connected with a glass syringe and pre-washed with 300 μl of acetonitrile and then with 500 μl of distilled water.

Sample application. An aliquot of a few millilitres of the treated water sample as above (*Sample pretreatment*) was applied onto the minicolumn through a tuberculin test glass syringe.

Direct injection of eluate into the HPLC system. After the application of water samples, the minicolumn was connected to the injection port of the HPLC system. The pesticides absorbed on the minicolumn were slowly injected into the loop of the chromatograph through a tuberculin test syringe over a period of 20 s with 130 μl of the elution solvents, which were the mixture of organic solvents and aqueous solution: acetonitrile–potassium phosphate buffer (pH 3.2) for asulam, methanol–potassium phosphate buffer (pH 3.0) for oxine-Cu and acetonitrile–water for thiram.

RESULTS AND DISCUSSION

Stability of pesticides in water

The effect of pH on the stability of asulam, oxine-Cu and thiram in water was investigated. Each pesticide was added at 50 μg/l to 50 m*M* potassium phosphate buffer at pH 3, 5, 7 and 9, and then an aliquot of the solution was injected into the HPLC system. Asulam and oxine-Cu were stable at all pH values examined. The residual amount of thiram was about 90% at pH 5, 7 and 9 after 120 min, whereas it was 98% at pH 3. Acidification of water samples, preferably to less than pH 3, is necessary to determine thiram at low levels.

Similarly, the stability of the pesticides in water samples of various origins was examined. Tap water

samples contain 0.3–0.4 mg/l free available chlorine and their pH values are 7.2–7.4. Asulam, oxine-Cu and thiram in tap water gradually disappeared without any treatment, probably by decomposition or structural transformation. The loss was overcome by the addition of sodium sulphite before spiking the pesticides. By treating sodium sulphite, more than 99% of asulam and oxine-Cu remained after 120 min, while the residual amounts of thiram after 15 and 120 min were 93.5 and 65.2%, respectively. Similar results were obtained when ascorbic acid instead of sodium sulphite was used to remove free available chlorine. Dechlorination is necessary if the water sample contains free available chlorine.

In river water samples, more than 99% of asulam and oxine-Cu were retained after 120 min. Residual amounts of thiram were 97.6 and 89.0% after 15 and 120 min, respectively.

In ground water samples, asulam and oxine-Cu were stable after 120 min. Thiram was retained at 98.8% after 120 min.

It is necessary to analyse thiram as quickly as possible no matter what the sample is.

Conditions of sample adsorption on the minicolumn

The optimum pH of adsorption on the minicolumn was investigated. More than 95% of asulam was retained on the minicolumn at pH values below 4, although the amount retained was drastically reduced at pH values above 4, as shown in Fig. 1A. This result corresponds to the fact that asulam is a weakly acidic compound with $pK_a = 4.82$ [11,12]. It is necessary to acidify water samples to less than pH 4 to detect asulam in water. The amount of oxine-Cu retained was more than 95% at pH above 4, although the amount retained was reduced at pH below 4, as shown in Fig. 1A. Because the dissociation constant (pK_1) of 8-hydroxyquinoline is about 4 in 50% dioxane [13], oxine-Cu partially dissociated to give rise to 8-hydroxyquinoline at lower pH. 8-Hydroxyquinoline could not be quantitatively adsorbed on the minicolumn at pH below 4. When the pH of water sample was below 4, it was necessary to adjust the pH of water sample to neutrality by adding appropriate base. More than 95% of thiram was retained at all pH values examined, as shown in Fig. 1A.

The effect of sample volume on retention of the pesticides on the minicolumn was investigated. At least 4 ml of oxine-Cu and thiram were retained, as shown in Fig. 1B. On the other hand, asulam passed through when the sample volume was more than 300 μl. Adsorption of asulam in aqueous solution by C_{18} bonded silica was relatively weak. There-

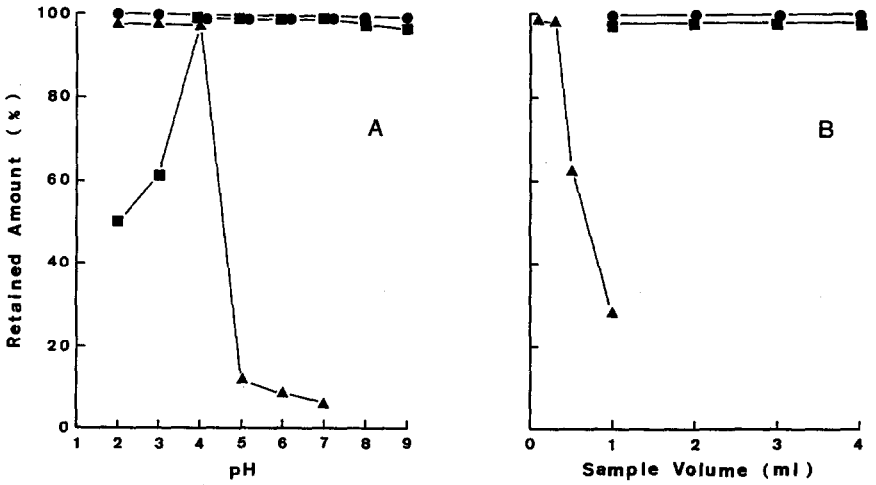

Fig. 1. Effects of pH (A) and sample volume (B) on retention of the pesticides. Each pesticide in 50 m*M* potassium phosphate buffer was spiked at 50 μg/l and the spiked solution was applied to the minicolumn. Each pesticide in the solution passed through the minicolumn was analysed by HPLC. (A) Sample volume was fixed at 200 μl for asulam (▲) and 1 ml for oxine-Cu (■) and thiram (●). The pH of potassium phosphate buffer was varied. (B) The pH of potassium phosphate buffer was fixed at pH 2 for asulam (■), at pH 7 for oxine-Cu (■) and at pH 3 for thiram (●). Sample volumes were varied.

fore, the minicolumn is suitable when the sample volume is less than 300 μl, but when a large-volume sample is to be analysed, other packing materials such as strong anion exchanger [14] have some advantages.

Conditions of eluting from the minicolumn

The elution solvent from the minicolumn was investigated. The recoveries of asulam at a concentration of 2 mg/l were more than 90% when the solvent contained more than 10% acetonitrile, as shown in Fig. 2A.

The recoveries of oxine-Cu from the minicolumn were 97.5, 65.0 and less than 5% with methanol–potassium phosphate buffer (20:80, v/v) at pH 2, 3 and 4, respectively. These results correspond to the data regarding the effect of pH on adsorption, as mentioned above. The recoveries of oxine-Cu were more than 95% when the solvent contained more than 20% methanol, as shown in Fig. 2B. When acetonitrile was used instead of methanol, the recoveries of oxine-Cu from the minicolumn were lower (data not shown).

For thiram, the recoveries were more than 95% when the solvent contained more than 50% acetonitrile, as shown in Fig. 2C. It is unnecessary to adjust the pH of eluting solvent for a neutral compound such as thiram.

In order to detect pesticides at low levels, the proportion of organic solvents in the eluting solvent from the minicolumn should be low, because the theoretical plate numbers of asulam, oxine-Cu and thiram on HPLC were reduced when the proportion of organic solvents was increased, as shown in Fig. 2. Moreover, reducing the proportion of organic solvents prevents compounds that are more hydrophobic than the pesticides from flowing into the system. From these results, the eluting solvents from the minicolumn were optimized as follows: acetonitrile–50 m*M* potassium phosphate buffer (pH 3.2) (10:90, v/v) for asulam; methanol–100 m*M* potassium phosphate buffer (pH 2) (20:80, v/v) for oxine-Cu; and acetonitrile–water (50:50, v/v) for thiram.

Recoveries of pesticides from water samples

The recoveries of asulam from ground, tap and river water samples were investigated at concentrations of 5 μg/l and 2 mg/l. The recoveries of asulam from each water sample were more than 90% at both levels, as shown in Table I. The average relative standard deviation (R.S.D.) was within 4% at

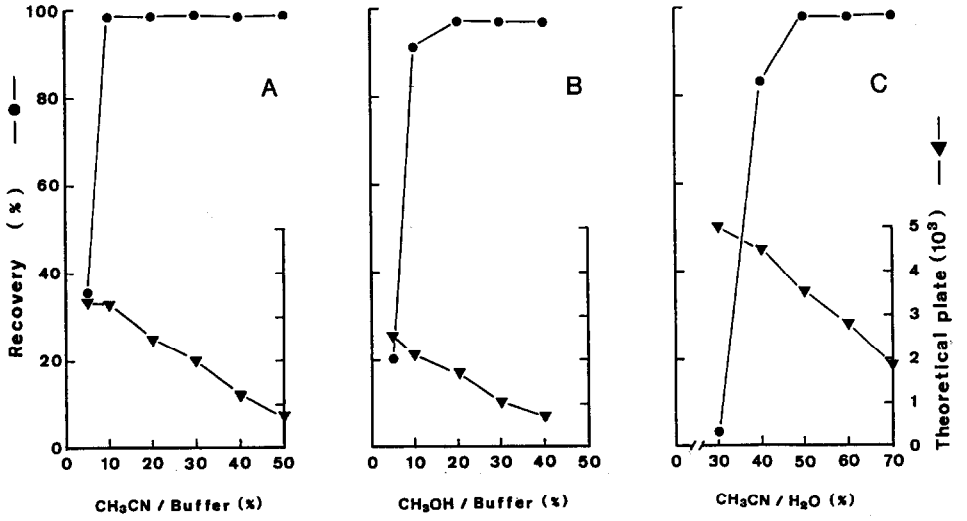

Fig. 2. Effects of the composition of the eluting solvent from the syringe-type minicolumn on the recoveries and theoretical plate numbers of the pesticides. Each pesticide was applied to the minicolumn and eluted with solvent of various compositions. (A) Asulam: spiked level, 2 mg/l; sample volume, 200 μl; buffer, 50 m*M* potassium phosphate buffer (pH 3.2). (B) Oxine-Cu: spiked level, 200 μg/l; sample volume, 1 ml; buffer, 100 m*M* potassium phosphate buffer (pH 2). (C) Thiram: spiked level, 200 μg/l; sample volume, 1 ml.

TABLE I

RECOVERIES OF ASULAM, OXINE-COPPER AND THIRAM IN SPIKED WATER

Values are mean ± S.D. (n = 5). ND = Not determined.

Pesticide	Sample[a]	Spiked level (μg/l)	Recovery (%)	
			Sodium sulphite pretreatment	No pretreatment
Asulam	G	5	92.1 ± 3.5	93.0 ± 3.0
		2000	96.6 ± 1.7	ND
	T	5	92.5 ± 2.4	<10
		2000	97.2 ± 2.0	ND
	R	5	90.0 ± 3.0	89.0 ± 2.5
		2000	92.7 ± 2.3	ND
Oxine-Cu	G	5	76.6 ± 4.0	78.0 ± 2.0
		500	95.9 ± 3.0	ND
	T	5	79.3 ± 2.7	<10
		500	99.2 ± 0.8	ND
	R	5	75.3 ± 2.9	76.2 ± 3.9
		500	97.8 ± 1.4	ND
Thiram	G	5	90.2 ± 4.0	91.2 ± 5.2
		500	99.2 ± 2.3	ND
	T	5	88.9 ± 5.0	<10
		500	97.3 ± 2.6	ND
	R	5	82.9 ± 4.8	81.5 ± 5.0
		500	99.1 ± 0.6	ND

[a] G = Ground water; T = tap water; R = river water.

both levels, and the lowest detection limit of asulam in 300 μl of water sample was 0.2 μg/l. Fig. 3A shows a chromatogram of asulam spiked at 5 μg/l in 300 μl of river water after treatment through the minicolumn.

The recoveries of oxine-Cu from ground, tap and river water samples were 75–79% at the spiked level of 5 μg/l and more than 95% at 500 μg/l, and the R.S.D. was within 4% at both levels examined. The lowest detection limit of oxine-Cu in 1 ml of water sample was 1 μg/l. Fig. 3B shows a chromatogram of oxine-Cu spiked at 5 μg/l in 1 ml of river water.

The effects of metal cations in the water samples on the recovery of oxine-Cu were examined. It is well known that 8-hydroxyquinoline is a typical chelating agent for metal cations [13]. Oxine-Cu was spiked at a concentration of 5 μg/l in 50 mM potassium phosphate buffer (pH 7) in the presence of either cadmium chloride, copper chloride, ferrous chloride, ferrous sulphate, manganese chloride, lead nitrate, aluminium chloride or nickel chloride, and extracted on the minicolumn and eluted by the eluent. In the case of nickel chloride, oxine-Cu recoveries were 81.8, 59.4 and 45.0% at nickel chloride concentrations of 0.8, 1.6 and 8 μM, respectively. The poor recoveries were improved by the addition of 27 μM disodium ethylenediaminetetraacetate. Metal cations other than nickel at a concentration of 1 mg/l had no effects on the recovery of oxine-Cu.

The recoveries of thiram from water samples were more than 90% for ground and tap water and 82% for river water at a spiked level of 5 μg/l. With fortified levels of 500 μg/l thiram, the recoveries were more than 97%. The R.S.D. values were within 5% at both levels examined, and the lowest detection limit of thiram in 1 ml of water sample was 0.5 μg/l. Fig. 3C shows a chromatogram of thiram spiked at 5 μg/l in 1 ml of river water.

Recently, Tsukioka *et al.* [14] utilized solid-phase

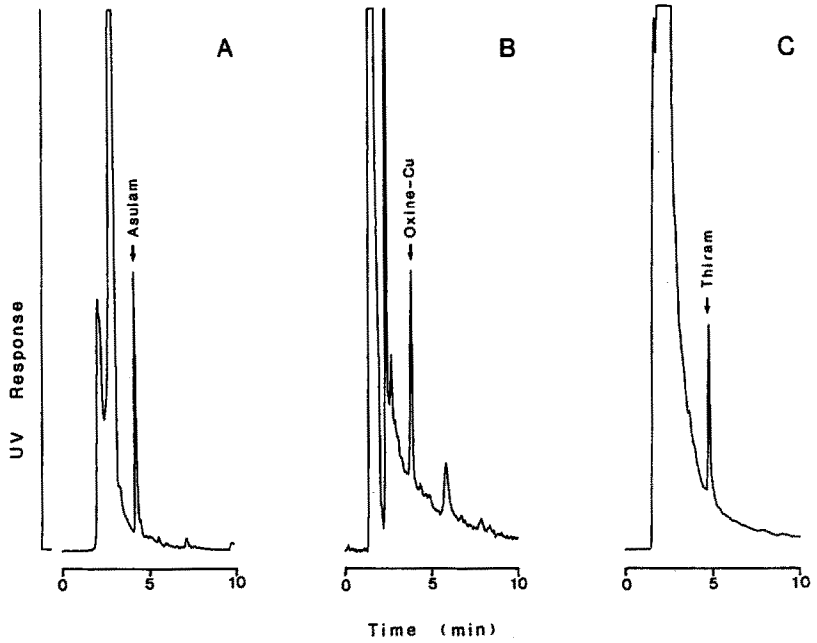

Fig. 3. Chromatograms of river water spiked with pesticides at a concentration of 5 μg/l. The water sample spiked with pesticides was applied to the syringe-type minicolumn, and adsorbed pesticides were directly injected into the HPLC system with 130 μl of eluent. (A) Asulam: sample volume, 300 μl; eluent, acetonitrile–50 m*M* potassium phosphate buffer (pH 3.2) (10:90, v/v). (B) Oxine-Cu: sample volume, 1 ml; eluent, acetonitrile–100 m*M* potassium phosphate buffer (pH 2.0) (20:80, v/v). (C) Thiram: sample volume, 1 ml; eluent, acetonitrile–water (50:50, v/v).

extraction with a strong anion exchanger (SAX) for the analysis of asulam in 100 ml of river water by HPLC. The average recovery of asulam spiked at a concentration of 10 μg/l was 80.6% and the R.S.D. was 3.0%, with the lowest detection limit of 1 μg/l. An analytical method for oxine-Cu in water by HPLC after liquid–liquid extraction with dichloromethane was presented by Ogawa *et al.* [2]. The average recovery of oxine-Cu in 1000 ml of distilled water at a concentration of 100 μg/l was 81.6% with an R.S.D. of 2.4%. Miles and Moye [15] reported on the analysis of thiram in water by HPLC, in which methylamine derived from thiram by UV photolysis was detected by fluorescence detection after reacting with *o*-phthalaldehyde–2-mercaptoethanol. In this method, the recoveries of thiram in 0.4 ml of ground water at a concentration of 10 μg/l were 86.7–96.3% with the lowest detection limit of 3.8 μg/l. In comparison with data from previously published reports, the recovery and R.S.D. of asulam, oxine-Cu and thiram in the present study utilizing syringe-type minicolumn with ODS were as good as and similar to those in large-scale analysis.

In Japan the maximum allowable contaminant levels of asulam, oxine-Cu and thiram are 200, 40 and 6 μg/l, respectively, for drinking water, and 2 mg/l, 400 and 60 μg/l, respectively, for river water. The analysis time for the whole procedure including sample pretreatment and detection on the chromatogram was only about 10 min. This method can be practically applied to estimate a low level of pesticides in water samples with only a few millilitres of samples.

Storage of minicolumn after sample application

The effects on recoveries of the pesticides of storage time of the minicolumn after sample application were evaluated. Water samples spiked with asulam, oxine-Cu and thiram at a concentration of 5 μg/l were passed through the minicolumn, and the pesticides were eluted with appropriate elution solvents

TABLE II

EFFECT OF 24 h STORAGE AT 4°C OF THE MINICOLUMN WITH SAMPLE AFTER APPLICATION

Values are mean ± S.D. (n = 5). Spiked levels of each pesticide were 5 μg/l.

Pesticide	Sample[a]	Recovery (%)
Asulam	G	91.5 ± 2.3
	T	91.5 ± 3.1
	R	95.0 ± 7.5
Oxine-Cu	G	61.5 ± 7.1
	T	67.7 ± 5.5
	R	62.4 ± 7.1
Thiram	G	6.9 ± 0.5
	T	8.2 ± 2.5
	R	4.6 ± 0.4

[a] G = Ground water; T = tap water; R = river water.

after storage for 24 h at 4°C (Table II). The recoveries of asulam and oxine-Cu were, respectively, more than 90% and 61–67% in any water sample, while those of thiram were less than 10%. When thiram was eluted with 130 μl of acetonitrile—10 mM phosphoric acid (50:50, v/v) immediately after application and analysed 24 h after storage of the eluate at 4°C, the recoveries of thiram in ground, tap and river water were 68.9 ± 9.0, 64.9 ± 6.8 and 47.0 ± 4.5%, respectively. These results suggest that the minicolumn may not be suitable for storing the pesticides on it, especially in the case of thiram. The pesticides adsorbed on the minicolumn should be eluted as soon as possible.

CONCLUSIONS

The use of the syringe-type minicolumn containing C_{18} bonded silica for the screening of asulam, oxine-Cu and thiram in water was established. The merits of this screening method with the minicolumn are: (1) a quick procedure that takes only 10 min for analysis; (2) a high sensitivity that enables detection at 1 μg/l so that only a few millilitres of water sample are required; (3) only a small amount of organic solvent is required; and (4) prepacked solid-phase minicolumns or cartridges with a versatile matrix could expand its usefulness for the analysis of chemicals in water.

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