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Note

Determination of Methyl Yellow, Sudan I and Sudan II in water by high-performance liquid chromatography

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Methyl Yellow (C.I. 11020), Sudan I (C.I. 12055) and Sudan II (C.I. 12140) are used as dyes in gasoline, shoe polish, special inks and plastics. However, Methyl Yellow has been reported to be a carcinogen[1,2]. If these dyes are present in the environment, they would be released slowly into river and sea waters, owing to their limited solubility in water. Therefore, a microanalytical method is required to detect these dyes in environmental waters.

There have been few papers concerning analytical methods for these dyes in environmental samples. This investigation was undertaken to establish a suitable method for their simultaneous determination in environmental waters at very low concentrations. After distillation, the dyes were extracted by modified mixing extraction as they are not readily soluble in water. Mixing extraction methods [3–5] using a very small amount of solvent permit simple and rapid operation, but the formation of emulsions often makes the extraction difficult. In this paper, the formation of the emulsion was improved by adding acetone. The separation of the dyes was possible by high-performance liquid chromatography (HPLC). The method established gives excellent sensitivity and recovery.

EXPERIMENTAL

Reagents

Methyl Yellow, Sudan I and Sudan II were obtained from Wako and recrystallized from acetone. The grades of hexane and acetone used were those suitable for the detection of pesticide residues. The grade of methanol used was that suitable for HPLC. Sodium chloride was of analytical-reagent grade. After 0.025 g of each dye had been dissolved in 50 ml of acetone, standard solutions were prepared by diluting each acetone solution with methanol.

Apparatus and measurement conditions

HPLC was carried out with a Shimadzu LC-4A instrument and an SPD-1 variable-wavelength UV–VIS spectrophotometric detector. The output of the detector was monitored with a Chromatopac C-R2AX data processor.

The HPLC conditions were as follows: column, LiChrosorb RP-18, 7 μm (25 cm × 4.0 mm I.D.); column temperature, 40°C; mobile phase, methanol–water

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(90:10, v/v); flow-rate, 1 ml/min; detection, 460 mm, 0.02 a.u.f.s.; and sample size, 20 μl.

Standard procedure

A 500-ml sample of water was placed in a 1-l distillation flask and three boiling stones were added. The distillation flask was heated with a mantle heater. Distillation was carried out into 20 ml of distilled water in a 500-ml measuring cylinder for 80–90 min until 400 ml of distillate had been collected. The distillate was transferred to a 500-ml measuring flask equipped with a PTFE stirring bar (3 cm × 7 mm O.D.). The water-cooled condenser and the funnelled adapter were washed with 10 ml of acetone and the 500-ml measuring cylinder was washed with 5 ml of acetone and 20 ml of distilled water. The wash liquids were transferred to the measuring flask containing the distillate and 5 g of sodium chloride were added and dissolved. The solution was diluted to volume with distilled water and 3 ml of hexane were added. After mixing extraction for 10 min with a magnetic stirrer, the hexane layer was transferred to a 10-ml test-tube using a Pasteur pipette. The mixing extraction procedure was repeated twice for a total of three extractions. In the measuring flask, the distance from the base of the neck to the calibration line should be as short as possible (less than 2 cm) to make mixing easy.

The extract in the test-tube was concentrated and dried on a water-bath (below 50°C) with a flow of nitrogen. The dyes were dissolved in 1 ml of methanol using an ultrasonic bath. The solution was passed through a Millipore membrane filter (pore size 0.45 μm), then 20 μl of the methanol-dissolved extract was injected into the HPLC column.

RESULTS AND DISCUSSION

Examination of distillation conditions

First, an examination was conducted to establish the most suitable pH for distillation. To a 1-l distillation flask were added 500 ml of water adjusted to pH 1–13 with sodium hydroxide solution or sulphuric acid, and three boiling stones and 2 μg of the standard mixture were added. The solution was distilled until 400 ml of distillate had been obtained and the distillate was treated according to the standard procedure. The recoveries were constant, being 87.3–91.7% in the pH range 5–13 for Methyl Yellow, 88.5–93.9% in the pH range 1–11 for Sudan I and 90.5–94.8% in the pH range 1–13 for Sudan II. Normally, the pH of river and sea water is in the range 5–11, here the pH of the water samples did not need to be adjusted.

Next, an examination was conducted to establish the most suitable distillate volume. The above procedure was employed. In this examination, the pH of the distilled water sample was not adjusted and the volume of distillate was varied between 100 and 450 ml. It was found that constant recoveries were obtained if the volume of distillate was in the range 400–450 ml. The recoveries were 87.6–87,7% for Methyl Yellow, 92.5–93.3% for Sudan I and 91.1–93.0% for Sudan II. Consequently, it was necessary to continue distillation until 400 ml of distillate had been collected.

Examination of extraction conditions

An examination of the effect of the pH of the distillate on the recovery was

carried out. To a 500-ml measuring flask equipped with a stirring bar were added 400 ml of distilled water adjusted to pH 1–13 with sodium hydroxide solution or sulphuric acid. After 2 μg of the standard mixture and 15 ml of acetone had been added, 5 g of sodium chloride were also added and dissolved. The solution was diluted to volume with distilled water. Subsequent treatments were conducted according to the standard procedure. The recoveries were constant, being 94.3–96.2% in the pH range 5–11 for Methyl Yellow, 92.8–98.8% in the pH range 1–13 for Sudan I and 100–102% in the pH range 1–13 for Sudan II.

In the mixing extraction, the amount of hexane used for the extraction was very small. Therefore, an examination of the effect of the amount of hexane added on the recovery was carried out. The above procedure was employed and the amount of *n*-hexane was varied between 1 and 5 ml. In this examination, the pH of the distilled water for the distillate was not adjusted. Extraction was carried out only once, and therefore the residual layer of hexane in the measuring flask was recovered by the addition of 5 ml of hexane and transferring the solution to a test-tube. The addition of more than 2 ml of hexane gave constant recoveries of over 90%.

The residual dyes in the water-cooled condenser, the funnelled adapter and the measuring cylinder should be recovered by washing with acetone. Therefore, an examination of the effect of the amount of acetone used on the recovery was carried out. The same procedure as used for the examination of the effect of the pH of the distillate on the recovery was employed and the amount of acetone was varied between 0 and 100 ml. In this examination, the pH of the distilled water for the distillate was not adjusted. It was found that the recoveries were constant and over 90% when the amount of acetone was in the range 0–100 ml. Moreover, the formation of emulsion was improved when acetone was used.

Examination of HPLC conditions

The absorption maxima of the dyes dissolved in the mobile phase consisting of methanol–water (90:10, v/v) were 405 nm for Methyl Yellow, 475 nm for Sudan I and 493 nm for Sudan II. Consequently, the dyes were detected at 460 nm.

An examination was carried out with three mobile phases, with different proportions of methanol and water (95:5, 90:10 and 85:15, v/v), to determine the opti-

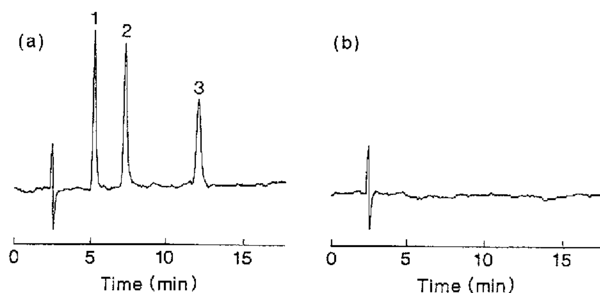

Fig. 1. Chromatogram of (a) a standard mixture containing 40 ng of each dye and (b) river water. Column, LiChrosorb RP-18, 7 μm (25 cm × 4.0 mm I.D.); column temperature, 40°C; mobile phase, methanol–water (90:10, v/v); flow-rate, 1 ml/min; detection, 460 nm. Peaks: 1 = Methyl Yellow; 2 = Sudan I; 3 = Sudan II.

TABLE I

RECOVERY OF DYES FROM RIVER AND SEA WATERS

c.v. = Coefficient of variation ($n = 5$).

Compound	*River water*			*Sea water*		
	Added (μg)	*Mean recovery (%)*	*C.V. (%)*	*Added (μg)*	*Mean recovery (%)*	*C.V. (%)*
Methyl Yellow	2	91.4	2.2	2	87.5	4.5
Sudan I	2	96.4	1.7	2	96.4	1.3
Sudan II	2	90.5	3.8	2	89.3	2.4

mum mobile phase composition for separation. Methanol–water (90:10) offered a good compromise between peak resolution and reasonably short analysis time. Fig. 1a illustrates the chromatogram.

Recovery experiments and detection limits

As shown in Table I, the recoveries from river and sea waters were over 87.5% with coefficients of variation below 5%.

The calibration graphs were linear in the ranges 4–80 ng for Methyl Yellow, 5–105 ng for Sudan I and 7–145 ng for Sudan II. The detection limits for a 500-ml water sample were 0.2 μg/l for Methyl Yellow and Sudan I and 0.3 μg/l for Sudan II.

Application

The proposed method was applied to several samples of river and sea waters. The dyes were not detected in any of the samples analysed and the chromatograms were free from interferences. A chromatogram of water from the Mikasa River in Fukuoka is shown in Fig. 1b.

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REFERENCES

1 International Agency for Research on Cancer, *IARC Monogr. Eval. Carcinog. Risk Chem. Man.*, 8 (1975) 125.
2 E. Longstaff, *Dye Pigm.*, 4 (1983) 243.
3 K. Grob, K. Grob Jr. and G. Grob, *J. Chromatogr.*, 106 (1975) 299.
4 T. Kodama and H. Ishida, *Kagaku Bussitsu Bunsekihou Kaihatsu Chousa Houkokusho*, Jap. Environ. Agency, Tokyo, 1983, p. 75.
5 T. Matsueda, Y. Kurokawa and Y. Osaki, *Water Purif. Liquid Waters Treatm.*, 29 (1988) 299.