Journal of Chromatography, 346 (1985) 450–452
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 950

Note

High-performance liquid chromatographic determination of melamine extracted from cups made of melamine resin

TAKIKO INOUE, HAJIMU ISHIWATA*, KUNITOSHI YOSHIHIRA and AKIO TANIMURA
National Institute of Hygienic Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158 (Japan)
(First received May 1st, 1985; revised manuscript received June 3rd, 1985)

Melamine (2,4,6-triamino-*s*-triazine) is an important material in the manufacture of thermosetting plastics used in housewares. Kitchenware and tableware made of melamine resin are widely used in homes, school and office cafeterias and restaurants, but the migration of melamine from these wares has not been well studied.

This paper describes a sensitive high-performance liquid chromatographic procedure for the determination of melamine based on the methods reported by Beilstein *et al.*[1] and the National Toxicology Program[2] and the application of the proposed method to migration solutions obtained from cups made of melamine resin.

EXPERIMENTAL

Apparatus

A Yanaco (Tokyo, Japan) Model L-2000 high-performance liquid chromatograph equipped with a Model 215 spectrometer set at 235 nm (range at 0.08) was used. A Yanapak (Tokyo, Japan) ODS-A column (250 × 4.6 mm I.D.) was employed. A Shimadzu (Kyoto, Japan) UV-240 spectrophotometer was utilised.

Materials and chemicals

Cups of volume 255 ml, made of melamine resin were used.

All chemicals and reagents were of Japanese Industrial Standards special grade. Melamine (Wako, Kyoto, Japan) was 99.0% pure.

Mobile phase. A 0.1 *M* phosphate buffer (pH 3.0) containing monopotassium phosphate and phosphoric acid was prepared. To determine the optimum pH, 0.1 *M* phosphate buffers of pH 8.0, 7.0, 6.0 and 5.0 containing disodium phosphate and monopotassium phosphate were prepared and buffers of pH 4.0 and 2.0 were prepared in the same manner as the buffer of pH 3.0. The flow-rate was set at 0.78 ml/min.

Sample preparation

A 220-ml portion of a food-simulating solvent, water or 4% (v/v) acetic acid, which had been preliminary warmed to 60°C, was poured into melamine resin cups so that the solvent surface was 5 mm below the rim. The cups were covered with watch-glasses, then stood at 60°C for 30 min. The migration solution (200 ml) was

0021-9673/85/$03.30

evaporated to dryness using a rotary evaporator at 65–70°C, the residue was dissolved in 2 ml of water and volumes of 10 μl of this solution were injected into the chromatograph.

Recovery study

A volume of 200 ml of the food-simulating solvents and 20 μg of melamine were placed in a round-bottomed flask, the mixture was allowed to stand at 60°C for 30 min and was then treated in the same manner as the migration solution.

RESULTS AND DISCUSSION

The pH of the mobile phase strongly affected both the retention time (Fig. 1a) and the absorbance of melamine (Fig. 1b). The retention time of melamine decreased with decrease in pH. The wavelength of maximum absorption ($\lambda_{max.}$) of melamine (235 nm) was not shifted by changes in pH, but the intensity of the absorption increased when the pH was lowered. The peak height of melamine increased with increasing acidic conditions, especially at pH 2.0 and 3.0. However, use of a mobile phase of pH 2.0 was not suitable because the retention time of melamine at pH 2.0 was close to the water peak and a pH of 2.0 represented the limit that could be tolerated by the column. Neither the retention time nor the peak height of melamine was affected when the concentration of buffers of pH 3.0 was varied from 0.05 to 0.5 *M*. Citric acid, which had been used to adjust the pH of the buffer to 3.0[2], was not suitable for obtaining a linear baseline with the proposed sensitivity.

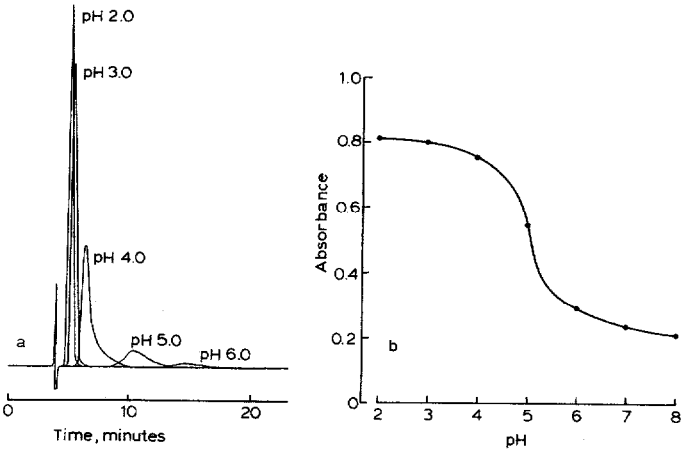

Fig. 1. Effect of pH on (a) retention time in HPLC and (b) absorbance in spectrophotometry. The concentration of melamine was 10 μg/ml in each buffer. Determination was carried out at 235 nm.

On the basis of these results, subsequent experiments were carried out using 0.1 *M* phosphate buffer of pH 3.0.

The recoveries of melamine from food-simulating solvents were more than 98.8% (Table I). Results of the determination of melamine in the migration solution are presented in Table II. The concentration of melamine in the migration solution

TABLE I

RESULTS OF RECOVERY STUDY

20 μg of melamine were added to 200 ml of the solvent. Results are averages of four determinations.

Solvent	*Recovery ± S.D. (%)*
Water	99.2 ± 0.9
4% acetic acid	98.8 ± 1.0

TABLE II

RESULTS OF ANALYSIS OF MELAMINE IN MIGRATION SOLUTION

Results are averages of four determinations.

Solvent	*Melamine (ng/ml ± S.D.)*
Water	19.0 ± 2.7
4% acetic acid	23.6 ± 5.2

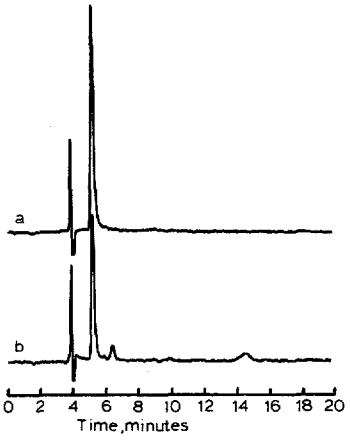

Fig. 2. Chromatograms of standard solution of (a) melamine and (b) migration solution in 4% acetic acid. Melamine standard solution corresponded to 50 ng/ml of migration solution.

was about 20 ng/ml. Higher standard deviations of the amount migrated than those given by the recovery tests may be caused by the differences in the surface roughness and the content of residual melanine in the individual cups. Formaldehyde, which could be present in the migration solution, did not interfere with the determination of melamine at a level of 4000 ng. A typical chromatogram is shown in Fig. 2.

The limit of determination of melamine by the proposed method was 2.5 ng/ml in the migration solution (signal-to-noise ratio = 5).

REFERENCES

1 P. Beilstein, A. M. Cook and R. Hutter, *J. Agr. Food Chem.*, 29 (1981) 1132.
2 National Toxicology Program, *Carcinogenesis Bioassay of Melamine in F344/N Rats and B6C3F$_1$ Mice*, U.S. Department of Health and Human Services, MD, 1983, p. 154.