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Determination of Melamine and Three Hydrolytic Products by Liquid Chromatography

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Melamine occurs as an environmental contaminant in foods (EPA 1985, 1987) and wastewater (Burrows et al. 1984; Mogami et al. 1986), and is considered to be released from melamine resin products into foods (Ishiwata et al. 1978). The compound is gradually hydrolyzed to ammeline, ammelide and cyanuric acid under acidic conditions or metabolized via the same pathway by certain microorganisms (Jutzi et al. 1982; Myskow et al. 1983). Ammeline is also produced from 2-substituted diamino-*s*-triazine compounds such as herbicides (Bakke et al. 1972; Khan et al. 1985). Gas chromatography (GC) after trimethylsilylation (Stoks & Schwarts 1979) and high-performance liquid chromatography (HPLC) (Beilstein et al. 1981) have been reported as analytical methods for melamine and its three hydrolytic products. Although HPLC shows a broad peak and low sensitivity for melamine and the separation of some peaks is incomplete (Beilstein et al. 1981), HPLC is more attractive than GC because no preliminary derivatisation procedures are required. This paper describes a sensitive HPLC method for the determination of melamine and its hydrolytic products, ammeline, ammelide and cyanuric acid. The method is used to analyse leachate solutions obtained from melamine tableware.

MATERIALS AND METHODS

A Shimadzu (Kyoto, Japan) LC-3A high-performance liquid chromatograph equipped with a spectrophotometric detector (SPD-1) set at 214 nm was used. A Zorbax NH_2 column (25 cm x 4.6 mm I.D., DuPont, Wilmington, DE, USA) containing 5-μm particles was employed. An integrator, C-R1A, (Shimadzu, Kyoto,

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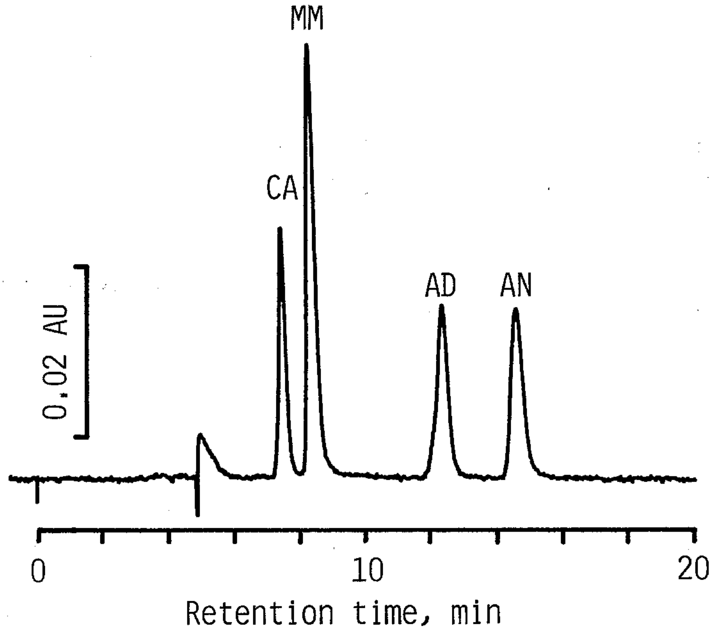

Figure 1. Chromatogram of cyanuric acid (CA), melamine (MM), ammelide (AD) and ammeline (AN).

Japan) was used. Quantitatiion was by peak area and by using the external standard method. Two sets of cups made of melamine resin were examined:cups, 155 mL in volume, which had been used for 10 months at the cafeteria at this Institute, and new cups, 240 mL in volume, which had been treated 20 times by filling of 4% acetic acid at 95°C for 30 min (Inoue et al. 1987). Melamine (special grade, more than 99.0%, Wako Pure Chemical Industries, Ltd., Osaka, Japan), ammeline (extra pure, 94.5%, Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan), ammelide (ICN Pharmaceuticals, Inc., N.Y., USA) and cyanuric acid (guaranteed grade, Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) were used. The mobile phase was a mixture of 78% (v/v) acetonitrile (HPLC grade, Wako Pure Chemical Industries, Osaka, Japan) and 22% (v/v) 5 mM phosphate buffer solution, pH 6.7, consisting of mono-potassium phosphate and di-sodium phosphate.

A 220- or 140-mL portion of 4% (v/v) acetic acid, which had been preliminarily warmed to 95°C, was poured into the melamine cups. The cups were covered with watch glasses, and allowed to stand at 95°C for 30 min in a water bath. A 100-mL aliquot of the acetic acid solution was removed from each set of cups, and the liquid was evaporated to dryness using a rotary

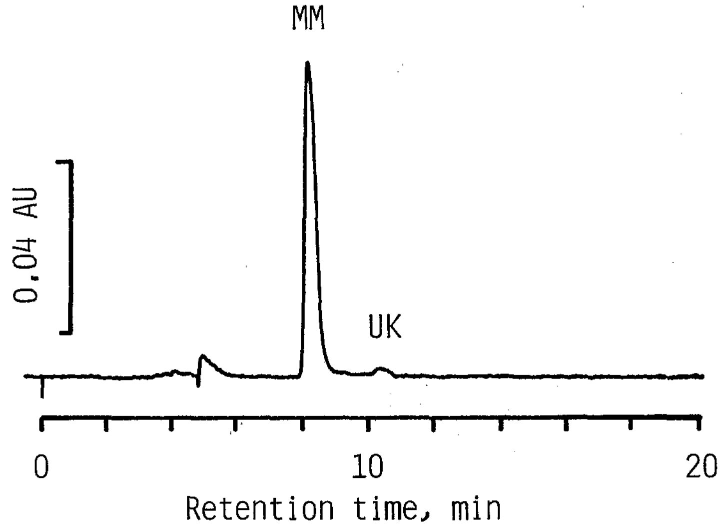

Figure 2. Chromatogram of a 4% acetic acid leachate solution obtained from melamine tableware. MM: melamine, UK: unknown

evaporator at 50-60°C. The nonvolatile residue was dissolved in known volume of water of about 5 mL. This solution was used as a test solution for HPLC. A 10-µL aliquot of this solution was analysed.

RESULTS AND DISCUSSION

A chromatogram of the four s-triazines is shown in Fig. 1. These four compounds were completely resolved and showed sharp peaks. Besides the column described in the METHODS section, ODS- and CN-columns were examined. The sharpest peaks and complete separation of the four s-triazines were obtained with the combination of a Zorbax or a Nucleosil NH_2 column and a mobile phase of the mixture of 78% (v/v) acetonitrile and the 5 mM phosphate buffer solution at pH 6.7. Calibration curves based on 5 concentrations of the s-triazines were linear between 1 and 20 ppm and can be described by the following equations: $y = 13112x + 2496$ for melamine, $y = 7283x - 1038$ for ammeline, $y = 7007x - 1088$ for ammelide, and $y = 8022x - 508$ for cyanuric acid, where y = area (mV x sec) and x = concentration (ppm). Correlation coefficients were 0.999 for all curves. The detection limits were 10 ng for melamine and 20 ng for the others. Recoveries of the compounds added to 4% acetic acid, heated at 95°C for 30 min, evaporated to dryness, and redissolved in water were between 98.7 and 101.1% (Table 1), indicating that these substances are stable under such conditions.

Table 1. Recoveries of four s-triazines*

Compounds	Recovery ± S.D. (%)
Melamine	98.7 ± 1.5
Ammeline	101.1 ± 0.4
Ammelide	99.4 ± 2.3
Cyanuric acid	101.0 ± 1.8

* A 4% acetic acid solution fortified at 0.1 ppm with each of the four analytes was heated at 95°C for 30 min. Results are mean±S.D. of triplicate determinations.

The proposed method was applied to the leachate solutions obtained using melamine tableware which had been used for 10 months or washed 20 times with 4% acetic acid at 95°C for 30 min for the acceleration of surface deterioration. A typical chromatogram of the leachate solution (Fig. 2) and concentrations of s-triazines in the solution (Table 2) are shown. From 3.0 to 9.7 ppm of melamine was detected in the leachate solution.

Table 2. Leaching of s-triazines into 4% acetic acid from cups made of melamine resin

Sample	melamine	ammeline	ammelide	cyanuric acid
	(Concentration, ppm)			
A	9.6	nd	nd	nd
B	9.5	nd	nd	nd
C	9.7	nd	nd	nd
D	5.5	nd	nd	nd
E	8.7	nd	nd	nd
F	3.0	nd	nd	nd

nd:less than 0.02 ppm, A-C: cups washed 20 times with 4% acetic acid, D-F:cups used for 10 months. Results are mean of duplicate determinations.

Although ammeline, ammelide and cyanuric acid were not detected (detection limit: 0.02 ppm), some unknown peaks regarded as intermediates or plastic additives were observed.

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