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Note

Improved method for the separation of methylolmelamines by high-performance liquid chromatography

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Exudation of formaldehyde and melamine from cups made of melamine resin has been reported using water or 4% acetic acid as a solvent for extraction[1–4]. On the other hand, it is well known that melamine reacts with formaldehyde to form methylolmelamines (N-hydroxymethylmelamines)[5–8] and the resulting methylolmelamines have been separated by high-performance liquid chromatography (HPLC)[5–7]. The techniques used were not suitable as regards the sensitivity and resolution for the determination of very small quantities of methylolmelamines. We now report a rapid and sensitive reversed-phase HPLC assay which permits the microanalysis of methylolmelamines.

EXPERIMENTAL

Materials

Formalin (37% formaldehyde solution), acetonitrile and acetic acid were obtained from Wako (Osaka, Japan), and melamine from Tokyo Kasei (Tokyo, Japan).

HPLC

A Model 5A high-performance liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with UV spectrophotometric detector set at 235 nm was operated at room temperature for qualitative and quantitative analyses. A stainless-steel reversed-phase HPLC column (250 mm × 4.6 mm I.D.) was prepared with Develosil ODS-5 (Nomura Chemical, Japan). Elution was carried out with acetonitrile–acetic acid–water (5:0.5:94.5, v/v) for highly substituted methylolmelamines and with acetonitrile–acetic acid–water (1:0.5:98.5, v/v) for the separation of low substituted methylolmelamines. The flow-rate was 0.8 ml/min.

Preparation of standard methylolmelamines mixture

A methylolmelamines mixture was prepared according to Tomita's method[5] as follows; 0.16 *M* formaldehyde and 0.033 *M* melamine in 0.05 *M* phosphate buffer pH 9.0, were mixed in equal quantities and the mixture was allowed to stand for 120 h at 28°C.

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Preparation of sample solutions

Cups of volume 200 ml, made of melamine resin, were used. An 100-ml portion of water or 4% acetic acid was poured into the cups. The cups were covered with watch-glasses, then heated in an electric range for 2.5 min and 10 μl of the extracts were injected into the chromatograph.

RESULTS AND DISCUSSION

All of the nine methylolmelamines, including two isomers of di-, tri- and tetramethylolmelamine, were separated by HPLC and each species isolated was identified by NMR according to Tomita[5]. An addition reaction between melamine and formaldehyde was carried out under the same conditions as those in his report and a similar chromatogram was obtained from the reaction mixture as shown in Fig. 1. Each peak was identified by comparing the corresponding peaks on both chromatograms, that reported by Tomita and ours. The retention time increased in the order of the number of methylol substituents on the amino groups of melamine.

The pH of the mobile phase was the first parameter studied. The intensity of the absorption of melamine at 235 nm increased when the pH was lowered[1]. The same phenomenum was observed for methylolmelamines. Addition of acetic acid suppressed tailing of the peaks on the chromatogram and improved their resolution. The peak height increased remarkably with increasing acidity, which made possible trace analysis. The detection limit was less than 10 ng/ml for melamine with a 20-μl injection. No significant variation in sensitivity was observed in the range of 0.1–1.0%

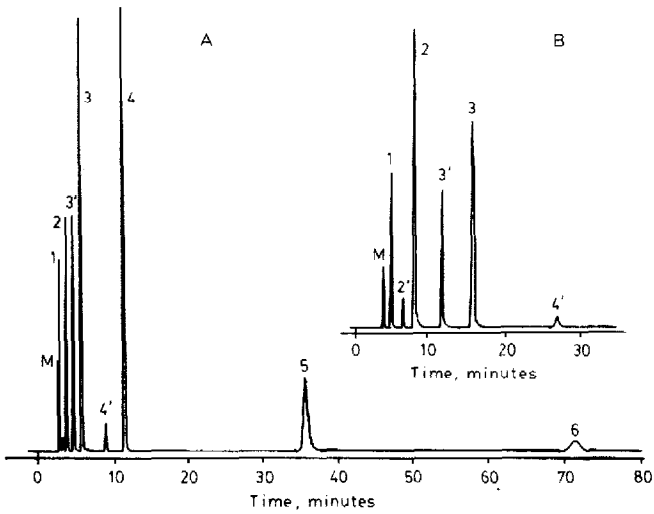

Fig. 1. Elution profile of an equilibrium methylolmelamines mixture obtained from the reaction between 0.16 *M* formaldehyde and 0.033 *M* melamine. HPLC conditions: column, ODS-5 (250 mm × 4.6 mm I.D.); detector, UV (235 nm); mobile phases: A, acetonitrile–acetic acid–water (5:0.5:94.5, v/v); B, acetonitrile–acetic acid–water (1:0.5:98.5, v/v); flow-rate, 0.8 ml/min. Peaks: M = Melamine; 1 = monomethylolmelamine; 2 = N,N′-dimethylolmelamine; 2′ = N,N-dimethylolmelamine; 3 = N,N′,N″-trimethylolmelamine; 3′ = N,N,N′-trimethylolmelamine; 4 = N,N,N′,N″-tetramethylolmelamine; 4′ = N,N,N′,N′-tetramethylolmelamine; 5 = pentamethylolmelamine; 6 = hexamethylolmelamine.

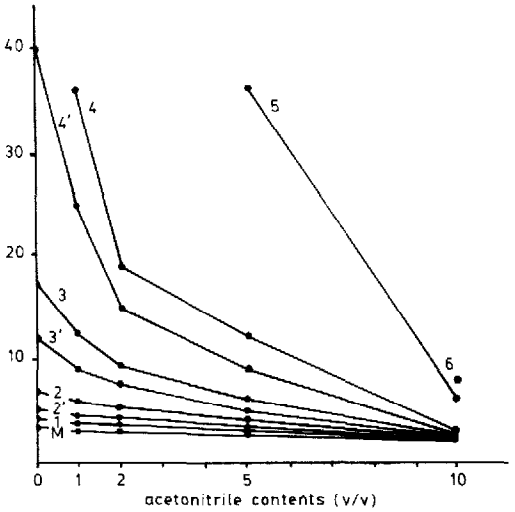

Fig. 2. Dependence of retention times on the acetonitrile content in the mobile phase containing 0.5% (v/v) of acetic acid. Compounds numbered as in Fig. 1.

(v/v) of acetic acid. Next, the dependences of the retention times of methylolmelamines on the acetonitrile content were studied between 0 and 10% (v/v), maintaining acetic acid content constant at 0.5% (v/v) for a good separation of interesting methylolmelamines. The results in Fig. 2 clearly show that the acetonitrile content had a strong effect on the retention times.

The sensitivity for melamine was of the same order of magnitude as that obtained by Inoue *et al.*[1], while their paper made no mention of methylolmelamines. In regard to methylolmelamines, the present method offered distinct advantages in sensitivity and resolution over Tomita's[5], where neutral media were used as the mobile phase and a differential refractometer was used as the detector.

The method proposed was applied to the determination of melamine and methylolmelamines in extracts from cups made of melamine resin and the results are presented in Table I. A typical chromatogram is shown in Fig. 3. Exudation of melamine and monomethylolmelamine was observed. The peak height was used for quantification and compared with that from a standard melamine solution. The

TABLE I

MELAMINE AND MONOMETHYLOLMELAMINE (ppm) IN SAMPLE SOLUTION

Sample solutions were prepared as described in the text.

Cup	*Solvent*	*Melamine*	*Monomethylolmelamine*
A	Water	0.85	0.03
B	Water	0.24	0.02
C	4% Acetic acid	4.20	0.32
D	4% Acetic acid	0.83	0.07

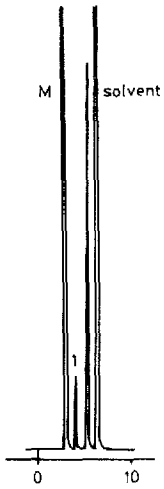

Fig. 3. A chromatogram of a sample solution in 4% acetic acid. Time scale in minutes.

amount of monomethylolmelamine was calculated by assuming that its absorption intensity is close to that of melamine. The presence of monomethylolmelamine was identified for the first time in extracts from cups made of melamine resin.

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