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DETERMINATION OF *s*-TRIAZINE DERIVATIVES AT THE NANOGRAM LEVEL BY GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

A gas–liquid chromatographic method for the determination of the triazines cyanuric acid, ammelide, ammeline and melamine at the nanogram level, using trifluoroacetic acid as solvent for the triazines and N-methyl-N-trimethylsilyltrifluoroacetamide as the silylation reagent, is described. Optimal derivatization and chromatographic conditions have been determined. The relative molar response with respect to phenanthrene as the internal standard was found to remain constant for amounts of sample in the range 0.025–10 μg and the minimum detectable amount for quantitative analyses was found to be 2.5 ng (*ca.* 20 pmole) of each triazine.

INTRODUCTION

Because of the importance of derivatives of *s*-triazine possessing herbicidal activity a number of papers have been published on their separation and detection, involving paper[1,2], thin-layer[3,4], gas–liquid[5,6] and high-performance liquid chromatography[7]. In contrast, only a few reports have dealt with the analysis of the parent *s*-triazines, which include the hydroxy and amino derivatives cyanuric acid, ammelide, ammeline and melamine.

Two methods have been developed[8,9] for the separation and detection of these triazines by thin-layer chromatography but, although these methods give satisfactory results for qualitative analyses, they are not entirely suitable for quantitative purposes. In the course of our investigations into the occurrence of N-heterocyclic compounds in carbonaceous meteorites, a technique was needed for the quantitative detection of sub-microgram amounts of these triazines.

We therefore investigated the possibility of using gas–liquid chromatography, which implies the use of a derivatization agent to increase volatility and reduce polarity. It was decided to use trimethylsilylation for this purpose, as this technique has been reported[10] to be especially suitable for the derivatization of compounds containing amino and hydroxy groups. N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was selected because of its high volatility and, consequently, narrower solvent peaks on gas chromatograms compared with other silylation reagents.

The technique presented here has the advantage over existing methods[8,9] of being a quantitative, highly sensitive and rapid technique for the simultaneous determination of cyanuric acid, ammelide, ammeline and melamine.

EXPERIMENTAL

Apparatus

Optimal gas chromatographic conditions were determined by using a Varian Model 1400 gas chromatograph equipped with a flame-ionization detector. Combined gas chromatographic–mass spectrometric (GC–MS) analyses were performed with a Finnigan Model 9500 gas chromatograph coupled to a Finnigan Model 3100 mass spectrometer through an all-glass jet separator. Both gas chromatographs were equipped with constant-flow controllers in the carrier gas lines. Peak areas and retention times were determined by using a Hewlett-Packard Model 3370 B integrator.

Reagents

MSTFA was purchased from Pierce (Rockford, Ill., U.S.A.), hexamethyldisilazane (HMDS) from Aldrich Europe (Beerse, Belgium) and trifluoroacetic acid (TFA), melamine (Mn), ammeline (An) and cyanuric acid (CA) from Fluka (Buchs, Switzerland). Ammelide (Ad) was synthesized via a literature method[11] and was shown to be homogeneous by mass spectrometry. All commercially obtained chemicals were of the highest purity available.

The systematic names of the triazines used in this study are 2,4,6-triamino-, 2-hydroxy-4,6-diamino-, 2,4-dihydroxy-6-amino- and 2,4,6-trihydroxy-*s*-triazine for melamine, ammeline, ammelide and cyanuric acid, respectively; their structures are shown in Fig. 1.

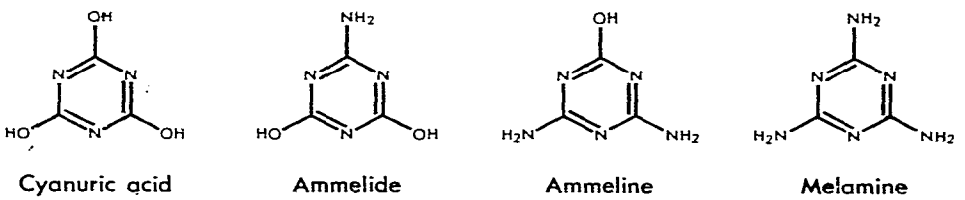

Fig. 1. Structures of the four *s*-triazines.

Chromatographic conditions

For both gas chromatographs 1.5 m × 3 mm I.D. glass columns were used, packed with 3% OV-17 on Chromosorb G AW (100–120 mesh). Helium was used as the carrier gas at a flow-rate of 40 ml/min. The injection block temperatures were 220° for both instruments; the detector temperature on the Varian gas chromatograph (hydrogen, 32 ml/min; air, 350 ml/min) and the jet separator oven temperature on the Finnigan instrument were both maintained at 280°.

Conditioning of both columns was performed by programmed heating (0.5°/min) to 270° while four 5-μl volumes of HMDS at 15-min intervals were injected in the temperature range 165–195°. Heating was continued at 270° for 10 days.

Sample preparation

A stock solution of the triazines was prepared by dissolving 5 mg of each triazine in 10 ml of TFA. This stock solution was stored at 4° and was prepared freshly twice a week.

Derivatization was achieved in the following manner. A 1-μl aliquot of the stock solution was transferred to the bottom of a capillary tube (3 cm × 2 mm I.D.), MSTFA (9 μl) was added and the capillary was immediately sealed with a flame. The contents were then thoroughly mixed and the capillary was immersed in an oil-bath kept at 150 ± 1°.

Routinely, 0.5 μl of the resulting clear solution was injected into the gas chromatograph. Relative molar responses (RMR) were determined with respect to phenanthrene (Ph) as the internal standard.

RESULTS AND DISCUSSION

Gas chromatographic conditions

Optimal separations were obtained by using temperature programming at 4°/min, with an initial column oven temperature of 140°. Under these conditions the triazine derivatives were eluted between 140° and 190°.

Stability of triazines

In order to determine accurately the amount of triazine to be silylated, a solvent was needed that would dissolve the triazines and from which aliquots could be taken for derivatization. TFA has been reported[12] to be a suitable solvent for silylation, especially for polar compounds, and it was tested for its properties with respect to the four triazines used in this work.

Separate stock solutions of each triazine were prepared with phenanthrene as the internal standard (5 mg of each in 10 ml of TFA) and aliquots were derivatized after storage for 0, 3, 4 and 5 days at 4°.

Gas chromatographic analysis of the silylated derivatives showed the triazines to be stable towards acid hydrolysis for 3 days. RMRs calculated for successive analyses after 3 and 4 days did not change significantly in comparison with the initial values. However, a slight hydrolysis resulted in a trace of ammelide appearing on the gas chromatogram of ammeline after 4 days (Fig. 2). Therefore, stock solutions were prepared freshly twice a week.

Silylation of triazines

For the determination of optimal derivatization conditions, the influence of reaction time at a specific temperature on the RMR was studied.

Initial investigation revealed that heating was necessary, because without heating the responses were low and not reproducible. The responses of the various triazines as a function of time at two different temperatures are presented in Table I.

The results demonstrate that, although at both temperatures prolonged heating results in a decrease in RMR, which is most prominent for melamine at 150°, the overall responses are higher on heating at 150°, especially for short periods of time. Therefore, heating for 5 min at 150° was selected for derivatization of the triazine samples. A typical gas chromatogram of the silylated triazines is depicted in Fig. 3.

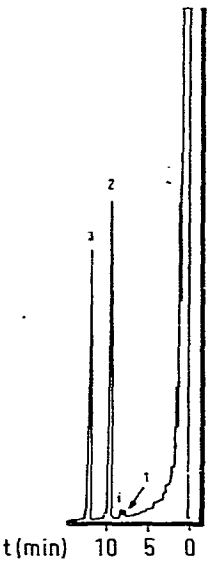

Fig. 2. Gas chromatogram of ammeline, silylated after storage for 4 days at 4°. $8 \cdot 10^{-11}$ A.f.s.d.: 1 μl injected (50 ng); 140–200° at 4°/min. Peaks: 1 = Ad (172°); 2 = An (179°); 3 = Ph (192°); i = impurity.

TABLE I

EFFECT OF REACTION TIME AND TEMPERATURE ON RMR

Compound	*RMR at 100°*				*RMR at 150°*			
	5 min	*15 min*	*30 min*	*60 min*	*5 min*	*15 min*	*30 min*	*60 min*
Mn	0.94	0.87	0.79	0.77	1.04	0.75	0.66	0.41
An	0.85	0.80	0.77	0.72	0.88	0.85	0.82	0.79
Ad	0.78	0.77	0.74	0.70	0.84	0.80	0.79	0.72
CA	1.17	1.17	1.13	1.01	1.25	1.20	1.20	1.18

Replacement of one, two or all hydroxyl groups on the triazine nucleus with amino groups (from cyanuric acid through ammelide and ammeline to melamine) results in an increased retention time, which can be explained by interaction of the remaining amino hydrogen atoms with the moderately polar stationary phase OV-17. That the amino groups are indeed monosilylated, leaving one hydrogen atom available for interaction, was confirmed by combined GC–MS analysis; the 70-eV mass spectra of the TMS derivatives of the four triazines showed intense peaks at *m/e* 345, 344, 343 and 342, corresponding to the molecular ions of O,O′,O″-tris(trimethylsilyl)-cyanuric acid, N,O,O′-tris(trimethylsilyl)ammelide, N,N′,O-tris(trimethylsilyl)ammeline and N,N′,N″-tris(trimethylsilyl)melamine, respectively. No evidence was found for N,N-bis(trimethylsilyl) derivatization. A similar observation has been made[13] with the pyrimidines uracil and cytosine and the purines hypoxanthine, adenine, xanthine and guanine.

Minimum detectable amount of triazines

Several dilutions of the triazine stock solution were made and 1-μl aliquots of these solutions were silylated as described above.

At an instrumental setting of $2 \cdot 10^{-11}$ A/mV, 2.5 ng of triazine injected appeared to be the minimum detectable amount for which RMR values could still be determined reliably (Fig. 4). For qualitative analyses the minimum detectable amount at the same instrumental setting was 0.5–0.7 ng at a signal-to-noise ratio of 5, with a 0.5-μl injection.

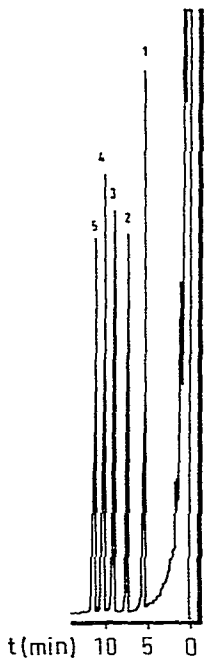

Fig. 3. Gas chromatogram of silylated triazines. $4 \cdot 10^{-11}$ A.f.s.d.; 0.5 μl injected; 140–200° at 4°/min; each peak represents 25 ng. Peaks: 1 = CA (164°); 2 = Ad (172°); 3 = An (179°); 4 = Mn (184°); 5 = Ph (192°).

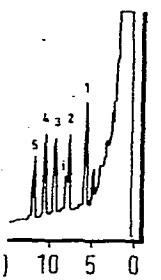

Fig. 4. Gas chromatogram of silylated triazines. $2 \cdot 10^{-11}$ A.f.s.d.; 0.5 μl injected; each peak represents 2.5 ng. Peaks as in Fig. 3.

Impurities in the silylation reagent

In initial studies the MSTFA was found to contain impurities that were eluted in the temperature range 190–210°. A final column oven temperature of 220° therefore had to be used with this MSTFA. In the course of the investigation another sample of MSTFA became available (purchased from Macherey, Nagel & Co., Düren, G.F.R.) that did not contain these impurities. However, once the protective Teflon seal had been penetrated, the MSTFA attacked the septum material, resulting in an impurity peak on the gas chromatograms eluting at 173° (Figs. 2 and 4).

Effect of triazine concentration on the RMR

The capillaries used for the silylation of nanogram amounts of triazine could

not be used for amounts larger than 1 μg, owing to the limited solubility of the triazines in TFA. Therefore, 10-μl aliquots of triazine solutions were silylated with 90 μl of MSTFA in sealed 1-ml glass ampoules. The silylation conditions were the same as described for the capillaries. The RMR was found to remain constant over the sample range 25 ng–10 μg.

Stability of the silylated triazines

Although many trimethylsilylated compounds are thermally stable[10], the decrease in RMR on prolonged heating even at 100° indicated that the TMS derivatives of the triazines used in this work are not thermally stable. Therefore, prolonged heating should be avoided. After heating at 150° for 5 min the sealed capillaries could be stored at 4° for 1 week without significant changes in the RMR. However, once a capillary had been opened a 10–20% decrease in RMR in less than 15 min was observed for samples containing $\leqslant$10 ng of triazine. The susceptibility towards hydrolysis by atmospheric humidity must therefore be greater for the TMS triazines than for MSTFA itself.

REFERENCES

1 A. Cee and J. Gasparič, *Mikrochim. Acta*, (1972) 823.
2 J. Perkavec, M. Perpar and D. Brodnik, *Mikrochim. Acta*, (1969) 1224.
3 W. Ebing, *J. Chromatogr.*, 65 (1972) 533.
4 J. Reichling, *Z. Anal. Chem.*, 278 (1976) 125.
5 G. T. Flint and W. A. Aue, *J. Chromatogr.*, 52 (1970) 487.
6 R. Delley, K. Friedrich, B. Karlsruber, G. Székely and K. Stammbach, *Z. Anal. Chem.*, 228 (1967) 23.
7 T. H. Byast, *Analyst (London)*, 100 (1975) 325.
8 A. Cee and J. Gasparič, *J. Chromatogr.*, 56 (1971) 342.
9 H. Milster and L. Meckel, *Text.-Rundsch.*, 17 (1962) 485.
C.A., 58 (1963) 1576b.
10 A. E. Pierce, *Silylation of Organic Compounds*, Pierce Chemical Co., Rockford, Ill., 1968.
11 O. Diels, *Chem. Ber.*, 32 (1899) 691.
12 M. Donike, *J. Chromatogr.*, 85 (1973) 1.
13 V. Miller, V. Pacáková and E. Smolková, *J. Chromatogr.*, 119 (1976) 355.