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Note

Determination of cyanuric acid by high-performance liquid chromatography

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The derivatives of *s*-triazine are widely used in agriculture and industry. The hydroxy derivative of *s*-triazine, 1,3,5-triazine-2,4,6(1H,3H,5H)-trione, commonly known as cyanuric acid (Fig. 1) is used in the synthesis of other compounds which serve as herbicides, dyes, resins and antimicrobial agents. Gas–liquid[1–3], high-performance[4], thin-layer[5,6] and paper chromatography have all been used in the detection of the more complex triazine compounds. Cyanuric acid can be detected quantitatively by gas–liquid chromatography, however this method requires derivatization[1]. All other methods are only qualitative or the sensitivity of the method is not acceptable for trace analysis.

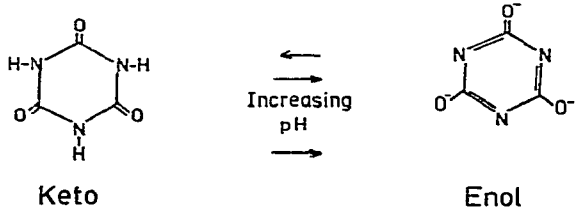

Fig. 1. Cyanuric acid (keto and enol forms).

In the investigation of the degradation of cyanuric acid under anaerobic conditions, we wished to develop an assay system that: (1) did not require derivatization; (2) was sensitive; (3) was rapid; and (4) allowed detection of cyanuric acid in a defined microbat growth medium. This paper describes a high-performance liquid chromatographic (HPLC) method which meets these requirements and has been routinely used for one year to analyze cyanuric acid in a defined cyanuric acid–inorganic salt medium.

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EXPERIMENTAL

A Varian (Palo Alto, CA, U.S.A.) Model 5000 liquid chromatograph fitted with a Varian Vari-Chrom variable-wavelength UV–VIS detector set at 214 nm was employed. The column (30 cm × 4 mm) was a Varian Micropak-NH_2 column. The mobile phase was acetonitrile–water (60:40). The flow-rate was 2.0 ml/min. Samples of 10 μl were injected onto the column. Recorder chart speed was 1.0 in./min.

Peak identification

The procedure for identifying the separate components was the collection of separated peak fractions, evaporation of the fractions to dryness using a rotary evaporator, and placing the dried sample into the mass spectrometer sample holder. A Varian-MAT 112 mass spectrometer (Bremen, G.F.R.) with an electron impact source was employed. A low temperature direct insertion probe at 45°C was used to record the background. At a sample probe temperature of 215°C the suspected cyanuric acid spectrum was recorded. The appropriate background spectrum was subtracted by use of the Varian data system.

A UV spectrum was generated to determine the wavelength of maximum absorbance ($\lambda_{max.}$) and the molar absorptivity for cyanuric acid. A Perkin-Elmer–Coleman (Maywood, IL, U.S.A.) Model 124 double-beam spectrophotometer was used. Cyanuric acid samples from evaporated peak fractions were first weighed and then placed in a 10-ml volumetric flask and brought to volume with HPLC grade water and buffered at pH 8.0 with tris(hydroxymethyl)aminomethane hydrochloride.

Reagents

Cyanuric acid was obtained from Eastman-Kodak (Rochester, NY, U.S.A.), HPLC-grade acetonitrile from EM Labs. (Cincinnati, OH, U.S.A.) and tris-(hydroxymethyl)-aminomethane hydrochloride from Sigma (St. Louis, MO, U.S.A.).

RESULTS AND DISCUSSION

The elution profile of cyanuric acid is shown in Fig. 2. Peak parameters are summarized in Table I. The mass spectrum obtained is shown in Fig. 3. Analysis of the mass spectra with a known standard and comparison with literature[7] values confirm that the spectrum is indeed cyanuric acid. The ion of mass 129 is characteristic of cyanyric acid.

The decomposition of cyanuric acid to cyanic acid at high temperature is responsible for the ions at mass 42 and 43. UV spectroscopic analysis of dried fractions

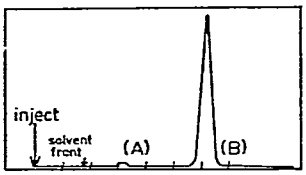

Fig. 2. Chromatogram of cyanuric acid (B) representing a concentration of 0.43 μg/10 μl; (A), unknown contaminant.

TABLE I

REPRODUCIBILITY OF PEAK HEIGHT AND CAPACITY FACTOR (k') (10 REPLICATE RUNS)

	$\bar{x}$	σ	σ (%)
k'	0.4335	0.0054	1.24
Peak height (mm)	61.59	1.4	2.27

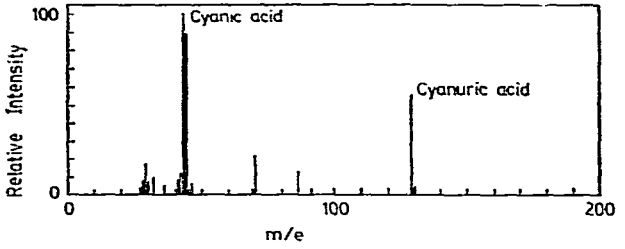

Fig. 3. Direct insertion probe mass spectrum (220–245°C background substracted) of cyanuric acid collected peak fractions.

indicated: (1) a $\lambda_{max.}$ at 214 nm and (2) a molar absorptivity of $\log_{10}$ 4.00 in an aqueous solution at pH 8.0. Both values are close to the reported literature[8] values.

The fact that cyanuric acid undergoes a pH dependent tautomeric shift between an enol and keto form was of concern. The tautomeric shift into the keto form could cause irreversible reaction with the amino groups of the stationary phase. Also the elution time of the enol and keto forms may differ. Attempts to separate the enol and keto forms however were not successful. Since the keto form of cyanuric acid has no absorbance at 214 nm, it can be inferred that the mobile phase of acetonitrile–water (60:40) favors the enol form. Minimum detectable quantities were less than 4.3 μg/μl liter samples.

Statistical analysis of peak height and k' values indicates that the assay is reproducible (see Table I).

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