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Note

Chromatographic trace analysis of guanidine, substituted guanidines and *s*-triazines in water

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Nitroguanidine is a frequent component of military propellants. To insure that Army production facilities remain in compliance with wastewater discharge limits, we were tasked with development of highly sensitive analytical methods for determination of the trace organics known or suspected to be present in nitroguanidine production wastewater. These included nitrosoguanidine, cyanoguanidine, guanidine, urea, cyanamide, melamine and ammeline, in addition to nitroguanidine.

Until the emergence of reversed-phase high-performance liquid chromatography (RP-HPLC), separation and analysis of these small, highly polar compounds was generally limited to thin-layer chromatography (TLC) on cellulose in aqueous systems. Relative to HPLC, TLC procedures are insensitive (frequently by several powers of ten) and difficult to apply quantitatively because the compounds can only be visualized by chromogenic spray or dip reagents. Recently, RP-HPLC separations of nitroguanidine and nitrosoguanidine[1] and of melamine and ammeline[2] have been desscribed. We here report two highly sensitive, reproducible, and rapid RP-HPLC methods for quantitative estimation of three substituted guanidines and the two triazines, and their application to trace analysis of wastewater. Guanidine, classically determined by fluorescence spectrophotometry of the ninhydrin complex[3], has insufficient UV absorbance alone for detection by HPLC. We have developed a novel ion chromatographic method for its estimation, which was not subject to interference by other cations present in the wastewater. Cyanamide and urea were determined by spectrophotometric procedures[4] described elsewhere[5].

EXPERIMENTAL

Chemicals

Nitroguanidine (NQ) was purchased (Aldrich) and purified by recrystallization from water. Nitrosoguanidine (NSQ) was synthesized according to a published procedure[6]. Cyanoguanidine (CNQ, Eastman Kodak), guanidine hydrochloride (Aldrich), cyanamide (Fisher), melamine (Chemical Service), ammeline (Pfaltz & Bauer) and *m*-phenylenediamine dihydrochloride (Fisher), were commercial products used without further purification.

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HPLC analyses

A Waters liquid chromatographic system (Waters Assoc., Milford, MA, U.S.A.) consisted of the following components: two Model 6000A solvent delivery systems, a Model 721 programmable systems controller, a Model 730 data module, a Lamda-max Model 480 LC spectrophotometer and a Model 710B Waters intelligent sample processor (WISP). A Zorbax C_8 reversed-phase stainless-steel column (25 cm × 4.6 mm I.D., particle size 6 μm, DuPont, Wilmington, DE, U.S.A.) was used.

Conditions for NQ, NSQ, and CNQ were as follows: mobile phase, glass-distilled deionized water; flow-rate, 0.8 ml/min. Effluent was monitored at 235 nm, 0.05 absorbance units full scale (a.u.f.s.). Injection volume was 20 μl. Standard solutions of concentrations 10, 5, 2, 1 and 0.5 mg/l were prepared by dilution of a stock solution freshly prepared each day of analysis.

Conditions for melamine and ammeline were as follows: mobile phase, methanol–0.005 *M* octanesulfonic acid (28:72, v/v) adjusted to pH 3 with acetic acid; flow-rate 1.5 ml/min. Effluent was monitored at 235 nm, 0.1 a.u.f.s., and injection volume was 200 μl. Standard solutions of concentrations 4, 2, 1, 0.4 and 0.2 mg/l were prepared as above.

Precision and accuracy data for the HPLC analyses are given in ref. 5. Correlation coefficients (r^2) were 0.9995.

Ion chromatographic analyses

A Dionex Model 16 ion chromatograph, interfaced with a Varian Vista 401 data station and equipped with a Dionex No. 30831 cation-exchange column in conjunction with a cation concentrator pre-column (Dionex No. 30830), was used. Eluent was 0.25 m*M* *m*-phenylenediamine dihydrochloride in 0.25 m*M* hydrochloric acid at a flow-rate of 2.5 ml/min. The hollow fiber suppressor (Dionex No. 35352, see Results and Discussion) was regenerated with 0.04 *M* potassium hydroxide at a flow-rate of 2–3 ml/min. Samples were injected manually via a 3-ml plastic Luer-Lok syringe into a 100-μl sample loop. The instrument was calibrated by injection of 50, 25, 10, 5 and 1 mg/l standard solutions, prepared from guanidine hydrochloride in water. Response was linear over this range with a typical correlation coefficient of 0.999, and the detection limit was 0.5 mg/l. Precision and accuracy data are listed in ref. 5.

RESULTS AND DISCUSSION

HPLC proved to be the method of choice for all UV-absorbing compounds, which included the three substituted guanidines and the two triazines, melamine and ammeline. Wastewater samples could conveniently be injected onto the column without extraction or pretreatment. Detection limits and retention times are summarized in Table I. Sensitivity for NQ at 235 nm was found comparable to that reported previously at 263 nm[1], while sensitivity for NSQ at 235 nm was ten-fold greater. The use of water alone as mobile phase afforded better resolution and more efficient yet rapid separation of the substituted guanidines. Fig. 1 displays a typical chromatogram.

The triazines were optimally separated by isocratic ion-pair chromatography

TABLE I

HPLC ANALYSES OF SUBSTITUTED GUANIDINES AND *s*-TRIAZINES

Compound	*Low standard (mg/l)*	*Detection limit (ppb*)*	*Retention time (min)*
Nitroguanidine	0.50	100	6.0
Nitrosoguanidine	0.50	42	4.6
Cyanoguanidine	0.51	170	5.4
Melamine	0.21	28	10.0
Ammeline	0.20	21	9.2

* The American billion (10^9) is meant.

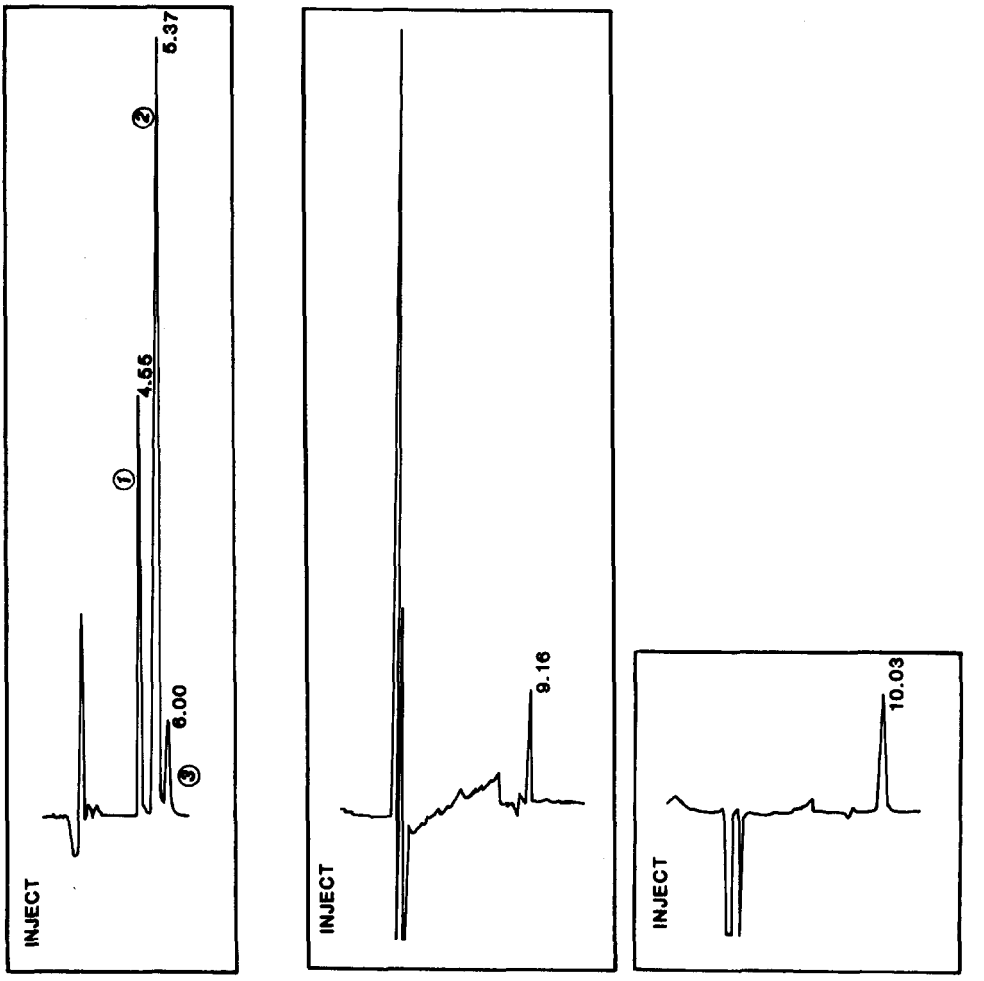

Fig. 1. HPLC of (1) nitrosoguanidine (4.55 min, 1.72 ppm), (2) cyanoguanidine (5.37 min, 5.09 ppm) and (3) nitroguanidine (6.00 min, 0.33 ppm).

Fig. 2. HPLC of ammeline (9.16 min, 0.100 ppm) and melamine (10.03 min, 0.217 ppm).

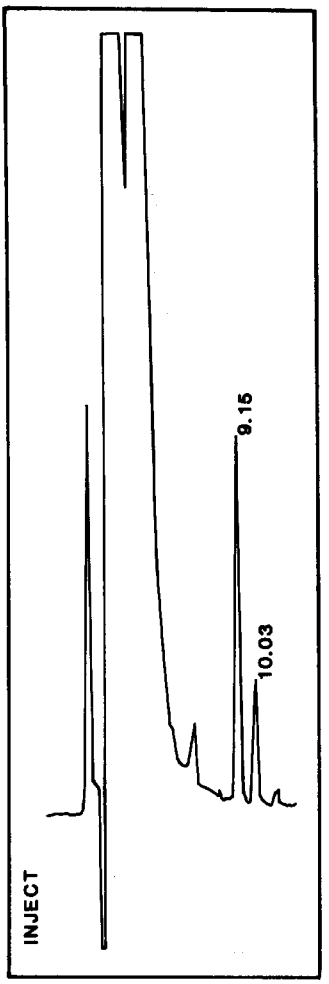

Fig. 3. HPLC analysis of wastewater effluent containing ammeline and melamine.

using octanesulfonic acid at pH 3 (Fig. 2). A typical analysis of NQ production wastewater containing ammeline at 0.38 mg/l and melamine at 0.23 mg/l is depicted in Fig. 3. The previously published HPLC method[2], which involved complex mixtures of triazines, utilized phosphate buffer–methanol gradients and required cryostatic maintenance of the column at 2°C. Nevertheless, widely skewed peaks and excessive baseline drift were apparent. Ion-pair chromatography, on the other hand, afforded a combination of high sensitivity, system simplicity, and excellent peak shape for more precise quantitation. With inclusion of gradients, the method is of potential utility for complex mixtures of triazines including the less polar herbicides.

Guanidine, not amenable to HPLC detection, was optimally determined conductimetrically as the cation by ion chromatography. The method necessitates utilization of a suppressor to reduce the background conductivity of the eluent which in turn enhances the conductivity signal of the analyte. During initial attempts using

a suppressor resin, successive sample injections resulted in increasingly longer retention times. This problem, attributed to possible interaction of guanidinium ion or nitroguanidine with the suppressor resin, was eliminated by replacing the suppressor resin with a fiber suppressor. With this system, anions are exchanged through a membrane wall, thus minimizing any undesirable interactions.

Under the previously described conditions, the retention time of guanidinium ion is 5.1 min. Common monovalent cations, *e.g.* Na^+, K^+ and NH_4^+, have shorter retention times (1.6–2.0 min) and do not interfere. Divalent cations, *e.g.* Ca^{2+} and Mg^{2+}, elute in excess of 30 min. In summary, the method appears to be highly reproducible, with few interferences and adequate sensitivity. It should be noted, however, that during development of the method the cation column began to turn pink. This was attributed to slow polymerization of *m*-phenylenediamine and attachment of the polymer to the resin. There was no immediate effect on the separations, and it was found that polymerization was minimal if air was excluded from eluent reservoirs and columns were covered with aluminum foil to exclude light. Under these conditions, cation columns should be expected to last six months or longer.

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