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Note

Determination of nitrate and nitrite in water using high-performance liquid chromatography

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The determination of nitrate in a wide range of waters is important because of its influence on such factors as biological activity, especially proliferation of algae, potability and public health, and the rapid detinning of tinpate containers in which foods or beverages are heat processed. Because of its intermediate position between ammonia and nitrate during biological conversion, nitrite is also of some interest. Sensitive chemical methods for the determination of these ions are available but these may be too complex and time consuming for laboratories that analyse large numbers of samples. As a consequence, rapid screening techniques employing UV spectrophotometry or ion-selective electrodes have been developed. However, these rapid methods are subject to interferences by organic matter and other ions[1].

Several workers[2–5] have described the separation and measurement of inorganic ions, including nitrate and nitrite, using high-performance liquid chromatography (HPLC). This technique offers simple sample preparation, minimal interference by other materials, high sensitivity and rapid analysis time. In this study we have used a radial compression C_{18} column and a mobile phase of aqueous tetramethylammonium phosphate to analyse the nitrate and nitrite ion levels in water samples collected from various sources. The nitrate levels were compared with those obtained from a UV screening method.

EXPERIMENTAL

Samples and chemicals

Water samples were obtained from the Water Laboratory, Division of Analytical Laboratories, Health Commission of New South Wales. They were collected and delivered to the laboratory by municipal council health inspectors throughout New South Wales. Samples were stored frozen at −18°C prior to analysis.

All chemicals were of analytical-reagent grade. Water was doubly distilled in glass.

Standard solutions

The stock standard solution was prepared by dissolving potassium nitrate (81.5 mg) and sodium nitrite (75.0 mg) in water and diluting to 1 l to give a solution of 50 mg/l for each ion. Working standard solutions of 1, 3, 5 and 10 mg/l were prepared by dilution of the stock solution.

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HPLC conditions

The equipment used was manufactured by Waters Assoc. (Milford, MA, U.S.A.) and consisted of a Model 201 liquid chromatograph equipped with Model 441 absorbance detector set at 214 nm, a Model RCM Z radial compression module and a Radial-Pak C_{18} μBondapak column. Peaks were integrated by a Model 730 data module integrator. The mobile phase was 0.005 *M* aqueous tetramethylammonium phosphate (Waters Assoc., Pic A UV grade reagent) which had been filtered through a Millex filter (Millipore HATF 01300) and degassed under vacuum. A flow-rate of 3.0 ml/min was used. Water samples (20 μl) were injected directly after filtration as for the mobile phase.

UV screening method

The method used is that published by the American Public Health Association[1]. Water samples were filtered as for HPLC analysis. To 50 ml of water was added 1 *M* hydrochloric acid (1 ml), the sample was mixed thoroughly and the ab-

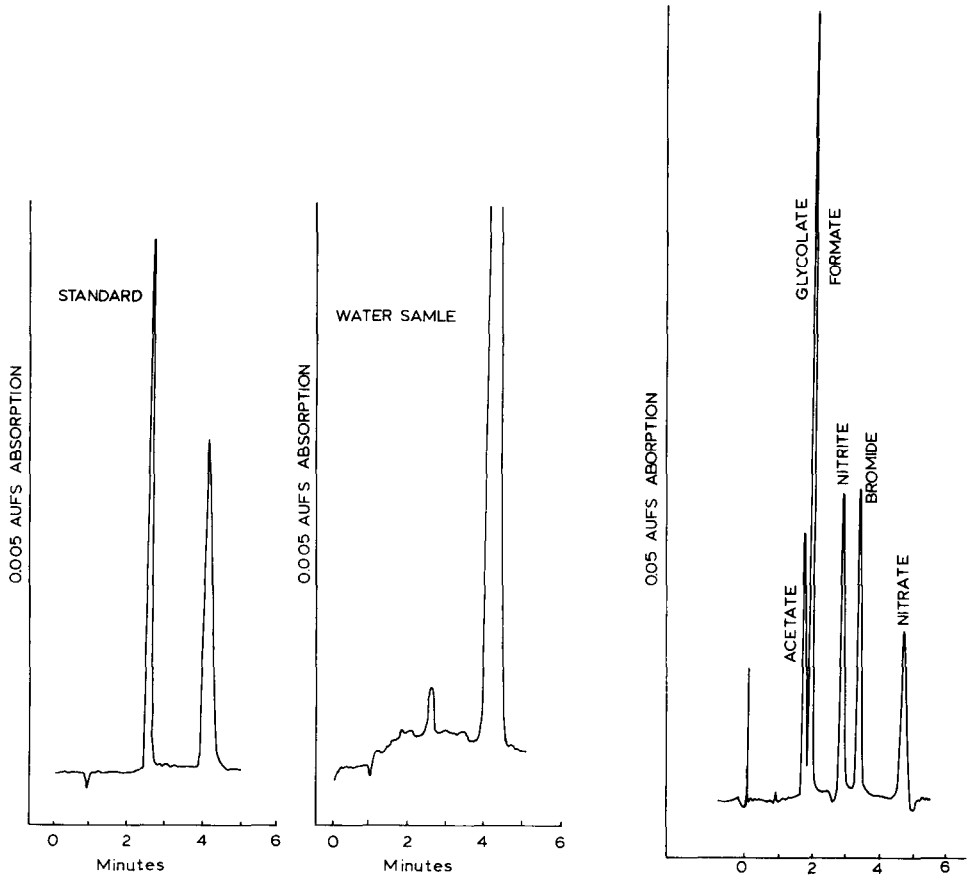

Fig. 1. Typical chromatograms of a standard nitrate nitrite solution and a water sample.

Fig. 2. Chromatogram of a standard mixture of acetate, glycolate, formate, nitrite, bromide and nitrate.

sorbance read at 220 and 275 nm in 10-mm cuvettes against distilled water as blank. The absorbance at 275 nm was doubled and subtracted from that at 220 nm to give the corrected nitrate absorbance. Nitrate was determined by reference to a calibration graph of nitrate concentration *versus* the corrected nitrate absorbance.

RESULTS AND DISCUSSION

Fig. 1 shows typical chromatograms of standard nitrate–nitrite solution and of a water sample. It can be seen that at a flow-rate of 3.0 ml/min each analysis was complete after 6 min. The detection limit for each ion using HPLC, calculated as

TABLE I

LEVELS OF NITRATE AND NITRITE FOUND IN WATER SAMPLES FROM VARIOUS SOURCES

Values in mg/l.

Sample No.	*Source*	*HPLC method*		*UV screening method: nitrate*
		Nitrate	*Nitrite*	
1	Bore	3.1	–*	1.8
2	Bore	2.2	–	1.1
3	Bore	1.3	–	1.7
4	Bore	2.7	–	4.9
5	Bore	0.8	0.03	3.1
6	Bore	1.4	–	2.2
7	Reticulated	32.1	–	14.6
8	River	0.4	–	–**
9	Dam	12.6	–	16.6
10	Dam	3.1	–	0.8
11	Bore	2.9	–	2.9
12	Creek	0.8	–	1.0
13	River	4.0	–	–
14	River	0.3	–	–
15	Spring	6.3	0.8	10.2
16	Rain tank	3.3	–	3.7
17	Roof	3.2	–	3.5
18	Bore	0.5	–	1.6
19	Dam	4.3	0.5	6.0
20	Bore	3.7	–	2.6
21	Bore	2.9	–	1.1
22	River	9.1	0.10	8.9
23	Dam	0.7	–	0.8
24	Dam	0.9	–	–
25	Dam	0.5	–	1.4
26	Roof	3.6	–	4.4
27	Reticulated	4.2	0.04	0.5
28	Reticulated	0.5	–	0.4
29	River	2.4	–	–
30	Roof	0.6	–	1.2
31	Roof	3.3	–	4.6

* < 0.1 mg/l of nitrite detected by HPLC.

** < 0.2 mg/l of nitrate detected by UV method.

three times baseline peak-to-peak random noise level, was 0.1 mg/l. Table I presents the nitrate and nitrite levels determined by HPLC and the nitrate levels found by the UV screening method in water samples from various sources. Low levels of nitrite were found in five of the samples analysed and were not detected in the others. Agreement between the nitrate concentrations found by HPLC and the UV screening method was poor, with the UV method giving substantially higher results for samples 4, 5, 9, 15 and 19 but substantially lower results for samples 1, 2, 7, 10, 13, 21, 27 and 29. Agreement between the nitrate results for most of the other samples was only fair. These discrepancies are probably a consequence of interference by other materials in the UV method, which was intended as a rapid screening method.

The advantages of the present HPLC method over those described earlier[2–5] include the ready availability of C_{18} or equivalent columns, the simple solvent system and the potential for more rapid flow-rates to decrease analysis times even further. The low back-pressure of the system (6890 kPa) offers considerable scope in this regard. Interference from other common anions appears unlikely, as these either do not absorb in the UV region (*e.g.*, SO_4^{2-}, PO_4^{3-}) or they are well resolved from NO_2^- and NO_3^-. This can be seen from Fig. 2, which is a chromatogram of a standard mixture of acetate, glycolate, formate, NO_2^-, Br^- and NO_3^- ions, even though glycolate and formate were not resolved. Chloride ion, not present in this mixture, had a retention time of 0.9 min well away from NO_2^- and NO_3^-.

CONCLUSIONS

The greater accuracy and sensitivity of this HPLC method for the determination of nitrate and nitrite in water, coupled with its simplicity and speed, recommend its adoption as a routine procedure for water analysis in place of the UV screening method. In further work in our laboratories we are investigating the application of this technique to the determination of nitrite and nitrate in foods, especially cured meats.

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