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NITRATE ANALYSIS BY GAS–LIQUID CHROMATOGRAPHY USING THE NITRATION OF 2,4-DIMETHYLPHENOL IN SULPHURIC ACID

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SUMMARY

Nitration of aromatic compounds in a sulphuric acid medium as a method for nitrate determination has been studied by gas chromatography. 2,4-Dimethylphenol was used as reactant because it is highly reactive and can only form a single nitro-derivative. Its concentration and that of sulphuric acid during nitration were optimized. The yield of the toluene extraction method employed was determined and found to be satisfactory. No degradation of the reaction product in the samples was found within 72 h. The detection limit was 0.1 ppm. Concentrations from 1 to 100 ppm and higher can be quantitatively detected without dilution.

INTRODUCTION

The determination of nitrate by nitration of aromatic compounds in a sulphuric acid medium is frequently used. After a purification step (extraction or distillation), the reaction product is usually measured photometrically[1–4]. Gas chromatographic (GC) methods have also been described[3,5–8]. Compared with photometric measurements, they are advantageous in eliminating errors caused by coloured samples or oxidation products of organic compounds formed during the action of the sulphuric acid[9,10] and therefore special purification procedures are not needed. Modern equipment and the use of an internal standard[3,8] have made the accuracy of GC analysis equivalent to that of photometric and polarographic methods for nitrate analysis. The high sensitivity and low detection limit are useful in minimizing the sample size.

The use of benzene[5,6] as reactant in GC nitrate determination is widespread, but it is only very slowly attacked by electrophilic substitution such as nitration. In comparison, alkyl-substituted aromatic compounds react readily because of the strong inductive influence of the alkyl substituent. Based on these considerations, toluene was used as a reactant[3], but it yields not a single derivative but a mixture of *ca.* 62% *o*-, 5% *m*- and 33% *p*-nitrotoluene. For similar reasons, other reactants, such as anisole, tested by Tesch *et al.*[7] are not recommended. A computer-aided interpretation of the results would be very difficult using these methods. Thus a reagent forming a single species of nitro compound was sought.

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Tesch *et al.*[7] and Tan[8] described a method using 1,3,5-trimethoxybenzene as a reactant, where the nitration reaction is followed by decomposition in the sulphuric acid medium, forming nitrobenzene as a single derivative. In the study described here a highly reactive dimethylphenol yields the same effect. From the six dimethylphenol isomers, 2,4-dimethylphenol (DMP) was chosen. It is readily available and can be purified by a single distillation. Furthermore it is liquid at room temperature and can directly pipetted with a micro-syringe. A single mononitro derivative, 6-nitro-2,4-dimethylphenol, is formed during nitration. Conditions for nitration of DMP and the subsequent distillation procedure have been described by Scharrer and Seibel[2]. This method was modified as described in the Experimental section.

By use of an internal standard[3] the reproducibility of GC analysis can be increased. In this work 2-naphthol was found to be suitable. 1,2,4-Trichlorobenzene[3] and hexamethylbenzene[8] which are commonly used in nitrotoluene analysis could not be employed because of their interference with the nitro-DMP peak.

EXPERIMENTAL

2,4-Dimethylphenol (pure; Fluka, Buchs, Switzerland) was purified by incubation with charcoal for 1 h and then distilled under atmospheric pressure. The pure product has a boiling point of 172°C. After this procedure no impurities were found by GC.

Assays were carried out in 10-ml volumetric flasks. Various amounts of DMP were added to 1-ml aqueous samples as described in Results and Discussion. DMP was handled with a Hamilton 725 syringe (Hamilton, Bonaduz, Switzerland). The sulphuric acid concentration and the nitration time were as indicated in the Results and Discussion. Nitration was terminated by making up to 10 ml with distilled water. The solution was then extracted with 0.2 ml toluene (GR; E. Merck, Darmstadt, F.R.G.) containing 1 mg/ml 2-naphthol (GR, Merck). After vigorous shaking, the organic phase was transferred to the sample vessels* used in the automatic sampling device.

GC analysis was done immediately or after defined intervals using a Hewlett-Packard 5835 A chromatograph with automatic sampler 7671 A and Hamilton 701 RN syringe. The column (6 ft. × 2 mm I.D.) was packed with 3% Dexsil 300 GC (Applied Science Labs., State College, PA, U.S.A.) on chromosorb W HP (100–120 mesh) (Johns-Manville, U.S.A.).

Temperature programme: initial, 165°C; then heated at 10°C/min, for 2 min, then at 30°C/min; final, 200°C. Injection port temperature: 200°C. Detector: flame ionization operated with an air–hydrogen flame, 320°C. Carrier gas: nitrogen (GR; AGA, Vienna, Austria); flow-rate 20 ml/min. Injected volume: 0.8 μl. The time required for the chromatographic analysis was 3.5 min; total analysis time (chromatography, cooling and equilibration), 6.5 min. Exhaust gases from the detector were removed by application of a slight vacuum.

The retention times of the components are given in Table I.

* Microvials made from ordinary glass tubing (5 × 0.5 mm) were inserted into the 2-ml flasks of the automatic sampler.

TABLE I

RETENTION TIMES

Component	*No. of tests*	*Mean retention time (min)*	*S.D.*
6-Nitro-2,4-dimethylphenol	136	1.71	0.002
2-Naphthol (internal std.)	136	2.49	0.005

RESULTS AND DISCUSSION

A high level of accuracy is one of the most important aims in trace analysis. For this reason it is necessary to test the sample preparation procedure as well as the instrument performance and the calculation mode. This was done by: (i) optimizing the stoichiometric proportions in the nitration procedure, (ii) introduction of a suitable internal standard (2-naphthol), (iii) checking the analytical column for maximum performance, and (iv) high accuracy in the peak area integration* and calculation of the statistical parameters so as to obtain a high-precision calibration curve. Each test was done four times or more. To calculate the amount of nitro-DMP extracted and the reproducibility of extraction under different conditions, a sample of 0.1 mg nitrate in a single assay (1 ml of a 100 ppm aqueous solution of KNO_3; GR, Merck) was chosen.

Dimethylphenol addition

The lowest amount of DMP needed for a reproducible nitration was tested under otherwise constant conditions. Nitration must be done in a homogeneous phase to maximize reaction rate and reproducibility. An upper limit was set by the solubility of DMP in sulphuric acid; in 65–80% acid as used here the solubility was found to be about 20 ml/l. Consequently the upper limit for 3–5 ml nitration assays described here was 60 μl DMP.

Tests were done under the following conditions: 4-ml nitration assay, 75% sulphuric acid (1 ml aqueous sample, 3 ml conc. sulphuric acid), nitration time, 15 min; DMP added, 20, 40 or 60 μl. Quotients, Q = area of nitro-DMP peak/area internal standard peak, were calculated from the integrated areas. Table II shows

TABLE II

ANALYTICAL REPRODUCIBILITY FOR DIFFERENT DIMETHYLPHENOL AMOUNTS

DMP addition (μl)	20	40	60
Number of tests	8	12	8
Mean Q	0.6433	0.6468	0.6459
S.D.	0.0382	0.0096	0.0087
S.D. (%)	5.94	1.48	1.35

* A simple peak height measurement[6,7] does not result in the desired accuracy.

TABLE III

EFFECTS OF SULPHURIC ACID CONCENTRATION AND REACTION TIME ON NITRATION OF DIMETHYLPHENOL

For %Q calculation the mean Q of the 75% sulphuric acid–15-min nitration assays was set equal to 100%.

	1-min nitration			*5-min nitration*			*15-min nitration*			*25-min nitration*		
Sample/H_2SO_4 (ml)	1:2	1:3	1:4	1:2	1:3	1:4	1:2	1:3	1:4	1:2	1:3	1:4
H_2SO_4 concentration (%)	67	75	80	67	75	80	67	75	80	67	75	80
Number of tests	4	4	4	4	4	4	12	12	12	4	4	4
Mean Q	0.6222	0.6358	0.5773	0.6377	0.6472	0.5805	0.6365	0.6468	0.5831	0.6413	0.6416	0.5843
Q (%)	96.20	98.30	89.25	98.59	100.06	89.75	98.41	100.00	90.15	99.15	99.20	90.34
S.D.	0.0227	0.0153	0.0199	0.0182	0.0147	0.0067	0.0126	0.0096	0.0137	0.0108	0.0106	0.0151
S.D. (%)	3.65	2.40	3.44	2.85	2.27	1.16	1.89	1.48	2.34	1.68	1.65	2.58

the mean values and standard deviations (S.D.). It is seen that the addition of 20 μl DMP is not enough to guarantee satisfactory analytical results. Volumes of 40 and 60 μl were found to yield nearly equal results; 40 μl were added in the subsequent tests.

Sulphuric acid concentration and nitration period

Different recommendations for the sulphuric acid concentration during nitration have been made[2,3]. For nitration of 1,3,5-trimethoxybenzene, Tesch *et al.*[7] found 50–60% sulphuric acid to be optimum. For other reactants the optimum concentration of H_2SO_4 is not yet known. In this investigation concentrations of 67, 75 and 80% were used (Table III).

The nitration time also might have a considerable effect on the analytical reproducibility and yield of reaction product. Nitration periods of 1, 5, 15 and 25 min were compared. It should be noted that 80% sulphuric acid yields the lowest amount of nitro-DMP extracted of all concentrations tested. This indicates that the distribution coefficient of nitro-DMP decreases with increasing sulphuric acid concentration. On the other hand, the reaction rate seems to decrease with decreasing H_2SO_4 concentration (this fact may be noted by comparison of the mean Q values given in Table III for 67% and 75% sulphuric acid at increasing nitration time). As the reproducibility of the results was found to be best in a 75% sulphuric acid medium (indicated by lowest S.D. values of all concentrations tested, see Table III) this concentration is preferable. The influence of the nitration time was unexpectedly small. Therefore the rate of reaction is satisfactory. When a nitration time of 1 min was employed the full yield of nitro-DMP was not attained, but in the other tests similar results were obtained.

TABLE IV

EXTRACTION YIELD OF NITRODIMETHYLPHENOL

	First extraction	*Second extraction*
Number of tests	4	4
Mean Q	0.6468	0.0309
S.D.	0.0096	0.00345
Amount of nitro-DMP extracted (%)	95.22	4.78

TABLE V

STABILITY OF NITRODIMETHYLPHENOL EXTRACTED

%Q as calculated from the analysis done immediately after preparation.

	Time between preparation and analysis (h)			
	0	*24*	*48*	*72*
Number of tests	12	12	12	12
Mean Q	0.6468	0.6470	0.6510	0.6459
Q (%)	100.00	100.03	100.65	99.86
S.D.	0.0096	0.0044	0.0109	0.0104
S.D. (%)	1.48	0.68	1.67	1.61

TABLE VI

COMPARISON OF THE DESCRIBED GC METHOD WITH OTHER ANALYTICAL PROCEDURES FOR NITRATE DETERMINATION IN VARIOUS WATER AND PLANT SAMPLES

Values are means and standard deviations for four separate analyses.

Sample	*Sample size*	*Nitrate concentration (mg/l)*			
		Method 2 of Scharrer and Seibel[12]	*Brucine–H_2SO_4 reaction*[11]	*GC*	*Calculated from the GC results*
River-water samples					
River Kamp, Sept. 9, 1980 Stift Zwettl	1 ml	20.09 ± 0.091	19.53 ± 0.048	19.94 ± 0.061	19.94
	0.5 ml + 0.5 ml of a 50 ppm nitrate standard	34.87 ± 0.069	35.01 ± 0.053	35.07 ± 0.036	20.14
	0.5 ml + 0.5 ml of a 100 ppm nitrate standard	59.32 ± 0.072	59.29 ± 0.050	59.22 ± 0.080	18.44
River Kamp, Sept. 12, 1980 Rosenburg	1 ml	21.99 ± 0.104	22.70 ± 0.094	22.12 ± 0.035	22.12
	0.5 ml + 0.5 ml of a 50 ppm nitrate standard	36.45 ± 0.072	36.18 ± 0.065	36.30 ± 0.041	22.60
	0.5 ml + 0.5 ml of a 100 ppm nitrate standard	60.78 ± 0.086	61.01 ± 0.100	60.87 ± 0.046	21.74

*Plant samples**				
Ranunculus fluitans Erlabach, Aug. 6, 1976	1 ml	75.35	74.28 ± 0.128	3.86
1.926 g dry weight in 100 ml hot water extract	0.5 ml + 0.5 ml of a 50 ppm nitrate standard		63.28 ± 0.0.092	3.97
Potamogeton perfoliatus Erlabach, Aug. 6, 1976	1 ml	46.61	45.04 ± 0.089	2.26
1.994 g dry weight in 100 ml hot water extract	0.5 ml + 0.5 ml of a 50 ppm nitrate standard		47.00 ± 0.113	2.21
Callitriche obtusangula Erlabach, Aug. 6, 1976	0.25 ml + 0.75 ml distilled water	82.78	85.16 ± 0.208	17.56
1.939 g dry weight in 100 ml hot water extract	0.25 ml + 0.75 ml of a 50 ppm nitrate standard		120.63 ± 0.987	17.14
Dry plant powder samples of about 4 mg	1 ml distilled water added			17.21

* Except for the last column (mg per g dry weight), values are mg/l in hot water extract.

All further investigations were done under the following conditions: 1 ml aqueous sample; 3 ml conc. sulphuric acid, yielding 75% aqueous sulphuric acid. 40 μl dimethylphenol; 15 min nitration time, terminated by making up to 10 ml with distilled water.

Extraction yield

Selected samples were twice extracted with 0.2 ml toluene–internal standard mixture. The results obtained in these tests are presented in Table IV. It is seen that a single extraction is sufficient. Increasing the distribution coefficient or multiple extraction steps are not needed.

Stability of the extracts

In the investigations mentioned above, GC analysis was done immediately after sample preparation. The long-term stability of the extracts was tested, by repeating the analysis after 24, 48 and 72 h. From Table V it is seen that no significant decrease in Q was found within a 72-h period.

Calibration curve and detection limit

A calibration curve was prepared from the following concentrations: 100, 50, 25, 10, 5, 2 and 1 ppm. Four samples of each concentration step were analysed. The detection limit was found to be *ca.* 0.1 ppm, consistent with results from photometric analysis[1]. The dependence of Q on concentration was found to be linear within the tested range. The following equation

$$C = aQ + b$$

was employed to fit the date by linear regression analysis. Here C is the concentration of the sample in mg (multiplying by 10^{-3} gives the amount in ppm for a 1-ml simple), $a = 0.154$ and $b = 3.85 \cdot 10^{-4}$. The correlation coefficient, R, was calculated from:

$$R = \frac{a\left(\frac{Q^2}{n} - \bar{Q}^2\right)}{\frac{C^2}{n} - \bar{C}^2}$$

When $n = 28$, a value of $R = 0.999$ was found.

Applications

The method was tested for quantitation of nitrate in various samples, and found to be satisfactory. Some results obtained with waste water and plant samples are compared those from other suitable methods in Table VI.

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REFERENCES

1 A. Gerlach, *Acta Oecologica, Oecol. Plant.*, 1 (1980) 185.
2 K. Scharrer and W. Seibel, *Z. Tierernähr. Futtermittelk.*, 11 (1956) 145.
3 H. Müller and V. Siepe, *Deut. Lebensm.-Rundsch.*, 75 (1979) 175.
4 *Official Methods of Analysis*, Association of Official Analytical Chemists, Washington, DC, 12th ed., 1975, p. 421.
5 D. J. Glover and J. C. Hoffsommer, *J. Chromatogr.*, 94 (1974) 334.
6 W. D. Ross, G. W. Buttler, T. G. Duffy, W. R. Rehg, M. T. Winninger and R. E. Sievers, *J. Chromatogr.*, 112 (1975) 719.
7 J. W. Tesch, W. R. Rehg and R. E. Sievers, *J. Chromatogr.*, 126 (1976) 743.
8 Y. L. Tan, *J. Chromatogr.*, 140 (1977) 41.
9 D. W. Hatcher and E. D. Schall, *J. Ass. Offic. Anal. Chem.*, 48 (1965) 648.
10 C. D. Usher and G. M. Telling, *J. Sci. Food Agr.*, 2 (1975) 1793.
11 *Die Untersuchung von Wasser*, E. Merck, Darmstadt, 7th ed., 1976.