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Note

Determination of aliphatic amines by gas and high-performance liquid chromatography

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Aliphatic amines, such as mono-, di- and trimethylamine (MMA, DMA, TMA) and trimethylamine N-oxide (TMAO), are produced by bacterial degradation of trimethylalkylammonium compounds, such as choline, acetylcholine, carnitine and γ-betaines. This degradation can be environmental, intestinal or faecal[1,2].

This paper describes an analytical investigation by gas chromatography (GC) with flame ionization detection (FID) or thermionic specific detection (TSD) and by high-performance liquid chromatography (HPLC) with conductimetric, refractive index (RI) and electrochemical detection (ED) for the simultaneous determination of the above amines, mainly applied to biological samples such as urine, and an assay procedure adopted consisting in chemical reduction of TMAO to TMA, followed by evaluation of the latter by GC in order to determine the TMAO.

EXPERIMENTAL

Materials

Solvents and chemicals were supplied by Merck (Bracco, Milan, Italy). The apparatus used was a Model 3400 gas chromatograph (Varian, Sunnyvale, CA, U.S.A.) equipped with FID and TSD instruments and a Model 4000i and Model BIO LC 4000 liquid chromatographs (Dionex, Sunnyvale, CA, U.S.A.).

Gases of high purity were supplied by SIAD (Cinisello Balsamo, Milan, Italy). The apparatus used for the TMAO reduction consisted of a dynamic thermal stripper and a Supelco thermal unit, from Supelco (Supelchem, Milan, Italy).

GC

A glass column (375 cm × 2 mm I.D.) packed with 4% Carbowax 20M + 0.8% KOH on Carbopack B (60–80 mesh), TSD, nitrogen at 40 p.s.i. as carrier gas, temperatures of 100°C for the column, 160°C for the injector and 250°C for the detector and monoisopropylamine (MIPA) as internal standard (I.S.) were used.

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Retention times were 1.76 min for MMA, 2.63 min for DMA, 3.24 min for TMA and 5.07 min for the I.S. (Fig. 1).

HPLC with conductimetric detection

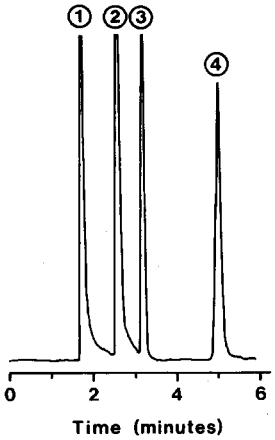
A Dionex Ionpac NS 1 (10 μm) column (250 × 4 mm I.D.) was used. The mobile phase was 2 m*M* hexanesulphonic acid (HSA) at a flow-rate of 1 ml/min. Retention times were 9.6 min for K^+, 13.1 min for MMA, 18.8 min for DMA and 27.5 min for TMA (Fig. 2).

HPLC with RI detection

A μBondapak C_{18} (7 μm) column (300 × 4.6 mm I.D.) (Waters Assoc, Milford, MA, U.S.A.) was used. The mobile phase was 0.05 *M* ammonium acetate in water–methanol (95:5) at a flow-rate of 1 ml/min. RI detection was used.

HPLC with electrochemical detection

A Dionex HPIC-CS3 (10 μm) cationic column (250 × 4 mm I.D.) was used. The mobile phase was 30 m*M* hydrochloric acid at a flow-rate of 1 ml/min. ED was



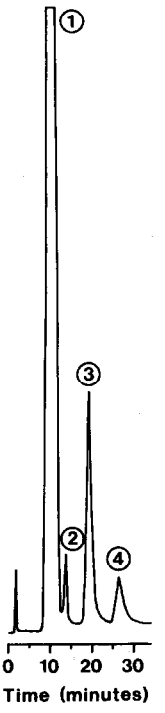


Fig. 1. GC of MMA, DMA and TMA. Column, 4% Carbowax 20 M + 0.8% KOH on Carbopack B, 60–80 mesh (375 cm × 2 mm I.D.); carrier gas, nitrogen at 40 p.s.i.; column temperature, 100°C; injector temperature, 160°C; detector temperature, 250°C; detection, TSD. Peaks: 1 = MMA; 2 = DMA; 3 = TMA; 4 = MIPA (I.S.).

Fig. 2. HPLC of aliphatic amines, with conductimetric detection. Column, Ionpac NS1 (250 × 4 mm I.D.); mobile phase, 2 m*M* HSA; flow-rate, 1 ml/min. Peaks: 1 = K^+; 2 = MMA; 3 = DMA; 4 = TMA.

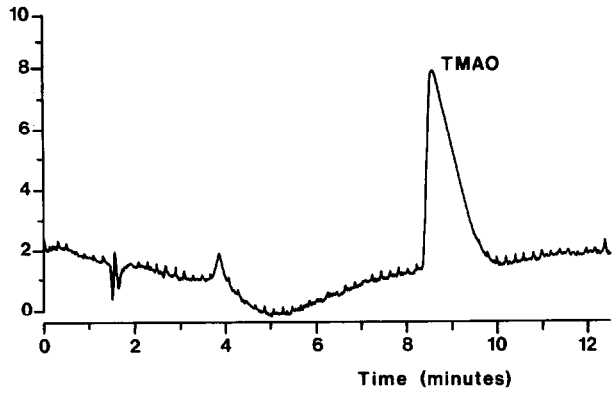


Fig. 3. HPLC of TMAO obtained with ED. Column, HPIC-CS3 (250 × 4 mm I.D.); mobile phase, 30 m*M* HCl; flow-rate, 1 ml/min.

effected with a pulsed amperometric detector from Dionex (PAD-2, Series 883818) equipped with a gold electrode (Dionex). The retention time of TMAO was 8.56 min (Fig. 3).

Chemical reduction of TMAO

Two approaches were attempted. The first, proposed by Lin and Hurng[3], consists in using 0.3% titanium(III) chloride in 10% (w/v) hydrochloric acid at 80°C as reducing agent. The other was reduction by hydrogen–palladium with a 5% Pd on charcoal catalyst, stripped under a flow of hydrogen at 100 ml/min for 30 min at 30°C. The gases were trapped on a Supelco Carbotrap 200 tube by using the dynamic thermal stripper. The tube, now containing TMA as a reduction product of TMAO, was transferred to the Supelco thermal unit to desorb the trapped compound into the

TABLE I

LINEARITY OF DETECTOR RESPONSE FOR MMA, DMA AND TMA WITH A FIXED ANALYTE-TO-INTERNAL STANDARD RATIO, ASCERTAINED BY A CONSTANT DETECTOR RESPONSE FACTOR (D.R.F.) FOR THE GC–TSD METHOD

Trials in quadruplicate for each amount; S.D. = standard deviation; C.V. = coefficient of variation.

Amount injected (ng)	*D.R.F. (mean ± S.D.)*		
	MMA	*DMA*	*TMA*
0.25	0.667 ± 0.012	0.443 ± 0.026	0.406 ± 0.134
0.5	0.617 ± 0.029	0.430 ± 0.023	0.389 ± 0.070
1	0.663 ± 0.012	0.459 ± 0.014	0.410 ± 0.036
2.5	0.634 ± 0.025	0.442 ± 0.032	0.406 ± 0.039
5	0.598 ± 0.024	0.427 ± 0.022	0.390 ± 0.023
10	0.625 ± 0.006	0.432 ± 0.004	0.417 ± 0.019
20	0.638 ± 0.011	0.440 ± 0.008	0.410 ± 0.034
Mean	0.634	0.439	0.404
S.D.	0.024	0.011	0.011
C.V. (%)	3.86	2.46	2.61

TABLE II

LINEARITY OF DETECTOR RESPONSE FOR MMA, DMA AND TMA WITH A VARIABLE ANALYTE-TO-INTERNAL STANDARD RATIO (1:4 → 4:1), ASCERTAINED BY A CONSTANT DETECTOR RESPONSE FACTOR (D.R.F.) FOR THE GC–TSD METHOD

Amine/I.S. (ng)	*D.R.F*		
	MMA	*DMA*	*TMA*
0.25/1	0.652	0.487	0.400
0.5/1	0.672	0.447	0.416
1/1	0.620	0.505	0.436
2/1	0.662	0.452	0.412
4/1	0.666	0.457	0.410
Mean	0.654	0.470	0.415
S.D.	0.021	0.025	0.013
C.V. (%)	3.14	5.36	3.19

TABLE III

RECOVERY OF TMA CONSIDERED AS EXPRESSION OF TMAO, AFTER HYDROGEN–PALLADIUM REDUCTION

TMAO added (μg)	*TMA theoretical (μg)*	*TMA recovered (μg)*	*Recovery (%)*
27.775	14.75	13.39	90.78
55.55	29.50	26.40	89.49
111.1	59.0	53.80	91.19
166.65	88.5	73.21	82.72
222.2	118.0	115.91	98.22
277.75	147.5	135.03	91.86
555.50	295.0	224.0	82.37
1111.0	590.0	522.21	88.51
Mean			89.39
S.D.			5.124
C.V. (%)			5.73

GC column. TMA was determined before and after reduction, the difference representing the TMAO originally present.

RESULTS AND DISCUSSION

HPLC with RI detection allowed all the amines to be evaluated but some interference from endogenous parent substances was observed.

The HPLC–ED technique produced negative results, because the potential needed for the TMAO reduction was very high (between −0.65 and −0.85 V) and the sensitivity was poor. The other approaches for the evaluation of TMAO as such proved to be unsatisfactory. GC and HPLC with conductimetric detection allowed the satisfactory simultaneous determination of MMA, DMA and TMA but not of TMAO.

Chemical reduction of TMAO to TMA appeared to be the only approach able to lead to the detection of all the amines. The method proposed by Lin and Hurng[3] was discarded because it also produced demethylation products (DMA and TMA). The hydrogen–palladium reduction of TMAO proved to give TMA as the only product.

GC with FID or TSD of the aliphatic amines carried out before and after hydrogen–palladium reduction of TMAO allowed TMA, DMA, MMA and TMAO to be determined in biological samples, such as urine, with satisfactory results in terms of linearity, reproducibility, specificity and recovery, as shown in Tables I–III and Fig. 1.

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