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Determination of aromatic amines as their N-dimethylthiophosphoryl derivatives by gas chromatography with flame photometric detection

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Abstract

A selective and sensitive method was developed for the determination of aromatic amines by gas chromatography (GC). Aromatic amines were converted into their N-dimethylthiophosphoryl derivatives and measured by GC with flame photometric detection using two-connected fused-silica capillary column containing DB-1 and DB-17, respectively. The derivatives were sufficiently volatile and stable to give single symmetrical peaks. The calibration graphs for aromatic amines in the range 25–2000 ng were linear and the detection limits at a signal-to-noise ratio of 3 were ca. 30–100 pg injected. This method was successfully applied to smoke samples without prior clean-up. Overall recoveries of aromatic amines added to cigarette smoke samples were 90–108%. Analytical results for the contents of aromatic amines in combustion smokes of various samples are presented.

Keywords: Derivatization, GC; Amines, aromatic

1. Introduction

Aromatic amines such as aniline and its substituted analogues are widely used as industrial intermediates [1–3] in the manufacture of carbamate and urethane pesticides, dyestuffs, cosmetics and medicines. These amines are also employed in the rubber industry as antioxidants and antiozonants [4] and as components in epoxy and polyurethane polymers [5]. Aromatic amines occur in a number of ambient environments such as air, water and soil [6–10], and are a source of serious social and hygienic problems as important occupational and environmental pollutants. Many of these amines are known to be highly mutagenic and carcinogenic [6,7,11–13] and to form adducts with proteins and DNA [8–10]. Therefore, the measurement of aromatic amines in the environment is very important.

The determination of aromatic amines has been carried out by capillary zone electrophoresis (CZE), high-performance liquid chromatography (HPLC), gas chromatography (GC) or GC–mass spectrometry with selected-ion monitoring (GC–MS-SIM). However, CZE with UV detection [14] lacks sensitivity and require preliminary clean-up of the samples by solid-phase extraction. HPLC analyses of aromatic amines by

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using UV [15,16] and fluorescence detection [17,18] require tedious and time-consuming procedures for the preparation of the sample. GC methods with electron-capture [18–21] and nitrogen–phosphorus thermionic detection [15,22,23] are specific and sensitive, but these methods require anhydrous conditions for derivatizations. Although GC–MS-SIM methods [21,24–26] are highly sensitive and specific, they require expensive equipment.

Recently, we have developed a selective and sensitive method for the determination of aliphatic secondary amines by GC with flame photometric detection (FPD), in which these compounds are analysed as their N-diethylthiophosphoryl derivatives [27,28]. By this method aromatic amines could be determined easily, rapidly and quantitatively in an aqueous medium without any further clean-up of the sample. In this paper, we report a method for the determination of aromatic amines as their N-dimethylthiophosphoryl derivatives by GC–FPD. By using this method, the contents of these amines in combustion smokes of several samples were also studied.

2. Experimental

2.1. Reagents

Aniline (AN), 2-toluidine (2-T) and 3-toluidine (3-T) were purchased from Nacalai Tesque (Kyoto, Japan) and 2,3-xylidine (2,3-X), 2,4-xylidine (2,4-X), 2,6-xylidine (2,6-X), 3-ethylaniline (3-E) and 4-ethylaniline (4-E) from Tokyo Kasei Kogyo (Tokyo, Japan). Each amine was dissolved in acetonitrile to make a stock solution of a concentration of 10 mg/ml and used after dilution with acetonitrile to the required concentration. 3-Phenyl-1-propylamine (Aldrich, Milwaukee, WI, USA) as an internal standard (I.S.) was dissolved in 0.01 *M* hydrochloric acid at a concentration of 1 μg/ml. Dimethyl chlorothiophosphate (DMCTP) (Tokyo Kasei Kogyo) was used as a 16% solution in acetonitrile after distillation. This solution was stable for at least 1 month. All other chemicals were of analytical-reagent grade.

2.2. Preparation of samples

Foods, cigarettes, wood chips and rubber were purchased at local retail markets and were treated for analyses on the same day. The samples (ca. 1–8 g) were smoked with a laboratory-made smoking machine described by Pieraccini et al. [24]. In this machine, the samples were heated with soldering copper and the resulting combustion smokes were bubbled through 25 ml of 5% hydrochloric acid containing 20 m*M* ascorbic acid in trapping bottles. The smoke samples were directly used for the derivatization after neutralization with 6 *M* sodium hydroxide.

2.3. Derivatization procedure

An aliquot of sample containing 25–2000 ng of aromatic amines was pipetted into a 10-ml Pyrex glass tube with a PTFE-lined screw-cap. To this solution was added 0.2 ml of 1 μg/ml I.S. solution and 0.3 ml of 30% potassium carbonate solution and the total volume was made up to 1 ml with distilled water. Immediately after addition of 0.25 ml of 16% DMCTP, the mixture was heated at 60°C for 30 min with the tube tightly capped. In order to remove the excess of reagent, the reaction mixture was heated again at 100°C for 5 min after addition of 0.1 ml of 0.5 *M* cysteic acid. Then the reaction mixture was extracted with 0.2–0.4 ml of *n*-hexane and 1 μl of this extract was injected onto the GC–FPD system. The derivatization process for aromatic amines is shown in Fig. 1.

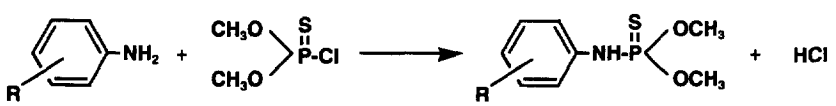

Fig. 1. Process for derivatization of aromatic amines. R = alkyl group.

2.4. Gas chromatography

GC analysis was carried out with a Shimadzu Model 14A gas chromatograph equipped with a flame photometric detector (526-nm interference filter inserted in the optical path). Two connected fused-silica capillary columns (J & W, Folsom, CA, USA) containing, respectively, DB-1 (15 m × 0.53 mm I.D., film thickness 1.5 μm) and DB-17 (15 m × 0.53 mm I.D., film thickness 1.0 μm) with a two-way press-fit fused-silica tube were used. The operating conditions were as follows: column temperature, maintained at 200°C for 10 min then programmed at 5°C/min from 200 to 250°C; injection and detector temperatures, 280°C; nitrogen flow-rate, 10 ml/min (flow velocity, 76 cm/s). The peak heights of aromatic amines and the I.S. were measured and the peak-height ratios against the I.S. were calculated.

2.5. Gas chromatography–mass spectrometry

A Hewlett-Packard Model 5890A gas chromatograph was operated in conjunction with a VG Analytical Model 70-SE mass spectrometer and a VG-11-250J mass data system. A fused-silica capillary column containing cross-linked OV-1 (Quadrex, New Haven, CT, USA) (12 m × 0.25 mm I.D., film thickness 0.25 μm) was used. The column temperature was programmed at 7°C/min from 150 to 240°C, the injection temperature was 240°C, ion-source temperature 250°C, ionizing voltage 40 eV and helium flow-rate 1 ml/min.

3. Results and discussion

Phosphorus-containing derivatives have been prepared with dimethylaminomethylphosphine [29,30], dimethylthiophosphinic chloride [31], diethyl chlorophosphate [32] and ethylphosphonothioic dichloride [33], and analysed by GC with alkali flame ionization detection. Although these methods are highly sensitive, the preparation of the derivatives requires a lengthy procedures and anhydrous conditions. We investigated a simple derivatization method using DMCTP as a phosphorus-containing reagent. The N-dimethylthiophosphorylation of aromatic amines with DMCTP proceeded in aqueous alkaline media. As shown in Fig. 2A–C, this reaction was completed within 30 min at 60°C by using 40 μl of DMCTP. Although aliphatic amines [27] and amino acids [34] rapidly reacted with diethyl chlorothiophosphate or DMCTP, the reaction of aromatic amines with these reagents required a relatively long time because of the lower reactivity of aromatic amino group. Excess DMCTP was removed by reaction with cysteic

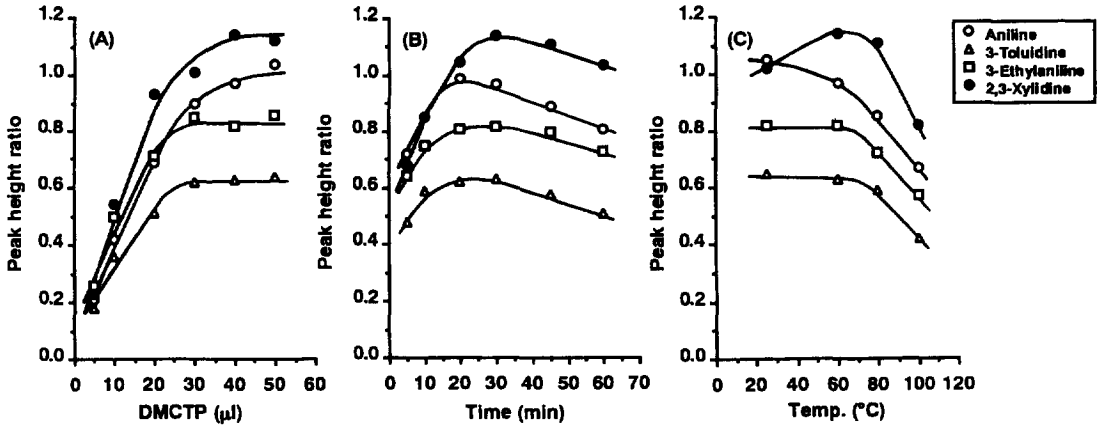

Fig. 2. Effects of (A) amount of dimethyl chlorothiophosphate, (B) reaction time and (C) temperature on the N-dimethylthiophosphorylation of aromatic amines.

acid. Dimethylthiophosphoryl derivatives of cysteic acid and other amino acids in the sample were not extracted into *n*-hexane in alkaline media. On the other hand, the N-dimethylthiophosphoryl derivatives of aromatic amines were quantitatively extracted into *n*-hexane.

The structures of the aromatic amine derivatives were confirmed by GC–MS analysis. As shown in Fig. 3, a molecular ion peak (M^+) was observed for each of the derivatives and other common ion peaks which were useful for structure elucidation were $[M-31]^+$ (OCH_3), $[M-62]^+$ $(OCH_3)_2$, $[M-125]^+$ $[S=P(OCH_3)_2]$, $[M-140]^+$ $[NHPS(OCH_3)_2]$ and *m/z* 125. The derivatives were stable under normal laboratory conditions and no decomposition was observed during GC analysis.

As shown in Fig. 4A, eight aromatic amines could be completely resolved as single and symmetrical peaks within 15 min by using two connected capillary columns containing DB-1 and DB-17, respectively, and gave an excellent response in the flame photometric detector, although the reagent peak was observed in front. The minimum detectable amounts of AN, 2-T, 3-T, 3-E, 4-E, 2,3-X, 2,4-X and 2,6-X at a signal-to-noise ratio of 3 under our instrumental conditions were 50, 100, 40, 30, 30, 50, 50 and 100 pg injected, respectively. The calibration graphs for these amines were constructed using as the I.S. 3-phenyl-1-propylamine, which showed a similar behaviour to other amines during the derivatization and was well separated from other amines on the chromatogram. As shown in Table 1, a linear response was obtained with correlation coefficients above 0.999 in the range 50–1000 ng for AN, 2-T, 2,3-X and 2,4-X, 25–550 ng for 3-T, 3-E and 4-E and 100–2000 ng for 2,6-X. The within-run R.S.D.s for these amines were 0.9–

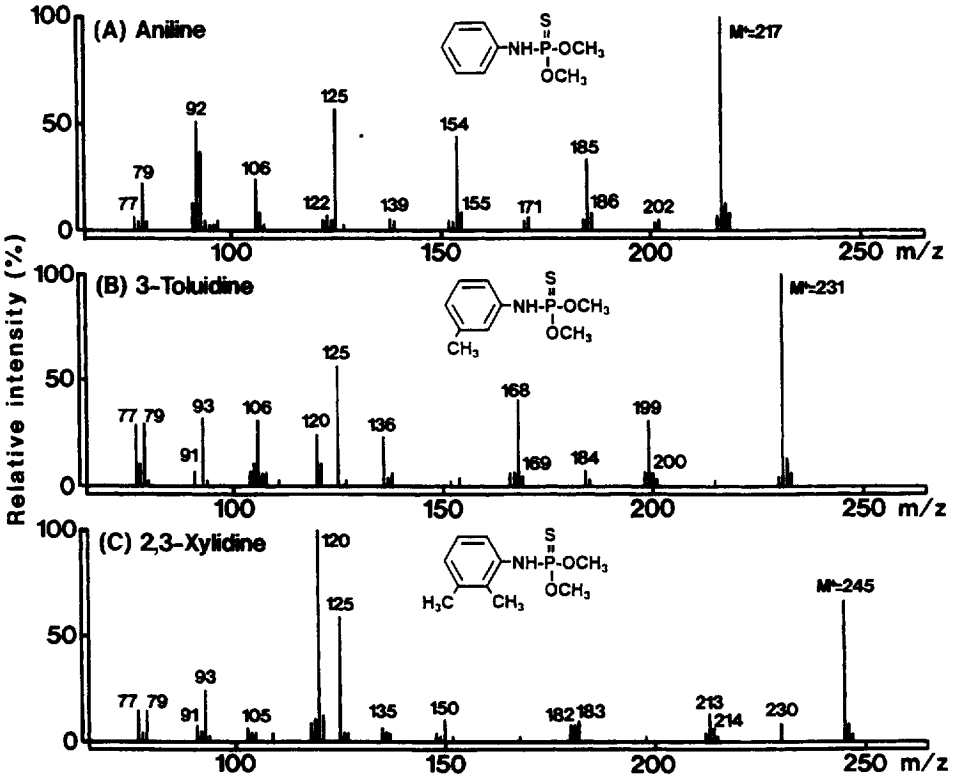

Fig. 3. Mass spectra of the N-dimethylthiophosphoryl derivatives of aromatic amines. (A) Aniline; (B) 3-toluidine; (C) 2,3-xylidine.

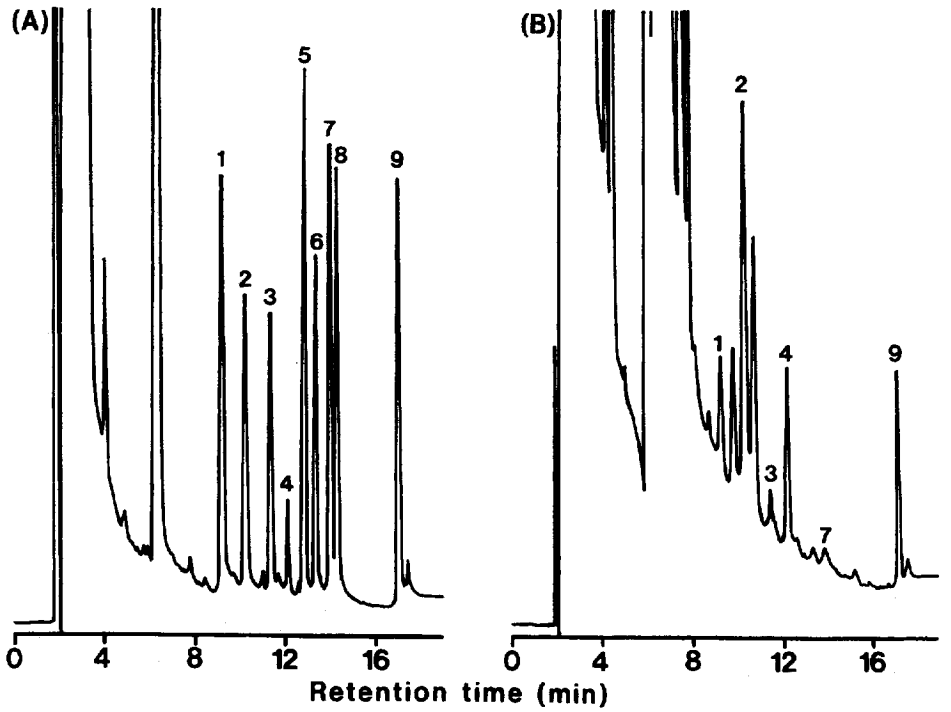

Fig. 4. Typical gas chromatograms obtained from (A) standard aromatic amines and (B) cigarette side-stream smoke. GC conditions are given in Experimental. Peaks: 1 = aniline (2.5 ng); 2 = 2-toluidine (2.5 ng); 3 = 3-toluidine (1.25 ng); 4 = 2,6-xylidine (5 ng); 5 = 2,4-xylidine (2.5 ng); 6 = 3-ethylaniline (1.25 ng); 7 = 2,3-xylidine (2.5 ng); 8 = 4-ethylaniline (1.25 ng); 9 = 3-phenyl-1-propylamine (I.S.) (1 ng).

8.3% ($n = 4$) and the between-run R.S.D.s over a period of 4 days were 1.5–9.7% ($n = 4$).

The method developed was successfully applied to combustion smoke samples. Combustion smoke was collected with a laboratory-made smoking machine [24] by bubbling in 5% hydrochloric acid containing 20 m*M* ascorbic acid as an antioxidant. The main- and side-stream smokes of cigarette could be separately collected by this apparatus. The collected smoke samples could be

Table 1
Linear regression data for aromatic amines

Amine	Range (μg)	Regression line[a]	Correlation coefficient (r)	Number of data (n)
Aniline	0.050–1.00	$y = 1.927x + 0.0537$	0.999	15
2-Toluidine	0.050–1.00	$y = 1.314x + 0.0006$	0.999	15
3-Toluidine	0.025–0.50	$y = 2.602x + 0.0015$	0.999	15
3-Ethylaniline	0.025–0.50	$y = 3.362x + 0.0008$	1.000	15
4-Ethylaniline	0.025–0.50	$y = 4.601x - 0.0022$	1.000	15
2,3-Xylidine	0.050–1.00	$y = 2.382x - 0.0093$	0.999	15
2,4-Xylidine	0.050–1.00	$y = 2.557x - 0.0096$	0.999	15
2,6-Xylidine	0.100–2.00	$y = 0.186x + 0.0058$	0.999	15

[a] y = peak-height ratio; x = amount of amine (μg).

Table 2
Recoveries or aromatic amines added to cigarette smoke samples

Smoke sample	Amine	Added (μg/g)	Amount found (μg/g)		Recovery (%)
			No addition	With addition	
Main-stream	Aniline	3.0	0.56 ± 0.04	3.65 ± 0.18	103
	2-Toluidine	3.0	0.56 ± 0.02	3.69 ± 0.19	104
	3-Toluidine	1.5	ND[a]	1.53 ± 0.04	102
	3-Ethylaniline	1.5	ND	1.36 ± 0.05	91
	4-Ethylaniline	1.5	ND	1.41 ± 0.02	94
	2,3-Xylidine	3.0	ND	3.23 ± 0.05	108
	2,4-Xylidine	3.0	ND	2.97 ± 0.06	99
	2,6-Xylidine	6.0	5.66 ± 0.23	11.89 ± 0.23	104
Side-stream	Aniline	3.0	2.63 ± 0.13	5.40 ± 0.23	92
	2-Toluidine	3.0	17.58 ± 0.87	20.69 ± 1.24	104
	3-Toluidine	1.5	0.65 ± 0.03	2.05 ± 0.10	93
	3-Ethylaniline	1.5	ND	1.47 ± 0.06	98
	4-Ethylaniline	1.5	ND	1.35 ± 0.02	90
	2,3-Xylidine	3.0	0.47 ± 0.02	3.43 ± 0.09	99
	2,4-Xylidine	3.0	ND	2.96 ± 0.16	99
	2,6-Xylidine	6.0	49.13 ± 1.38	55.13 ± 1.80	100

[a] ND = not detectable.

directly derivatized after neutralization without any further clean-up procedure. As shown in Fig. 4B, the aromatic amines in cigarette side-stream smoke could be detected without any interference from co-existing substances, although several unidentified peaks (probably derived from aliphatic amines in the sample) were observed. As shown in Table 2, the overall recoveries of aromatic amines added to cigarette smoke samples were 90–108% ($n = 4$) and the reproducibility was found to be satisfactory. The contents of aromatic amines in the combustion smokes of several samples are given in Table 3. AN, 2-T, 2,3-X and 2,6-X were present at higher concentrations in some smoke samples. The contents of these amines in cigarette side-stream smoke were found to be more than five times higher than those in the main-stream smoke sample. These results were similar in tendency to those obtained by Pieraccini et al. [24], although they reported the presence of 4-toluidine, 2-ethylaniline and 2,5-xylidine in cigarette smokes. However, these amines, which are co-eluted between 3-T and 2,6-X in our GC system, were not detected at all in any of the smoke samples investigated in this study. These results suggest that compounds which may be harmful to the health can be formed during the combustion process.

4. Conclusion

These experiments have conclusively demonstrated that aromatic amines can be accurately and precisely determined by GC–FPD as their N-dimethylthiophosphoryl derivatives. This method is selective and sensitive, and combustion smoke samples can be analysed directly without prior clean-up and any interference from other substances. We believe that this method provides a useful tool for environmental analysis.

Table 3
Aromatic amine contents in combustion smokes from various samples

Sample	Content[a] (μg/g)							
	AN	2-T	3-T	3-E	4-E	2,3-X	2,4-X	2,6-X
Chicken	1.86 ± 0.09	1.45 ± 0.00	ND[b]	0.57 ± 0.03	ND	1.55 ± 0.04	0.53 ± 0.04	4.02 ± 0.16
Sausage	1.13 ± 0.04	1.38 ± 0.00	ND	0.38 ± 0.00	0.25 ± 0.01	1.66 ± 0.05	0.29 ± 0.01	4.72 ± 0.15
Sardine	0.10 ± 0.00	ND	0.45 ± 0.02	0.89 ± 0.04	2.42 + 0.05	9.26 ± 0.35	0.93 ± 0.00	13.19 ± 0.57
Dried squid	1.84 ± 0.09	1.22 ± 0.06	ND	0.53 ± 0.03	ND	7.54 ± 0.12	1.43 ± 0.05	ND
Bread	1.73 ± 0.05	5.48 ± 0.05	0.38 ± 0.01	0.31 ± 0.02	ND	ND	ND	7.61 ± 0.27
Black pepper	2.45 ± 0.08	6.33 ± 0.16	ND	2.79 ± 0.14	ND	2.75 ± 0.07	ND	19.51 ± 0.46
Soy sauce	0.36 ± 0.02	3.92 ± 0.06	0.36 ± 0.02	0.60 ± 0.01	ND	5.93 ± 0.11	0.24 ± 0.01	4.44 ± 0.00
Wood chips	ND	8.24 ± 0.39	ND	ND	0.75 ± 0.05	ND	ND	ND
Rubber	10.30 ± 0.37	ND	ND	4.50 ± 0.10	ND	0.98 ± 0.04	1.08 ± 0.02	ND
Cigarette (main-stream)	0.56 + 0.04	0.56 ± 0.02	ND	ND	ND	ND	ND	5.66 ± 0.23
Cigarette (side-stream)	2.63 ± 0.13	17.58 ± 0.87	0.65 ± 0.03	ND	ND	0.47 ± 0.02	ND	49.13 ± 1.38

[a] Mean ± S.D. ($n = 3$).
[b] ND = not detectable.

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