CHROM. 16,100

TRACE ANALYSIS OF AMINES AND ISOCYANATES USING GLASS CAPILLARY GAS CHROMATOGRAPHY AND SELECTIVE DETECTION

II. DETERMINATION OF AROMATIC AMINES AS PERFLUOROFATTY ACID AMIDES USING NITROGEN-SELECTIVE DETECTION

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SUMMARY

A method for the trace analysis of aromatic amines is presented. It involves derivatization of the amines to the corresponding amides by reaction with a perfluorofatty acid anhydride. The amides were separated by glass capillary gas chromatography, and picogram amounts were quantitated using on-column injection and nitrogen-selective detection with a Varian thermionic specific detector.

The method was applied to amines of interest from work environment health aspects and to polyurethane pyrolysis products. Detection limits were of the order 10–20 pg amine. Comparison with a recent gas chromatographic amine analysis method from this laboratory utilizing electron-capture detection was made, and differences between the two detection methods are discussed.

INTRODUCTION

Gas chromatographic (GC) trace analysis of amines has met with considerable difficulties owing to the tendency of these compounds to become adsorbed in the analytical system, *i.e.* in sample vessels, injection system and chromatographic column. Hence primary and secondary amines are generally derivatized[1,2] before GC separation in order to produce compounds less susceptible to adsorption. Oxidation of free amines is also a problem that can often be avoided by derivatization. Monitoring of sub-nanogram amounts of amine derivatives in GC trace analysis has usually been performed with electron-capture detection (ECD), and both packed and capillary columns have been used[3,4].

Nitrogen-selective detection of underivatized amines has been investigated in
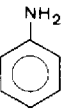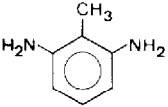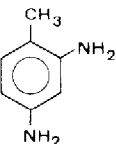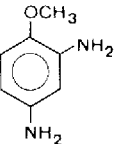
TABLE I

LIST OF INVESTIGATED AMINES
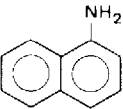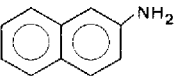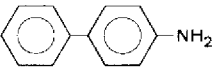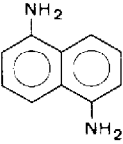
No.	Amine	Formula
1	Aniline CA* No. 62-53-3, benzeneamine	
2	2,6-Toluenediamine (2,6-TDA) CA No. 823-40-5 2-methyl-1,3-benzenediamine	
3	2,4-Toluenediamine (2,4-TDA) CA No. 95-80-7, 4-methyl-1,3-benzenediamine	
4	2,4-Diaminoanisole (2,4-DAA) CA No. 615-05-4, 4-methoxy-1,3-benzenediamine	
5	α-Naphthylamine CA No. 134-32-7, 1-naphthaleneamine	
6	β-Naphthylamine CA No. 91-59-8 2-naphthaleneamine	
7	4-Aminobiphenyl CA No. 92-67-1, (1,1'-biphenyl)-4-amine	
8	1,5-Naphthalenediamine CA No. 2243-62-1	

TABLE I *(continued)*

No.	Amine	Formula
9	Benzidine CA No. 92-87-5, (1,1'-biphenyl)-4,4'-diamine	H_2N—⬡—⬡—NH_2
10	Methylenedianiline (MDA) CA No. 101-77-9, 4,4'-methylenebis-benzeneamine	H_2N—⬡—CH_2—⬡—NH_2
11	*o*-Tolidine (TODA) CA No. 119-93-7, 3,3'-dimethyl-(1,1'-biphenyl)-4,4'-diamine	H_3C⬡CH_3 H_2N—⬡—⬡—NH_2
12	Methylene-bis-*o*-chloroaniline (MOCA, MBOCA) CA No. 101-14-4, 4,4'-methylenebis-(2-chlorobenzeneamine)	Cl⬡Cl H_2N—⬡—CH_2—⬡—NH_2
13	*o*-Dianisidine (DADA) CA No. 119-90-4, 3,3'-dimethoxy-(1,1'-biphenyl)-4,4'-diamine	H_3CO⬡OCH_3 H_2N—⬡—⬡—NH_2

* CA = *Chemical Abstracts.*

combination with both packed[5,6] and capillary[7,8] GC columns, using alkali treatment of the support to minimize adsorption. For packed columns, sub-nanogram detection limits have been achieved[6], but for capillary columns this has not been realized, undoubtedly because amines are strongly absorbed on to the glass wall. In this work, capillary GC combined with nitrogen-selective detection (thermionic specific detection, TSD) for the trace analysis of derivatized aromatic amines is investigated.

For mere detection purposes, derivatization of amines offers no advantages for the nitrogen-selective detection, but the vast improvement in the chromatographic properties and increased stability of sample compounds often warrants the extra preparative work involved. The problem of analysing free amines in picogram amounts using capillary GC and TSD will be dealt with in a future paper.

In the present work, aromatic amines of interest because of safety aspects in the work environment (see Table I) were derivatized to their corresponding amides by reaction with a fatty acid anhydride, preferably a perfluorofatty acid anhydride:

$$RNH_2 + (C_nF_{2n+1}CO)_2O \rightarrow RNHCOC_nF_{2n+1} + C_nF_{2n+1}COOH \qquad (1)$$

The amides formed were separated on an OV-73 capillary column and detected with a Varian thermionic specific detector. To ensure quantitative determinations, the on-column injection technique was applied.

EXPERIMENTAL

Apparatus

Chromatographs and detectors. A Varian Model 3700 gas chromatograph equipped with a Carlo Erba on-column injection system and a Varian thermionic specific detector was employed for the nitrogen-selective detection. The detector was surrounded by a gauze net cage to minimize external influences, such as air draughts. For ECD a Carlo Erba Model 4160 gas chromatograph equipped with a Model HT-25 ECD and ECD Control Module 251 was used. Chromatograms were recorded on Servogor Model 310 recorders, and a Hewlett-Packard Model 3390A "reporting integrator" was used for peak evaluation.

Gases. The carrier gas used for the TSD measurements was helium, and the make-up gas was helium or nitrogen. They were dried over molecular sieve 5A and deoxygenated using an "indicating oxytrap" (Chrompack, Middelburg, The Netherlands). For ECD measurements helium carrier gas and argon–methane (95:5) make-up gas were treated likewise. Hydrogen and air for the thermionic specific detector were used without extra purification.

Materials

Chemicals. Aromatic amines, heptafluorobutyric acid anhydride (HFBAA) and pentafluoropropionic acid anhydride (PFPAA) were as previously described[4]. Monochloroacetic acid anhydride (MCAAA) was obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.) and acetic acid anhydride (AAA) from E. Merck (Darmstadt, F.R.G.). Octamethylcyclotetrasiloxane (D_4) and OV-73 stationary phase were from Ohio Valley Specialty Chemicals (Marietta, OH, U.S.A.).

Solvents and solutions. A pH 7.0 phosphate buffer was prepared as described previously[4]. Solvents used were all of p.a. grade (see also ref. 4).

Procedure

Standard solutions of amines were prepared by dissolving accurately weighed amounts of each amine in acetonitrile. This solution was diluted to appropriate concentrations with toluene, as described in ref. 4.

Derivative preparation. Amides of HFBA, PFPA and MCAA were obtained by addition of 20 μl (*ca.* 20 mg for MCAAA) of the reagent to 1 ml of amine standard solution, which resulted in quantitative formation of the amides within 5 min at room temperature. For MCAAA gentle heating was required to dissolve the reagent. For the AAA reagent, quantitative reaction was achieved by heating to 70°C for 1 h. After reaction, excess reagent and liberated acids were extracted with 1 ml of pH 7.0 phosphate buffer.

Column preparation. Duran 50 borosilicate glass capillary columns were drawn on a Carlo Erba GCDM Model 60 glass capillary drawing machine and leached according to Grob[9]. The columns were dried by nitrogen purging for 2 h at 250°C. Deactivation was achieved by dynamic coating with pure D_4 followed by flame-seal-

ing and thermal treatment at 400°C over night. After rinsing with toluene, methanol and diethyl ether, OV-73 stationary phase was applied by static coating from pentane solutions.

Detection. The thermionic selective detector was optimized for maximum sensitivity to nitrogen by adjusting hydrogen, air and make-up gas flow-rates, bead heating current and bias voltage, whilst repeatedly injecting standard solutions of PFPA derivatives. Typical settings were gas flow-rates of 4.0 ml/min of hydrogen, 180 ml/min of air and 22 ml/min of helium make-up gas, bead heating current of 5.5 A, a bias voltage of −10 V and a detector base temperature of 300°C.

Quantitative analysis. With TSD quantitative analysis was based on peak area measurement. The linear response range was established by plotting peak area against concentration for injected standard solutions.

RESULTS AND DISCUSSION

Formation of amides and their chromatographic properties
　　Four acylating agents, AAA, MCAAA, PFPAA and HFBAA were investigated with regard to yield, stability, separation properties and TSD sensitivity of the amides formed. Without a catalyst all but the AAA gave rapid and quantitative reactions after 5 min at room temperature. For AAA, 1 h reaction time at 70°C gave quantitative yields for the two compounds tested, α- and β-naphthylamine. The use of an amine catalyst[1,2] was avoided because it resulted in increased noise levels in the chromatograms, owing to impurities in the catalyst. Investigated derivatives were stable for several days at room temperature, and repeated extraction of derivative solutions with pH 7.0 phosphate buffer gave no loss of derivatives.

　　Fig. 1 shows a chromatogram of α- and β-naphthylamine derivatives formed on reaction with the four acylating agents. It is seen that AAA and MCAAA give amides with appreciably higher elution temperatures than the two fluoro-containing acid anhydrides. This would mean that some of the diamines in Table I, *e.g.* MDA and MOCA, would form diamides with AAA and MCAAA requiring elution temperatures at or above 300°C. It should be noted that MCAA and especially AA derivatives tend to show somewhat tailing peaks at picogram levels, which is not the case for HFBA and PFPA derivatives. The separation characteristics of the HFBA and PFPA derivatives were discussed in Part I of this series[4]. Fig. 2 demonstrates the separation of PFPA derivatives of all investigated amines on an OV-73 column.

　　It is evident from Fig. 1 that halogen substitution in the acylating agent has little or no influence on the TSD sensitivity of the amides formed; the response is mainly dependent on the nitrogen content in sample compounds, as discussed below.

　　Thus AAA is unsuitable as an acylating agent for GC analysis of aromatic amines, because of high elution temperatures and the tendency of derivatives to give tailing peaks at picogram levels. MCAAA is attractive for trace analysis of not too high-boiling amines, while PFPAA and HFBAA are useful for general application. In the case of low-boiling amines, such as aniline, the MCAAA reagent is preferred owing to better separation between resulting amides and the solvent peak.

Chromatographic system
　　Injection system. The use of cold on-column injection in capillary GC has now
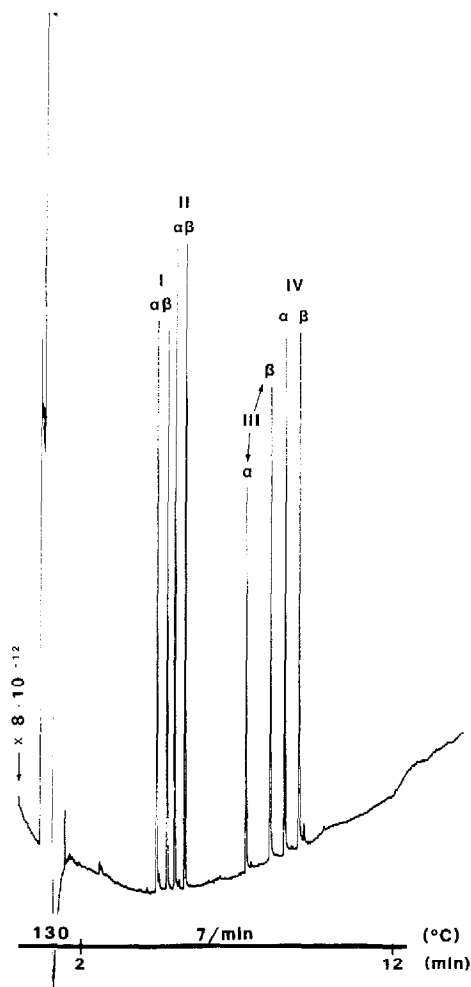
Fig. 1. Comparison between different acylating reagents. On-column injection of 2-μl aliquots of a 500 pg/μl solution of α- and β-naphthylamine derivatives with PFPAA (I), HFBAA (II), AAA (III) and MCAAA (IV). Column, 20 m × 0.32 mm I.D. Duran 50 glass capillary column with OV-73 stationary phase, film thickness 0.4 μm. Temperature programming as shown. Carrier gas, helium at 1.0 kg/cm². Thermionic specific detector; bead-heating current, 6.5 A; bias voltage, −10 V; temperature, 290°C; hydrogen flow-rate, 4 ml/min; air flow-rate, 180 ml/min; make-up gas, nitrogen (flow-rate 22 ml/min).

become widely accepted[10], despite peak-splitting anomalies recently described by Grob[11]. In Part I of this series[4] it was demonstrated that injection of 2-μl aliquots of 25 pg/μl solutions of PFPA derivatives of aromatic amines in toluene could be performed with a reproducibility of 0.8–1.7% for samples over a wide elution temperature range (150–300°C). These results were obtained with a Carlo Erba on-column injector in its original mounting on a Carlo Erba Model 4160 gas chromatograph. In this work the same injector was combined with a Varian Model 3700 gas chromatograph, where the benefits of a reliable thermionic specific detector could be utilized. Initial fears of adverse effects due to different injector surroundings, resulting
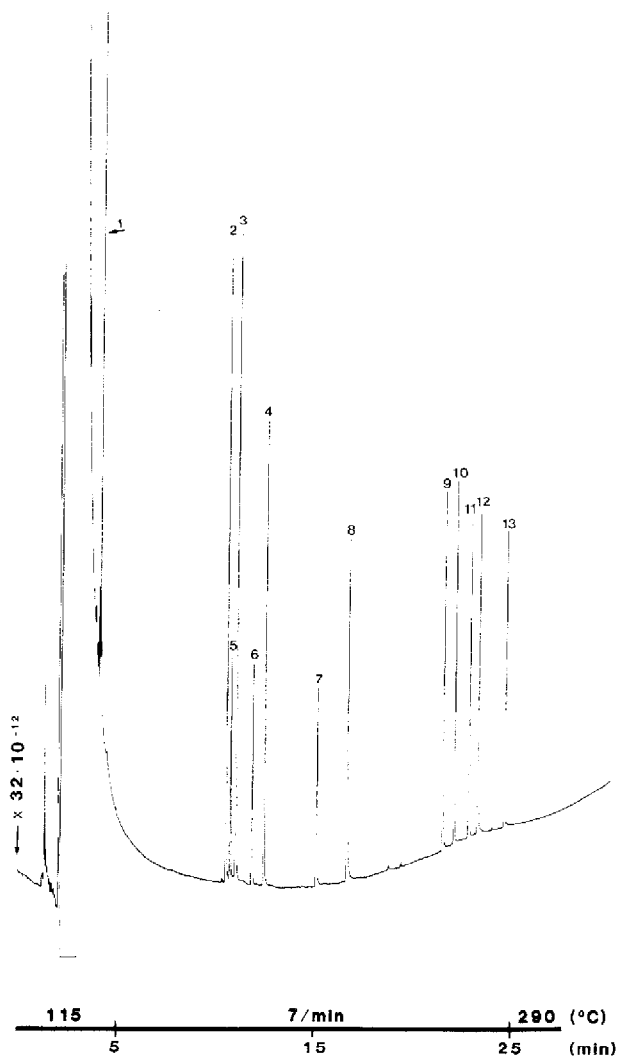
Fig. 2. Chromatogram of PFPA derivatives of all investigated amines. On-column injection of 2 μl of a 200 pg/μl solution. Peak identities according to Table I. Column, 30 × 0.32 mm I.D. Duran 50 glass capillary column with OV-73 stationary phase, film thickness 0.2 μm. Temperature programming as shown. Carrier gas, helium at 1.0 kg/cm². Thermionic specific detector; bead-heating current, 5.5 A, bias voltage, −12 V; temperature, 300°C; hydrogen flow-rate, 4 ml/min; air flow-rate, 180 ml/min; make-up gas, helium (flow-rate 20 ml/min).

in inappropriate temperature gradients in the injector body, were unfounded, and a comparable injection reproducibility was achieved.

   Capillary columns. As pointed out in Part I[4], trace analysis of perfluoroacylated amines requires columns with high inertness and good temperature stability. It was concluded that non-polar columns, *e.g.* with OV-73 stationary phase, and prepared by the persilylation method[9] were the best choice. It was also emphasized that the comparatively thick film of stationary phase used (1 μm) contributed to the in-

ertness of the columns. In this work, the persilylation has been exchanged for a more effective deactivation procedure utilizing D_4[12], which has made possible the reduction of the film thickness without impairment of the column separation characteristics or inertness. The columns obtained are not only suited for the quantitative trace analysis of fluoroamides in the picogram range, but can even be used for the analysis of traces of certain underivatized aromatic amines. Use of more polar columns, such as OV-1701 and OV-225, resulted in higher elution temperatures and reduced resolutions. In addition, these phases gave rise to increased bleeding signals when used with TSD, owing to the presence of nitrogen-containing cyano groups in these phases.

Carrier gas. Of the two carrier gases feasible in conjunction with TSD, *viz.* helium and nitrogen, the former is preferred because of its advantages in connection with capillary GC[13], although no major differences in detector behaviour with these two gases were noted.

Thermionic specific detection. The Varian thermionic specific detector used in this work is based on an electrically heated alkali ceramic bead as ion source. It was found that the bead-to-bead quality varied considerably, with some beads cracking or deteriorating after only a few weeks use, whereas others could be used for several months with only small variations in working characteristics. On a day-to-day basis, only small variations in the detector response were observed, some of which may depend on fluctuations in the electrical power circuit affecting the bead-heating current.

The response characteristics are dependent on several factors, *viz.* kind of sample, bead identity, bead-heating current, detector base temperature, bias voltage and hydrogen, air, make-up and carrier gas flow-rates. This multitude of conditional factors, and the fact that the detection mechanism is not fully known[14], make the explanation of apparent anomalies difficult. As evident from Figs. 2 and 3, a certain baseline drift occurs with temperature programming and operation at picogram detection levels.

Several attempts to reduce this by changing the carrier and make-up gases and their flow-rates or by preheating the make-up gas had little effect. Nor did thermal insulation between detector base and the chromatographic oven change the situation.
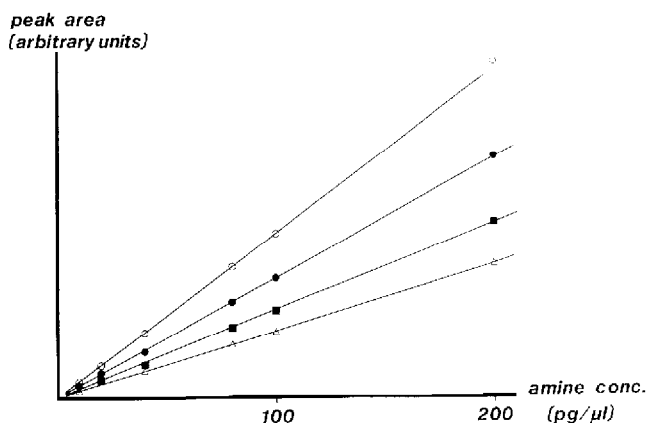

Fig. 3. Calibration curves for PFPA derivatives of 2,6-TDA (○), 2,4-DAA (●), MOCA (■) and α-naphthylamine (△). Chromatographic conditions as in Fig. 2.

The baseline drift cannot be explained by column bleeding, as temperature programming with an empty glass column gave identical results.

The Varian detector is furthermore not primarily constructed for operation with capillary columns. Although the column end can be inserted into the detector jet, thereby minimizing the dead volume, it was noted that the detector response could vary considerably with the actual positioning of the column in the jet.

TSD response to fluoroamides is mainly dependent on the relative nitrogen content of the compounds, but some structural influence is also noticeable (see Figs. 2 and 3). Thus the response to a diamine derivative is higher than to a comparable monoamine derivative, but generally not twice as high, *cf.* Nos. 5 and 8 and Nos. 7 and 9. The structural influence on sensitivity is usually slight, *cf.* 2,4- and 2,6-TDA (Nos. 2 and 3) and α- and β-naphthylamines (Nos. 5 and 6). However, the distinct decrease in sensitivity resulting when the methyl group of 2,4-TDA (No. 3) is exchanged for a methoxy group giving 2,4-DAA (No. 4) shows that TSD is not entirely independent of structure.

As will be demonstrated in a future paper, some aromatic amines may be chromatographed and detected with TSD in picogram amounts without prior derivatization, and without any significant differences in sensitivity compared with the derivatized compound.

Quantitative analysis. Calibration plots for some of the investigated PFPA derivatives on the OV-73 column are shown in Fig. 3. They demonstrate linear response in the 20–400 pg range (2-μl samples injected). Above this range, the response is increased and the plot is accordingly non-linear. The PFPA aniline derivative cannot be quantitatized on this column, owing to interference with the solvent peak. In this case a higher stationary film thickness is recommended (*cf.* ref. 4, Fig. 1). Its is also possible to use the MCAAA reagent instead, or to change to a more polar stationary phase such as OV-1701.

Detection limits. For the PFPA derivatives investigated these are in the order of 10–20 pg amine, the limit being set by the noise level of the detector rather than by the column characteristiscs. This is shown by the fact that considerably lower detection limits can be attained for the same compounds and columns using ECD (see below). Increased noise levels in combination with baseline drift have adverse effects on the detection limits for compounds with high elution temperatures. As previously discussed, various attempts to reduce this drift were unsuccessful

Comparison between TSD and ECD. The ECD sensitivities of aromatic PFPA and HFBA fluoroamides are considerably greater than those of TSD. Thus, while detection limits with the latter detector are in the 10–20 pg range, the former permits detection of fractions of a picogram[4]. However, the greater ECD sensitivity is not always an advantage. This fact is demonstrated in Fig. 4, where a mixture of compounds obtained on pyrolysis of a polyurethane specimen, after treatment with HFBAA, has been separated on an OV-73 capillary column and monitored by both TSD and ECD.

Pyrolysis of a polyurethane polymer leads to the formation of a variety of compounds, such as isocyanates, amines, phenols and hydro- and halocarbons. The gases formed were absorbed in acidic solution and the solution was extracted with toluene after alkalization. This procedure should ensure that only basic and neutral compounds are extracted. However, traces of acidic compounds, *e.g.* phenols, also

occur in the toluene phase. On treatment with HFBAA, ECD-sensitive derivatives of phenols as well as of amines are formed. As halocarbons can also be liberated on pyrolysis, the chromatograms obtained by ECD are considerably more complex than those given by TSD. In the latter case, mainly amine derivatives and other nitrogen-containing compounds are monitored, and more easily interpreted chromatograms are obtained.

It is interesting to note that whereas TSD is about as sensitive to aromatic fluoroamides as to aliphatic, ECD is considerably less sensitive to the latter. Accordingly, aliphatic fluoroamides are monitored by the two detectors with comparable sensitivities. The analysis of aliphatic fluoroamides by capillary GC will be dealt with in a forthcoming paper.
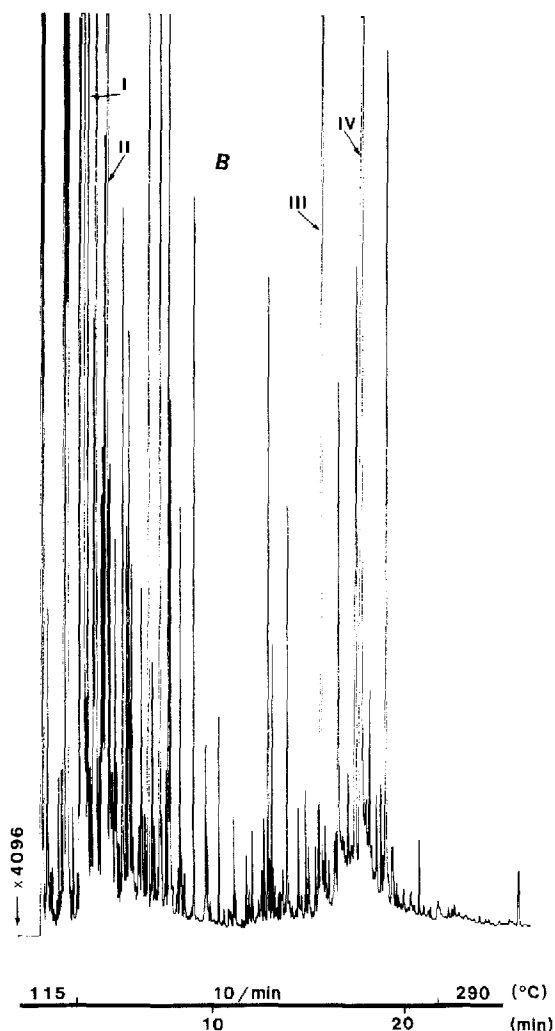

Fig. 4.

Fig. 4. Chromatograms of HFBAA-treated polyurethane pyrolysis products with (A) TSD (1 μl injected) and (B) ECD (0.2 μl injected) systems. Column and thermionic specific detector settings as in to Fig. 2. Temperature programming as shown. Carrier gas, helium at 0.5 kg/cm²; ECD, constant current mode; voltage, 50 V; pulse width, 0.1 μsec; temperature, 300°C; make-up gas, argon methane (95:5), flow-rate 60 ml/min; standing current, 1.9 nA. Peaks: I = aniline; II = p-methylaniline; III = 2,4'-MDA and IV = 4,4'-MDA.

CONCLUSIONS

Trace analysis of aromatic amines using capillary GC and TSD detection is a useful method for the assay of amines of interest from the aspect of work environmental health. The amines are preferably derivatized to perfluoroamides, but as will be discussed in a forthcoming paper, certain aromatic amines can be determined in picogram amounts without derivatization.

Although the sensitivity of TSD for PFPA derivatives of aromatic amines is

lower than that of ECD, the detection limits are still in the picogram range (10–20 pg) and for complex samples TSD tends to give simpler chromatograms. The complementary use of both detectors in the trace analysis of aromatic amines is often useful as increased information on samples can be obtained.

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