Journal of Chromatography, 479 (1989) 135–143
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 664

CHROMATOGRAPHIC DETERMINATION OF AMINES IN BIOLOGICAL FLUIDS WITH SPECIAL REFERENCE TO THE BIOLOGICAL MONITORING OF ISOCYANATES AND AMINES

II. DETERMINATION OF 2,4- AND 2,6-TOLUENEDIAMINE USING GLASS CAPILLARY GAS CHROMATOGRAPHY AND SELECTED ION MONITORING

J. F. SANDSTRÖM, G. SKARPING* and M. DALENE
Department of Occupational and Environmental Medicine, University Hospital, S-221 85 Lund (Sweden)
(First received March 21st, 1989; revised manuscript received June 2nd, 1989)

SUMMARY

A capillary gas chromatographic method, using selected ion monitoring in the electron impact mode, was developed for the analysis of 2,4- and 2,6-toluenediamine (TDA), (*C.A.* Nos. 95-80-7 and 823-40-5) in aqueous solutions and human urine. The method is based on basic extraction of TDA from 2 ml of hydrolysed urine into toluene. The TDA was derivatized to an amide using a perfluorofatty anhydride. Three anhydrides were investigated, trifluoroacetic anhydride, TFAA (*C.A.* No. 407-25-0), pentafluoropropionic anhydride, PFPA (*C.A.* No. 356-42-3) and heptafluorobutyric anhydride, HFBA (*C.A.* No. 336-59-4). Trideuterated 2,4- and 2,6-TDA, ($C^2H_3C_6H_3(NH_2)_2$), were synthesized and used as internal standards. The molecular ions of TDA-amides ($m/z = 314$, 414 and 514) and the trideuterated TDA ($m/z = 317$, 417 and 517) were monitored. The correlation coefficient was 0.999 for 2,4-TDA and 0.998 for 2,6-TDA (0.3–8 μg/l) for the monitoring of molecular ions, and the correlation coefficient was 0.999 for 2,4-TDA and 0.998 for 2,6-TDA (0.3–8 μg/l) for the monitoring of $m/z = 295$ and 298 fragments of TDA-PFPA and trideuterated TDA-PFPA. The detection limit of TDA in human urine was *ca.* 0.1 μg/l. Hydrolysed urine from an exposed worker was found to contain 4 μg of TDA/l, with a workroom atmosphere of 1–10 μg/m^3 TDI. The overall recovery for PFPA derivatives was found to be 96 ± 4 for 2,4- and 106 ± 5% for 2,6-TDA from water and 100 ± 4 and 109 ± 6% from urine.

INTRODUCTION

Quantification of isocyanates and amines in trace levels in air has been of great interest for many years due to their possible environmental and occupational hazards. Toluene diisocyanate (TDI) is one of the main components in the manufacture of polyurethane foams and coatings. The related aromatic amine, toluenediamine

0021-9673/89/$03.50

(TDA), is used as an intermediate in polyurethane foam production, in elastomer production and in dyes. TDA has also been detected in urine hydrolysate after occupational exposure to TDI[1].

The analysis of aromatic amines in human body fluids is of great interest in order to develop analytical methods for the biological monitoring of exposure to these compounds[2–5].

Several analytical techniques have been used for the determination of aromatic amines in air and in biological fluids. The methods most frequently employed are liquid chromatography (LC) with ultraviolet (UV)[6–10] and electrochemical (ED)[10–14] detection, and gas chromatography (GC) with electron-capture[15–17] and thermionic specific detection (TSD)[16,17] or selected ion monitoring (SIM)[1].

Glass capillary GC and TSD of toluenediamines has been investigated at our laboratories. The adsorptive behaviours of the aromatic amines and the corresponding derivatives have been studied[17–20].

In this work we present a method for the determination of TDA in the low μg/l level in human urine using capillary GC and SIM in order to investigate the exposure to TDI during manufacture of polyurethanes. The choice of derivatization reagent and the internal standard is discussed in some detail.

EXPERIMENTAL

Apparatus

A Varian 3500 (Varian Associates, Walnut Creek, CA, U.S.A.) gas chromatograph equipped with a Varian thermionic specific detector and a Varian 8035 automatic on-column injector was employed. The injector starting temperature was 120°C for 5 s and thereafter the temperature was increased at 150°C/min to 250°C, where it was kept for 7 min. The injector was cooled with air or liquid nitrogen. The column was held isothermal at 115°C for 1 min and then increased at 15°C/min to 270°C, where it was held for 1 min. The carrier gas, helium at a flow-rate of 3 ml/min, was dried over molecular sieve 5A and deoxygenated using an "indicating Oxytrap" (Chrompack, Middelburg, The Netherlands).

A Shimadzu GC–MS QP1000 EI/CI (Shimadzu, Kyoto, Japan) quadrupole mass spectrometer connected to a Shimadzu GC-9A gas chromatograph, equipped with a Shimadzu autosampler (AOC-9), was used for identification and quantitative analysis. An Hamilton 701RN syringe, point style 5 needle, with a conical point and sidehole to minimize the septum coring was used. The autosampler was used in connection with a Shimadzu split/splitless injection system (SPL-G9). The capillary column outlet was mounted directly in the ion source. The starting temperature of the column oven was 95°C for 2 min. It was then raised at 20°C/min to 260°C, where it was kept for 2 min. The split exit valve was kept closed for 1 min after the injection. The temperatures of the ion source and the interface were 250°C. The capillar inlet pressure was 0.2 kg/cm^2 for capillary columns with an I.D. of 0.32 mm and 0.5 kg/cm^2 in the case of 0.24 mm I.D. The pressure of the ion source was *ca.* $1.5 \cdot 10^{-5}$ and $1.0 \cdot 10^{-5}$ Torr.

The instrument was used in the electron impact (EI) mode (20 and 70 eV) and set as follows: the molecular ions (M^+) of the three derivatives were monitored at $m/z = 314$ (toluenediamine trifluoroacetyl derivative, TDA-TFA), m/z 414 (toluenediamine pentafluoropropionyl derivative, TDA-PFP) and m/z 514 (toluenediamine

heptafluorobutyryl derivative, TDA-HFB). The molecular ions of trideuterated derivatives of TDA were monitored at $m/z = 317, 417$ and 517. The ion source filament was turned on after *ca.* 5 min and off after *ca.* 8 min. Ten measurements were made per second (rate 4). The tuning of the instrument (autotune) was performed using a standard sample inlet system and nonafluorotributylamine as a calibrant, all according to a standard procedure (70 eV). The resolution was optimized and no further centring was necessary.

For enrichment and evaporation of solvent, a vacuum desiccator connected to an aspirating pump was employed. The apparatus was equipped with an electrically heated oven, designed and manufactured at our laboratory.

To centrifuge the samples a Sigma 3E-1 (Harz, F.R.G.) centrifuge was used.

Columns

Four types of fused-silica capillary columns with chemically bonded stationary phases were used: Nordion® (Helsinki, Finland) NB-54 (25 m × 0.32 mm I.D.), with film thickness of 0.50 μm, Chrompack (Middelburg, The Netherlands) CP-Sil 8 CB (25 m × 0.32 mm I.D.) with film thickness of 1.2 μm, and CP-Sil 8 CB (25 m × 0.25 mm I.D.) with a film thickness of 0.12 μm and J & W Scientific (Folsom, CA, U.S.A.) DB-5 (30 m × 0.243 mm I.D.), with a film thickness of 0.25 μm.

Chemicals

Chemicals used were 2,4- and 2,6-TDA from Fluka (Buchs, Switzerland), heptafluorobutyric anhydride (HFBA), pentafluoropropionic anhydride (PFPA) and trifluoroacetic anhydride (TFAA) from Pierce (Rockford, IL, U.S.A.), toluene from Lab-Scan (Dublin, Ireland), nonafluorotributylamine, HCl, NaOH and K_2HPO_4 from Merck (Darmstadt, F.R.G.), trideuterated 2,4- and 2,6-TDA from Synthelec (Lund, Sweden) and ethanol from Kemetyl (Stockholm, Sweden).

Sampling

A 5-ml volume of 6 *M* HCl was added to *ca.* 100 ml of urine. The acidic urine samples were stored in a refrigerator. The samples were stable for at least 3 months without noticeable degradation.

Synthesis of perfluorofatty anhydride derivatives of TDA

A 2-g amount of TDA was dissolved in *ca.* 90 ml of toluene. The perfluorofatty anhydride (PFPA, TFAA or HFBA, *ca.* 12 g) was added gradually to the solution with stirring, and the mixture was allowed to stand with continuous stirring for 5 min. The molar ratio between the perfluorofatty anhydride and TDA was 2.2:1 (w/w). The reaction mixture was gradually heated to *ca.* 90°C and then extracted with 1 *M* phosphate buffer (pH 7.5) in order to remove excess of the reagent and acid formed. The organic phase was separated and evaporated to dryness using a rotating evaporator connected to an aspirating pump (temperature of the water-bath was kept at 30°C). The residue was recrystallized from ethanol–water (87:13, v/v), whereafter the TDA derivative was filtered off (Mesh G4), washed with cold distilled water and dried in a vacuum desiccator.

Preparation of standard solutions

Standard solutions of TDA in toluene were prepared by using the work-up procedure. The completeness of the reaction was checked by GC–TSD, and compared with solutions containing the synthesized standard. Standard solutions of TDA derivatives were prepared by dissolving the 2,4- and 2,6-TDA derivatives in toluene and further dilution to appropriate concentrations. Standard solutions of TDA and trideuterated TDA were prepared by dissolving the amines in 0.1 *M* HCl and then further dilution in aqueous solutions such as acidified human urine. Amine standards in acidic solutions (0.1 *M* HCl) or acidified human urine were stable for at least 3 weeks at room temperature.

Work-up procedure

To a 2-ml urine sample, 3 ml of 6 *M* HCl, containing the trideuterated TDA as internal standard (*ca.* 100 μg/l), were added. The acidified sample was then hydrolysed at 100°C overnight. A 2-ml aliquot of the acidified sample was transferred to a test-tube. A 4-ml volume of saturated NaOH and 3 ml of toluene were added. The mixture was then shaken for *ca.* 10 min and centrifuged (1500 *g*) for 5 min. A 2-ml volume of the organic layer was transferred to a new test tube and 20 μl of anhydride reagent were added. The mixture was immediately shaken vigorously for *ca.* 10 min. The excess of anhydride reagent and acid formed were removed by extraction with 2 ml of 1 *M* phosphate buffer solution (pH 7.5). A 1-ml volume of the toluene layer containing the amide formed was then transferred to a new test-tube. The samples were enriched by evaporation of the toluene solution in a vacuum desiccator at controlled temperature (30°C). The dry residue was finally diluted in 50 μl of toluene and then analysed.

The same procedure was used for aqueous samples but in this case the hydrolysis step was omitted.

RESULTS AND DISCUSSION

Standards

Standards of perfluorofatty anhydride derivatives of 2,4-TDA, 2,6-TDA, trideuterated 2,4- and 2,6-TDA were identified by GC–mass spectrometry (MS) and the purity was checked by GC–TSD and elemental analysis. The purity was found to be better than 99%. The isotopic purity was checked for all the fragments investigated. The ratios of the mass fragment $m/z = 417$ to that of $m/z = 414$ of the 2,4- and 2,6-TDA amide derivatives were $< 0.3\%$. The ratios of the mass fragment $m/z = 414$ to that of $m/z = 417$ of the trideuterated 2,4- and 2,6-TDA amide derivatives were $< 0.3\%$.

Internal standard

The determinations with GC–MS were performed using a trideuterated TDA as the internal standard. The three hydrogen atoms at the methyl group were exchanged to deuterium. There are several advantages of using deuterium labelled substances as internal standards. The chemical, physical and chromatographic properties of the trideuterated TDA are similar to those of TDA. The behaviour of trideuterated TDA is similar to that of TDA during storage and the work-up procedure. Trideuterated TDA is not naturally present in the sample and no interferences have been found for the mass fragments monitored. The similar fragmentation patterns of TDA and trideuterated TDA are favourable for easier calibration of the mass spectrometer.

Work-up procedure

GC–TSD was used to optimize the work-up conditions. The concentrations of the two aromatic amines studied were *ca.* 180 μg/l in urine. The isobutyl chloroformate derivative of di-*n*-butylamine was used as the internal standard. The internal standard diluted in toluene was added after the evaporation step. The procedure was studied by comparison of standard solutions of the derivatives in toluene and the dissolved residue after the enrichment step. The basic properties of the aromatic amine, TDA, determine the choice of extraction solution. The conditions of extraction of TDA in urine into toluene were optimized by variation of the pH and ionic strength. The use of 5 *M* NaOH saturated with NaCl gave a recovery of less than 70%. However the use of saturated NaOH yielded *ca.* 100% recovery. For aqueous test solutions a two-phase derivatization system can also be employed.

The enrichment step was studied. Evaporation of the solvent with a gentle flow of nitrogen gave losses of about 70–80%. No losses and excellent reproducibility were found in the evaporation step, when a vacuum desiccator connected to an aspirating pump equipped with an electrically heated oven was used.

Chromatography

TFAA, PFPA and HFBA derivatives of 2,4- and 2,6-TDA were studied. All compounds showed excellent chromatographic behaviour using GC–MS. However baseline separation of the isomers was not complete for the HFBA derivatives and the isomers of the TFAA derivatives showed somewhat broader peaks. However baseline separation of the 2,4- and 2,6-TDA PFPA derivatives was found for all capillary columns tested, no endogenous peaks from the urine extracts interfered in the analysis.

Detection

TSD. The limit of detection for the PFPA derivative of TDA with GC–TSD is *ca.* 10 μg/l. An aqueous solution of TDA can be further enriched, giving detection limits of less than 0.5 μg/l, and several μl of the solvent can be injected onto the column. The molar sensitivity for all the derivatives of TDA studied was virtually the same. However, when analysing urine samples considerable interferences were found, and *ca.* 50 μg/l can be quantitatively analysed without a further clean-up step.

Mass-selective detection. GC–MS and SIM was used for the determination of TDA-amides in the low μg/l range. A 1–4 μl volume of the sample solution (toluene) was injected into the chromatographic system. This amount may damage and contaminate the ion source and the high vacuum system. These problems were eliminated when the filament was switched off during the entrance of the solvent and switched on during the chromatography. Several hundreds of analyses were hence possible.

Several mass fragments were suitable for quantitative analysis. Mass spectra were obtained for the purpose of identification, and to choose the suitable fragment ions for quantitative analysis. Fig. 1 shows mass spectra of the derivatives of PFPA. The molecular ions (M^+) at $m/z=414$ and 417 and fragment ions ($M^+ - C_2F_5$) at $m/z=295$ and 298 are suitable for quantitation of TDA. The molecular ions were however chosen due to the sensitivity, the matrix and the relatively more significant information. An ionization voltage of 20 eV was chosen due to the better signal-to-noise ratio.

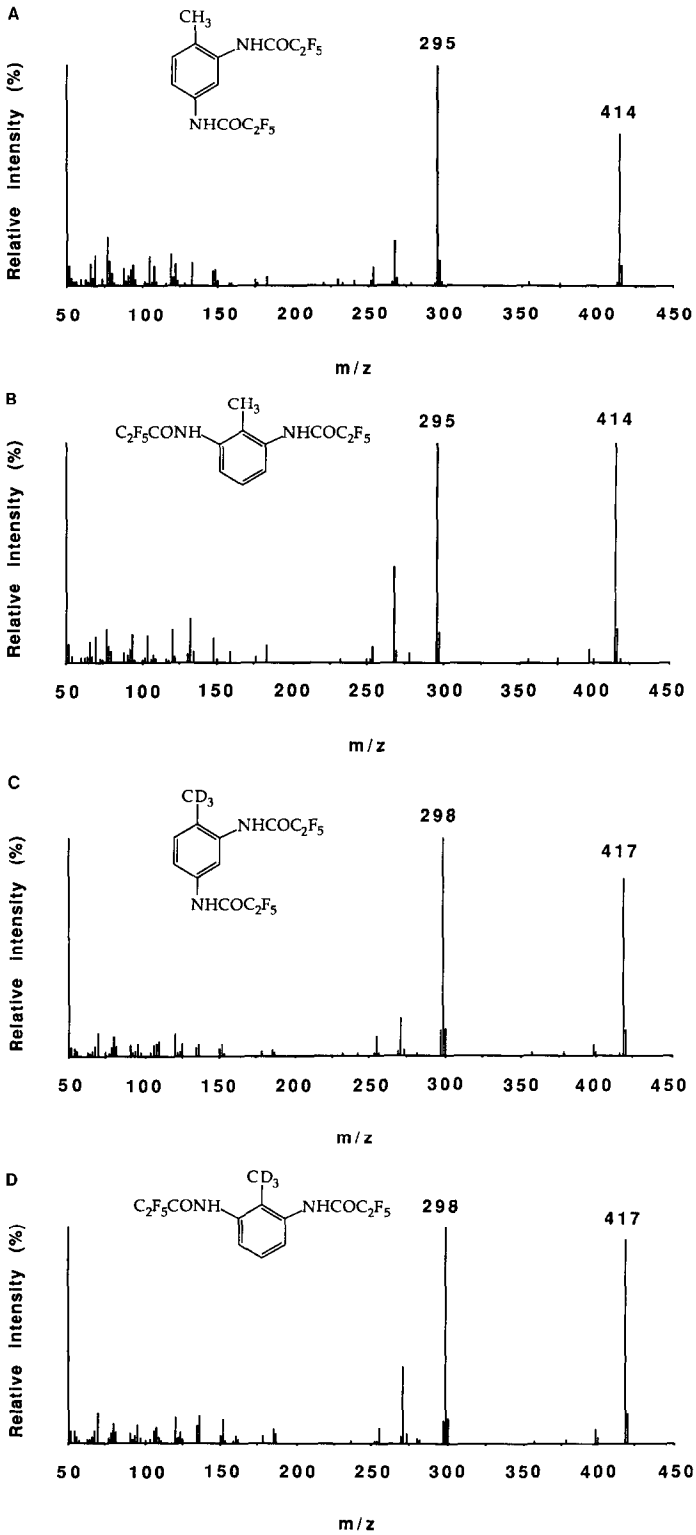

Fig. 1. Mass spectra obtained with EI at an ionization potential of 70 eV: (A) 2,4-TDA-PFPA derivative; (B) 2,6-TDA-PFPA derivative; (C) trideuterated 2,4-TDA-PFPA derivative; (D) trideuterated 2,6-TDA-PFPA

Quantitative analysis

Calibration graphs. Human urine was spiked with 2,4- and 2,6-TDA and the work-up procedure was performed. For each concentration two determinations with duplicate injections were made. The average values of the peak area ratio between TDA ($m/z = 414$ and 295) and trideuterated TDA ($m/z = 417$ and 298) were plotted. The calibration graphs were linear and passed through the origin. The calibration graph using SIM of the molecular ions for the concentration range 0.3–8 μg/l (peak area ratio 0–0.3) gave a correlation coefficient of 0.999 ($n = 9$) for 2,4-TDA and 0.998 ($n = 9$) for 2,6-TDA. The correlation coefficients using SIM of the ($M^+ - C_2F_5$) ions were 0.999 ($n = 9$) for 2,4-TDA and 0.998 ($n = 9$) for 2,6-TDA. No noticeable differences were found for peak height measurements when using HFBA as the derivatization reagent.

Recovery and precision. The overall recovery was studied for ten spiked urine samples containing *ca.* 145 μg/l of the two TDA isomers using GC–TSD. The urines were worked up according to the procedure, but without the presence of trideuterated TDA as the internal standard. Comparison was made by analysing the amide standards in toluene. The overall recoveries for 2,4- and 2,6-TDA was found to be 100 ± 4 and 109 ± 6% ($n = 10$). The recoveries for TDA-spiked urine in the hydrolysis step studied by using GC–SIM 15 μg/l) were 100 ± 4% ($n = 10$) for both 2,4- and 2,6-TDA. The values are given with a 95% confidence range.

An enrichment step using a vacuum desiccator connected to an aspirating pump was employed to increase the detection limit. Twentyfive samples were simultaneously evaporated within an hour. No carryover and no artefact were found during this procedure. More than a 40-fold enrichment of the sample was possible. The recovery in the enrichment procedure and 20-fold enrichment was found to be 100 ± 3 ($n = 10$) for 2,4-TDA and 104 ± 4% for 2,6-TDA ($n = 10$). The overall recovery for aqueous solutions was similar. The values are given with a 95% confidence range.

The usefulness of a trideuterated internal standard is demonstrated by the high precision in the GC–SIM analysis at the low μg/l level. Ten different spiked urines at *ca.* 2 μg/l and ten different spiked urines at *ca.* 15 μg/l were analysed. The relative standard deviations for the ten samples for TFAA, PFPA and HFBA derivatives were determined. The samples were enriched ten times and the mean values for duplicate injections for both the peak area and peak height ratio were measured by monitoring the molecular ions and the ratio between the amine and internal standard varied between 2 and 5% (Table I). The values in the table are given with a 95% confidence range.

Detection limit

The detection limit, according to Miller and Miller[21], for TDA in human urine with GC–MS using the EI mode was *ca.* 0.1 μg/l for PFPA derivatives. The samples were treated according to the work-up procedure and with a 40-fold enrichment (the dry residue was dissolved in 25 μl of toluene).

Applications

The method is applicable to the monitoring of isocyanates and amines in air, collected in an impinger flask containing 10 ml of diluted hydrochloric acid. The potential of the method is illustrated by the high selectivity and sensitivity, less than

TABLE I

PRECISION IN THE GC-SIM ANALYSIS OF 2,4- AND 2,6-TDA IN HYDROLYSED HUMAN URINE AS THEIR PERFLUOROFATTY ANHYDRIDE DERIVATIVES

Injected volume: 1 μl. Internal standard (IS): trideuterated TDA. An autoinjector was used for splitless injections. Ten different urine samples spiked with 2,4- and 2,6-TDA with duplicate injections were determined. Values are relative standard deviations (%) in peak area and peak height, respectively. TFAA = trifluoroacetic anhydride derivative (M^+ at $m/z = 314$); PFPA = pentafluoropropionic anhydride derivative (M^+ at $m/z = 414$) and HFBA = heptafluorobutyric anhydride derivative (M^+ at $m/z = 514$).

Amine	*Conc. (μg/l)*	*TFAA*		*PFPA*		*HFBA*	
		DD[a]	*IS*[b]	*DD*	*IS*	*DD*	*IS*
2,4-TDA	14.4	7, 10	3, 3	19, 20	3, 4	17, 17	3, 3
	2.2	17, 19	4, 3	17, 17	2, 3	13, 14	4, 5
2,6-TDA	14.5	15, 19	4, 4	6, 6	2, 2	6, 6	2, 2
	2.2	5, 7	4, 5	12, 12	2, 3	9, 9	4, 4

[a] Direct determination without internal standard.

[b] The ratio between the amine and the internal standard.

1/500 of the present Swedish threshold limit value (TLV) (40 $\mu g/m^3$) with a sampling time of 15 min and with a flow-rate of 1 l/min.

Urine from a worker exposed to TDI at a flexibe foam factory was analysed, the workroom atmosphere containing 1–10 $\mu g/m^3$. The work-up procedure was performed and the sample was enriched 20 times and analysed with GC–SIM. The corresponding diamines of TDI, 2,4- and 2,6-TDA were found in the hydrolysed urine (*ca.* 4 μg/l). The chromatograms are shown in Fig. 2.

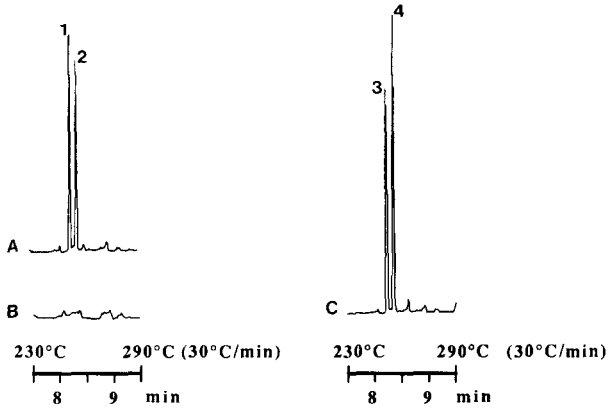

Fig. 2. SIM of hydrolysed urine samples using the EI mode (20 eV). (A) The molecular ion of the TDA-PFPA derivative ($m/z = 414$) from a worker exposed to TDI, with an air concentration of 1–10 $\mu g/m^3$. The peaks correspond to a concentration of *ca.* 4 μg/l in hydrolysed urine of 2,4- and 2,6-TDA. (B) The molecular ion of the TDA-PFPA derivative ($m/z = 414$) from a non-exposed subject. (C) The molecular ion of the trideuterated TDA-PFPA derivative ($m/z = 417$) used as the internal standard. Peaks: 1 = PFPA derivative of 2,6-TDA; 2 = PFPA derivative of 2,4-TDA; 3 = PFPA derivative of trideuterated 2,6-TDA; 4 = PFPA derivative of trideuterated 2,4-TDA. Column: J&W fused silica coated with DB-5 bonded stationary phase (30 m × 0.243 mm I.D.), film thickness 0.25 μm. Inlet pressure of the carrier gas (helium): 0.5 kg/cm^2. Splitless injection (1 min) of 1 μl toluene. Temperature programming: isothermal at 95°C (2 min), increased at 10°C/min to 110°C, then at 30°C/min to a final temperature of 300°C.

CONCLUSIONS

A GC–SIM method is demonstrated for determination of TDA in hydrolysed human urine. Due to the high selectivity and sensitivity no special clean-up procedure is necessary. Several samples can be worked up simultaneously. The use of trideuterated 2,4- and 2,6-TDA results in accurate and precise determinations. The combination of high-resolution chromatography and selective detection is demonstrated to be appropriate for the analysis of TDA in biological matrices.

ACKNOWLEDGEMENTS

We are indebted to Professor Staffan Skerfving, head of the department of Occupational and Environmental Medicine, for his interest in this work. We also gratefully acknowledge the Swedish Work Environment Fund (AMFO-88-0161) for financial support.

REFERENCES

1 C. Rosenberg and H. Savolainen, *J. Chromatogr.*, 367 (1986) 385–392.
2 *Bioassay of 2,4-Diaminotoluene for Possible Carcinogenicity, Technical Report Series no. 162, CAS No. 95-80-7*, National Cancer Institue, Bethesda, MD, 1979.
3 *Bioassay of 2,6-Diaminotoluene Dihydrochloride for Possible Carcinogenicity, Technical Report Series no. 200, CAS No. 15481-70-6*, National Cancer Institue, Bethesda, MD, 1980.
4 B. B. Furlong, R. P. Weaver and J. A. Goldstein, *Carcinogenesis*, 8 (1987) 247–251.
5 P. H. Grantham, L. Mohan, T. Benjamin, P. P. Roller, J. R. Miller and E. K. Weisburger, *J. Environ Pathol. Toxicol.*, 3 (1980) 149–166.
6 C. Sangö, *J. Liq. Chromatogr.*, 2 (1979) 763.
7 S. Sardas, A. E. Karakaya and B. Sener, *Gazi Univ. Eczacilik Fak. Derg.*, 3 (1985) 89–94.
8 R. C. Snyder, W. C. Brumley, C. V. Breder and T. Fazio, *J. Assoc. Off. Anal. Chem.*, 65 (1982) 1388–1394.
9 E. H. Nieminen, L. H. Saarinen and J. T. Laaksalo, *J. Liq. Chromatogr.*, 6 (1983) 453–469.
10 M. Dalene, L. Mathiasson, G. Skarping, C. Sangö and J. F. Sandström, *J. Chromatogr.*, 435 (1988) 469–481.
11 C. J. Warwick, D. A. Bagon and C. J. Purnell, *Analyst (London)*, 106 (1981) 676–685.
12 D. A. Bagon and C. J. Purnell, *J. Chromatogr.*, 190 (1980) 175–182.
13 D. A. Bagon, C. J. Warwick and R. H. Brown, *Am. Ind. Hyg. Assoc. J.*, 45 (1984) 39–43.
14 M. S. Varney and M. R. Preston, *J. Chromatogr.*, 348 (1985) 265–274.
15 T. Hirayama, M. Ono, K. Uchiyama, M. Nohara and S. Fukui, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 746–748.
16 C. Rosenberg and H. Savolainen, *J. Chromatogr.*, 323 (1985) 429–433.
17 G. Skarping, L. Renman and M. Dalene, *J. Chromatogr.*, 270 (1983) 207–218.
18 G. Skarping, C. Sangö and B. E. F. Smith, *J. Chromatogr.*, 208 (1981) 313–321.
19 G. Skarping, L. Renman, C. Sangö, L. Mathiasson and M. Dalene, *J. Chromatogr.*, 346 (1985) 191–204.
20 L. Renman and G. Skarping, in preparation.
21 J. C. Miller and J. N. Miller, *Statistics For Analytical Chemistry*, Ellis Horwood, Chichester, 1984.