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Biological monitoring of hexamethylene diisocyanate by determination of 1,6-hexamethylene diamine as the trifluoroethyl chloroformate derivative using capillary gas chromatography with thermoionic and selective-ion monitoring

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Abstract

A GC method using a novel derivatization reagent, 2',2',2-trifluoroethyl chloroformate (TFECF), for the derivatization of primary and secondary aliphatic amines with the formation of carbamate esters is presented. The method is based on a derivatization procedure in a two-phase system, where the carbamate ester is formed. The method is applied to the determination of 1,6-hexamethylene diamine (HDA) in aqueous solutions and human urine, using capillary GC. Detection was performed using thermionic specific detection (TSD) and mass spectrometry (MS)–selective-ion monitoring (SIM) using electron-impact (EI) and chemical ionization (CI) with ammonia monitoring both positive $(CI)^+$ and negative ions $(CI)^-$. Quantitative measurements were made in the chemical ionization mode monitoring both positive and negative ions. Tetra-deuterium-labelled HDA (TDHDA; $H_2NC^2H_2(CH_2)_4C^2H_2NH_2$) was used as the internal standard for the GC–MS analysis. In CI^+ the m/z 386 and the m/z 390 ions corresponding to the $[M + 18]^+$ ions (M = molecular ion) of HDA–TFECF and TDHDA–TFECF were measured; in CI^- the m/z 267 and the m/z 271 ions corresponding to the $[M - 101]^-$ ions. The overall recovery was found to be $97 \pm 5\%$ for a HDA concentration of 1000 μg/l in urine. The minimal detectable concentration in urine was found to be less than 20 μg/l using GC–TSD and 0.5 μg/l using GC–SIM. The overall precision for the work-up procedure and GC analysis was *ca.* 3% ($n = 5$) for 1000 μg/l HDA-spiked urine, and *ca.* 4% ($n = 5$) for 100 μg/l. The precision using GC–SIM for urine samples spiked to a concentration of 5 μg/l was found to be 6.3% ($n = 10$).

1. Introduction

Isocyanates are used in industry for the production of polyurethane polymers (PUR). Aliphatic isocyanates are mainly used in the production of PUR coatings and paint. Isocyanates have been associated with respiratory health hazards [1]. They are considered to be very toxic and the threshold limit values (TLVs)

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are therefore low. In Sweden the TLVs (threshhold limit values) are 5 ppb for all isocyanates.

An open surface of hexamethylenediisocyanate (HDI) results in high air concentrations of HDI and extreme precautions must be taken. For practical reasons HDI is not used as such but as adducts and prepolymerized technical compounds which have a much lower vapour pressure. The structure of the isocyanate has a great influence on its toxicity. Furthermore, aliphatic isocyanates are regarded as more toxic than the aromatic ones [1]. In the industry exposure not only occurs to HDI but also to 1-amino-6-isocyanato-hexamethylene which has been found in air after thermal decomposition of HDI based PUR [2,3].

Biological monitoring of exposure to HDI has been done by the determination of HDA in urine hydrolysates [4–7]. HDA has been determined with GC and selective-ion monitoring (GC–SIM) of the perfluoro fatty acid amides and carbamate esters [8,9]. In an exposure study the urinary half-life ($t_{1/2}$) was found to be very short (*ca.* 1.2 h) [5,7]. Leuenberger *et al.* [6] have exposed workers to HDI in an exposure chamber and studied changes in lung function as well as excretion of HDA in urine.

Two derivatization methods have been developed at our laboratory [5,9]. One method is based on the determination of HDA as its perfluorofatty acid amides and the other is based on carbamate esters formation. Tetradeuterium labelled HDA was used as the internal standard in both cases. The amide method is based on hydrolysis of the urine sample followed by basic extraction of the liberated HDA into toluene. Sensitive determinations were possible but the extraction recovery was low. The carbamate method is based on hydrolysis of the urine sample followed by a two-phase derivatization procedure. Compared to the amide method determination was less sensitive but extraction recovery was much better.

The aim of this study was to develop a derivatization method based on a novel reagent (TFECF) offering sensitive and selective determinations with improved recovery for the analysis of HDA in biological fluids after exposure to HDI.

2. Experimental

2.1. Apparatus

A Varian 3500 gas chromatograph equipped with a Varian thermionic specific detector (TSD) and a Varian 8035 automated on-column injector was employed (Varian, Walnut Creek, CA, USA). The injector starting temperature was 105°C for 1.2 s, thereafter it was programmed with 150°C/min to a final temperature of 280°C, maintained for 7 min. The starting temperature of the column oven was isothermal at 105°C for 1 min, then it was raised at 25°C/min to 280°C where it was kept for 2 min. Typical settings for the detector were: bead heating current, 2.85 A; bias voltage, −4.0 V; detector temperature, 280°C; gas flow-rates, 3 ml/min of hydrogen, 175 ml/min of air, and 20 ml/min of nitrogen as the make-up gas. The carrier gas was helium at a constant flow-rate of 2 ml/min. Chromatograms were recorded on a Shimadzu CR3A integrator.

A VG-Quattro triple quadrupole mass spectrometer (Fisons Instruments, VG-Biotech, Altringham, Cheshire, UK) connected to a Carlo Erba GC 8000 gas chromatograph equipped with a Grob type cold on-column injector (Fisons Instruments, Carlo-Erba, Milan, Italy) was employed. Injection was performed using on-column injection. The starting temperature of the column oven was isothermal at 100°C for 1 min; then it was raised at 25°C/min to 300°C where it was kept for 2 min. The temperature of the ion source was 250°C and the GC–MS interface temperature was 250°C. The capillary inlet pressure of helium was 1.0 kg/m^3. The solvent delay was set to 3 min. The instrument was used in the electron impact and chemical-ionisation mode using ammonia as the reagent gas monitoring both positive (GC–PCI) and negative ions (GC–NCI).

Quantitative measurement were made in the chemical-ionisation mode monitoring positive and negative ions. The dwell time for each of the ions was 0.1 s and the inter-scan delay was 0.02 s. The pressure in the ion source was, in the case of chemical ionisation with ammonia, kept at *ca.* $1.5 \cdot 10^{-4}$ mbar. The emission current was 100 mV and the electron energy 70 eV. Mass spectra

of *ca.* 50 ng of each derivative were obtained by scanning ions between 50 and 500 amu for 0.5 s with an inter-scan delay of 0.2 s. A Sigma 3E-1 centrifuge (Sigma Harz, Germany) was employed for phase separation. The samples were evaporated in a Speed-Vac 290 centrifuge (Savant, Farmingdale, NY, USA).

2.2. Columns

Fused-silica capillary columns with chemically bonded stationary phases, DB-5 (J & W Scientific, Folsom, CA, USA) 30 m × 0.25 mm I.D., with a film thickness of 0.25 μm were used.

2.3. Chemicals

Chemicals used were 1,6-hexamethylene diamine (HDA), isobutyl chloroformate, tributyl amine and toluene from Janssen (Beerse, Belgium). Tetra-deuterium labelled HDA (TDHDA; $H_2NC^2H_2(CH_2)_4C^2H_2NH_2$) from MSD Isotopes (Merck Frosst Canada, Montreal, Canada). Di-*n*-butyl amine (DBA) from Fluka (Buchs, Switzerland). HCl, NaOH, NH_3 and K_2HPO_4 from Merck (Darmstadt, Germany). The 2′,2′,2-trifluoroethyl chloroformate (TFECF) and the corresponding HDA derivative were synthesised and obtained by Synthelec (Lund, Sweden).

2.4. Internal standard

The internal standard for the GC–TSD determinations was the isobutyl chloroformate derivative of di-*n*-butyl amine (1 ng/μl). A solution of toluene containing the internal standard was added to the dry residue from the work-up procedure before the GC analysis.

For the GC–MS determinations TDHDA was diluted in 6 *M* HCl at a concentration corresponding to 200 ng/ml urine. This solution was added to the sample before the hydrolysis step.

2.5. Preparation of standard solutions

Standard solutions of the HDA– and TDHDA–TFECF were prepared by dissolving accurately weighted amounts of each derivative (*ca.* 60 mg/100 ml) in toluene. The solutions were then further diluted with toluene to the appropriate concentrations. Standard solutions of HDA and TDHDA were prepared and stored in 6 *M* HCl solutions.

2.6. Work-up using basic hydrolysis

To a 1-ml urine sample 1.5 ml of 5 *M* NaOH and 50 μl of 6 *M* HCl containing TDHDA (10 ng/ml), were added (for the GC–TSD method 1.5 ml of 5 *M* NaOH were added). The mixture was heated at 100°C for 4 h (hydrolysis). To the sample was added 1 ml of water, 10 μl of tributylamine and 2 ml of toluene. The mixture was shaken for 30 s before 40 μl of trifluorinated ethyl chloroformate were added. The mixture was then shaken for 20 min at room temperature. The toluene phase was separated and shaken for 0.5 min with 2 ml of 1 *M* aqueous phosphate buffer (pH 7.5) and then evaporated to dryness in a Speed Vac centrifuge at 40°C. In the case of GC–MS determinations the dry residue was dissolved in 100 μl of toluene. For GC–TSD determinations the dry residue was dissolved in 1 ml of toluene solution containing *ca.* 1 ng/μl of the isobutyl chloroformate derivative of di-*n*-butyl amine used as internal standard. The toluene layer was then analysed. The same procedure was used for aqueous samples where the hydrolysing step could be eliminated.

2.7. Work-up procedure using acidic hydrolysis

To a 1-ml urine sample 1.5 ml of 6 *M* HCl and 50 μl of 6 *M* HCl containing TDHDA (10 ng/ml) were added (for the GC–TSD method 1.5 ml of 6 *M* HCl were added). The mixture was heated at 100°C overnight (hydrolysis). To the sample 1 ml of water, 10 μl of tributylamine, 3 ml of 5 *M* NaOH and 2 ml of toluene were added. The procedure was then the same as for the work-up procedure using basic conditions.

2.8. Sampling and storage of biological samples

The urine samples were collected in polyethylene bottles. The density, creatinine concentration, pH and total volume of the urine

samples were determined. The urine samples were stored in a freezer, at −18°C until analysis. All analyses were done after acid or basic hydrolysis of the urine samples. The amounts given for HDA in Fig. 1 therefore reflect the sum of free and hydrolysable compounds.

3. Results and discussion

3.1. Standards

The structure of the HDA and the tetradeuterium labelled HDA derivatives were confirmed using mass spectrometry (see Fig. 1). The purity was checked using GC–TSD and GC–FID (flame-ionization detector). The purity was found to be higher than 98%.

3.2. Storage and treatment of samples

HDA spiked urine samples were found to be stable after acidification. No noticeable change in sample composition was found even after storage in the dark at room temperature for several weeks.

Urinary compounds originating from exposure to HDI or HDA consist of several metabolites and conjugates, free HDA probably constituting only a minor portion. The hydrolysis of the HDA conjugates obviously influences the possibility to separately determine these compounds, and consequently the described method only gives the sum of free HDA and hydrolyzable HDA-conjugates.

3.3 Derivatization

Derivatization of amines using perfluoro fatty acid anhydrides and chloroformates has earlier been studied in some detail at our laboratory [10]. The choice of pH depends on the basicity of the amine. For the reaction to occur at a reasonable rate at room temperature unprotonated amines must be present. For the two-phase chloroformate derivatization of piperazine ($pK_a = 9.8$), we found that with a phosphate buffer solution (pH 10) and with ammonia as a catalyst the reaction is completed in less than 5 min. For HDA ($pK_a = 11.9$) this procedure gave unsatisfactory results. The reaction is quantitative only at pH > 12.

Ammonia, pyridine and tributylamine were tested as catalysts for the HDA–TFECF derivatization. Only tributylamine gave a recovery of more than 80%. The added amount of the catalyst tributylamine was varied between 0 and 40 μl. The influence of the added amount was small but a 10-μl volume was chosen due to the relatively better reaction recovery and precision achieved.

The volume of TFECF was varied and the optimal volume for the basic work-up procedure, corresponding to a *ca.* 100% recovery, was found to be *ca.* 40 μl. The lower recovery found with increasing amounts of reagent is probably due to the increasing solubility of the derivative in the aqueous phase.

When injecting the sample with the reagent and its degradation products present the sensitivity of the TSD was greatly influenced and a low reproducibility was observed. The evaporation step is therefore essential to remove excess reagent.

3.4. Chromatography

In the GC–TSD system using automated on-column injection the carbamate ester derivative elutes at 200°C as a symmetric peak. Peak splitting was observed when injecting the sample at injection temperatures deviating more than *ca.* 4°C from 105°C. When analysing the carbamate ester on a different GC column, or changing the injection volume or the helium inlet pressure, the injection temperature had to be adjusted by *ca.* ±10°C. The injection volume was varied between 0.5 and 10 μl (with lower injection speed with a larger volume injected). Each injection consumes *ca.* 100 μl of the sample due to the dead volume in the autosampler. The use of an apolar stationary phase with a relatively low film thickness is preferred, due to the fact that the HDA–diurethane thermally decomposes at elevated temperatures. Analysis of the HDA–diurethane by GC–MS showed that this compound actually decomposed

to HDI. A HDI peak shows up as a tailing peak early in the chromatogram, and increases with increasing elution temperatures. Due to the decomposition at elevated temperatures, higher film thicknesses should be avoided. When using film thicknesses of less than 0.8 μm no problems occurred. Initial attempts to perform the determinations using the splitless injection technique resulted in loss of the HDA–CF_3 peak obviously due to thermal degradation.

Chromatograms of urine samples originating from an HDI-exposed person, are shown in Fig. 1. No interfering peaks are seen in the chromatogram.

3.5. Mass spectrometry

Electron impact (EI) and chemical ionization (CI) with ammonia monitoring positive (CI^+) and negative ions (CI^-) were studied for HDA- and TDHDA–TFECF. As can be seen in Fig. 2, EI produces many fragments at lower m/z values. No molecular ion was found for the aliphatic HDA-derivative. Comparing the spectra of the two compounds, significant differences due to the deuterium atoms in the molecule are seen. Chemical ionization monitoring positive ion shows rather simple and easy to interpret spectra and little fragmentation is observed. The m/z $[M+18]^+$, $[M+1]^+$, $[M-82]^+$, $[M-99]^+$ and $[M-182]^+$ fragments are the most abundant ions (amu > 180) for the two studied compounds. Even less complicated fragmentation patterns are seen when monitoring negative ions. The m/z $[M-101]^-$, $[M-169]^-$ and $[M-183]^-$ fragments are the most abundant ions (amu > 180). The relative abundance of the molecular ion was less than 0.1%. Ammonia as the reagent gas can be continuously used for weeks giving reproducible results without the necessity to clean the ion source.

3.6. Quantitative analysis

For each concentration two determinations with duplicate injections were made. Using GC–TSD peak areas of HDA–TFECF and the isobutyl chloroformate derivative of di-*n*-butyl amine used as internal standard were measured and the ratios were calculated. In the GC–SIM studies peak area measurements were made and the ratio between the HDA–TFECF and TDHDA–TFECF areas were calculated.

Recovery

The recovery was studied by spiking hydrolysed human urine and performing the derivatization procedures as described above. Peak heights were compared to standards of the derivatives by GC–TSD. For hydrolysis under basic conditions the recovery for spiked human urine was $90 \pm 6\%$ ($n = 5$) for a concentration of 1000 μg of HDA per liter at a 95% degree of confidence. The recovery for acidic hydrolysis conditions was $79 \pm 6\%$ ($n = 5$).

Calibration graphs

The calibration graph using GC–TSD was obtained by adding different amounts of HDA to urine and performing the work-up procedure as described above. The investigated concentration range of 100–400 μg/l urine, gave a correlation coefficient of 0.9982 for plotting the area ratio for 6 investigated concentrations in human urine ($y = 0.926x + 0.013$).

GC–SIM with ammonia as the reagent gas was investigated for urine samples ($n = 5$) spiked with HDA in the range of 1–10 μg/l containing 500 μg/l of the internal standard. The monitoring of the negative ions m/z 267 and 271 gave a virtually linear calibration graph, passing through the origin with the correlation coefficient of 0.988 ($y = 0.0034x + 0.0027$). Monitoring the positive ions m/z 386 and 390 the correlation coefficient was 0.992 ($y = 0.0031x + 0.0017$).

Precision

The precision was studied using GC–TSD. The internal standard chosen behaves similarly to the HDA derivatives in the chromatographic system. The overall precision with the work-up procedure and GC analysis was *ca.* 3% ($n = 5$) for 1000 μg HDA per litre spiked urine, and *ca.* 4% ($n = 5$) for 100 μg/l. The precision using GC–SIM and monitoring negative ions was in-

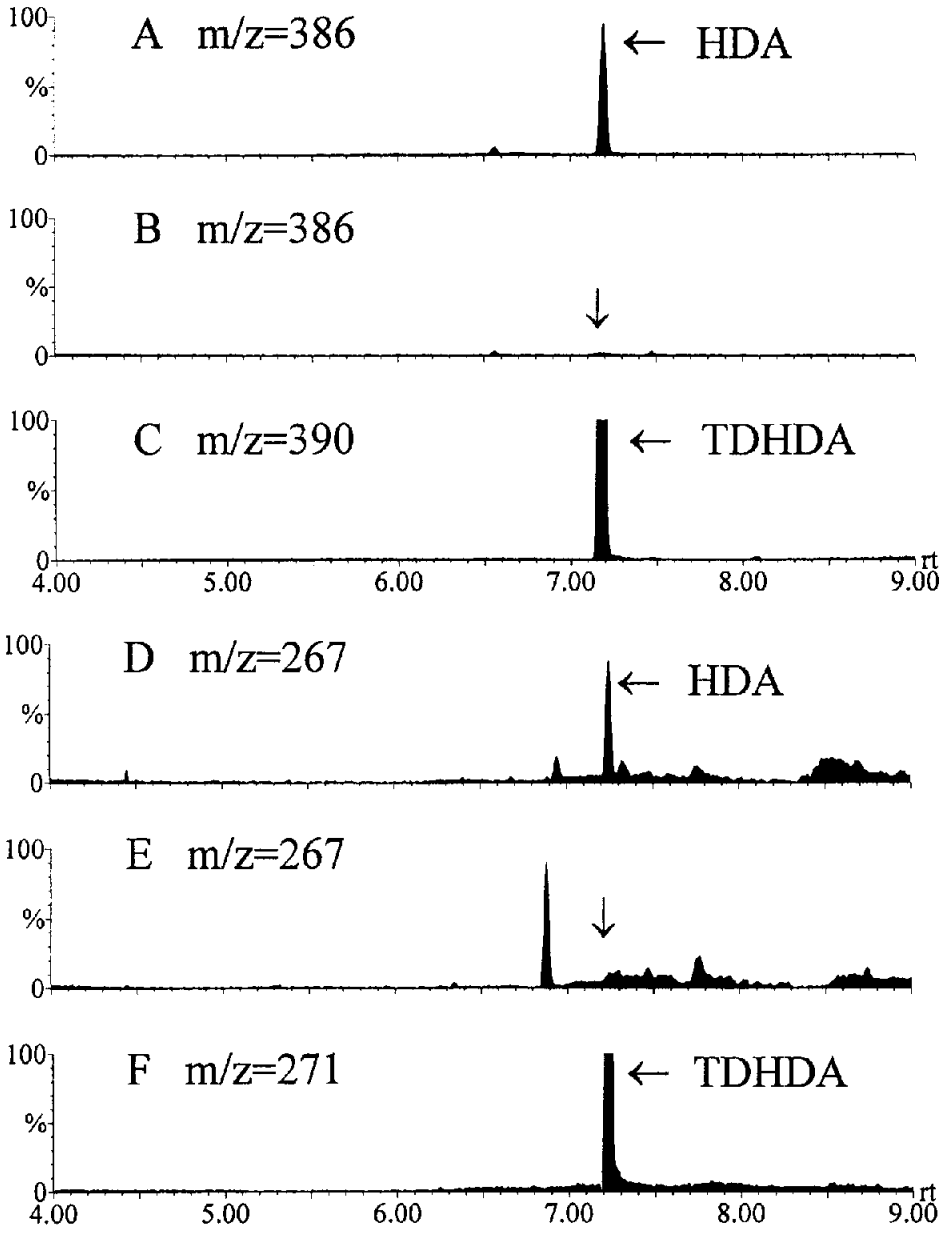


Fig. 1. A hydrolysed urine sample from a male person exposed to *ca.* 30 μg HDI per m^3 during 4 h. The urine sample was sampled shortly after the HDI exposure. Selected-ion monitoring using chemical ionisation with ammonia and monitoring positive ions ($[M + 18]^+$) and negative ions ($[m/z = M - 101]^-$) was performed. GC–PCI; Chromatogram A shows the m/z 386 ion of the HDA–TFECF derivative for the urine sample and B is the blank. Chromatogram C shows the m/z 390 ion of the TDHDA–TFECF. GC–NCI; Chromatogram D shows the m/z 267 ion of the HDA–TFECF derivative for the urine sample and E is the blank. Chromatogram F shows the m/z 271 ion of TDHDA–TFECF. The HDA peak corresponds to a concentration of *ca.* 20 μg of HDA per liter of urine and the I.S. peak corresponds to 1000 μg I.S. per liter of urine. The scales in the GC–PCI chromatograms (A–C) are the same and the scales in the GC–NCI chromatograms (D, F) are the same. The HDA peak in chromatograms A and D defines 100%. Column: J&W fused-silica coated with DB-5 bonded stationary phase (30 m × 0.25 mm I.D.); film thickness 0.25 μm; inlet pressure of the carrier gas (helium): 1.0 kg/cm^2; on-column injection (1 min) of 1 μl toluene; temperature programming: isothermal at 100°C (1 min), raised at 25°C/min to 300°C.

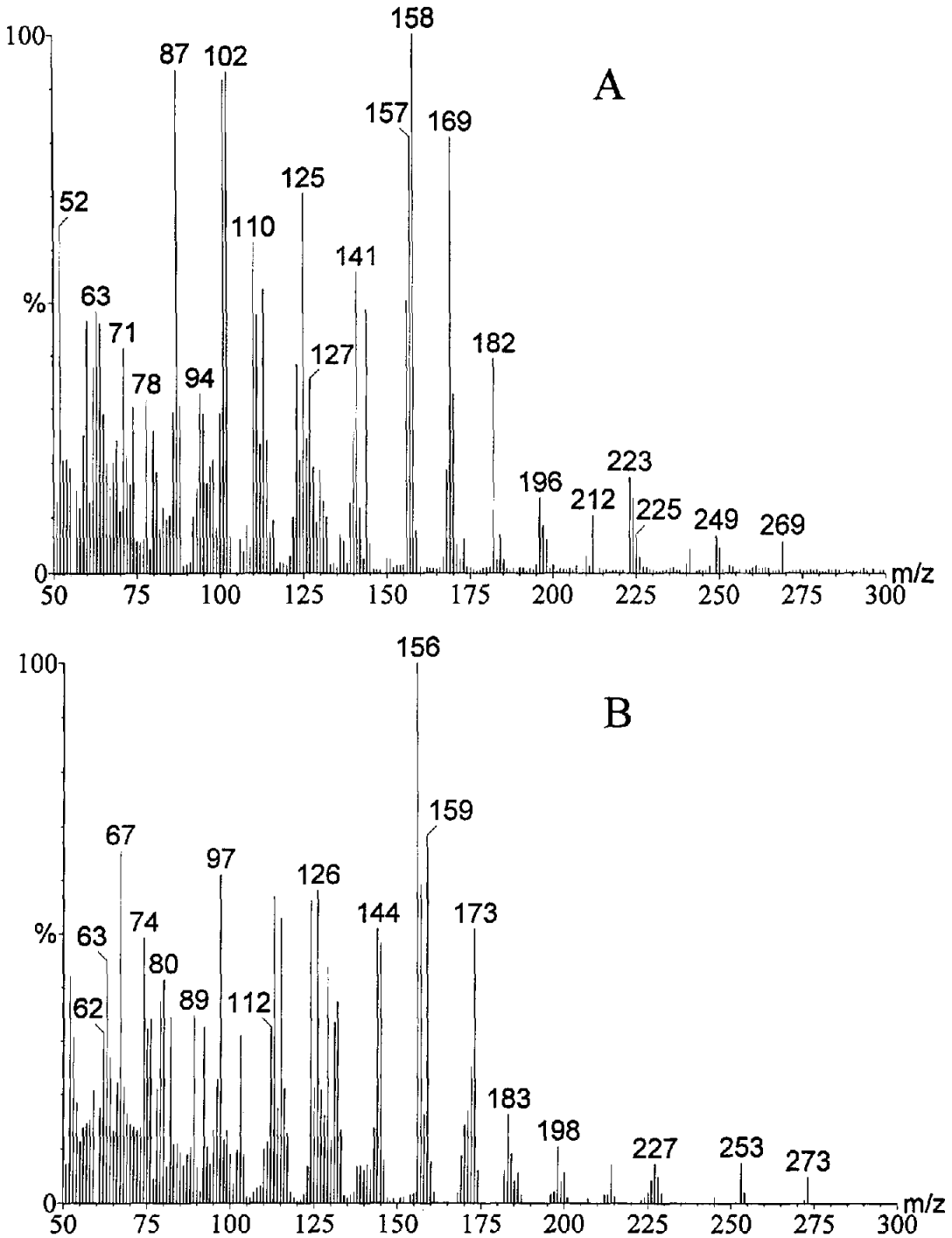


Fig. 2. Mass spectra of 1,6-hexamethylene diamine (HDA) and tetra-deuterium labelled 1,6-hexamethylene diamine TDHDA as the trifluoroethyl chloroformate (TFECF) derivatives obtained with electron-impact (EI) and chemical ionisation with ammonia monitoring positive (CI^+) and negative ions (CI^-). (A) HDA, EI; (B) TDHDA, EI; (C) HDA, CI^+; (D) TDHDA, CI^+; (E) HDA, CI^-; (F) TDHDA, CI^-.

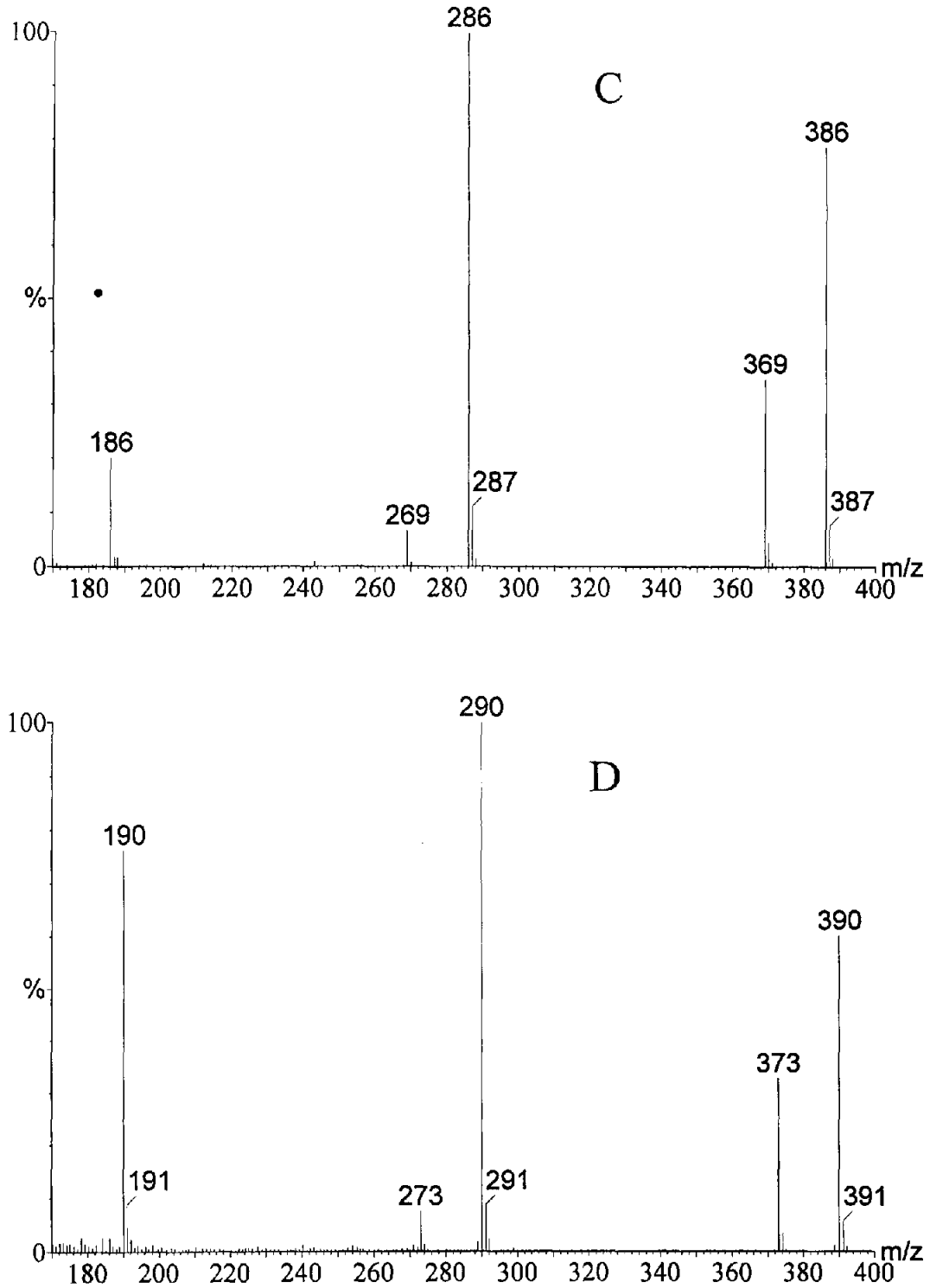
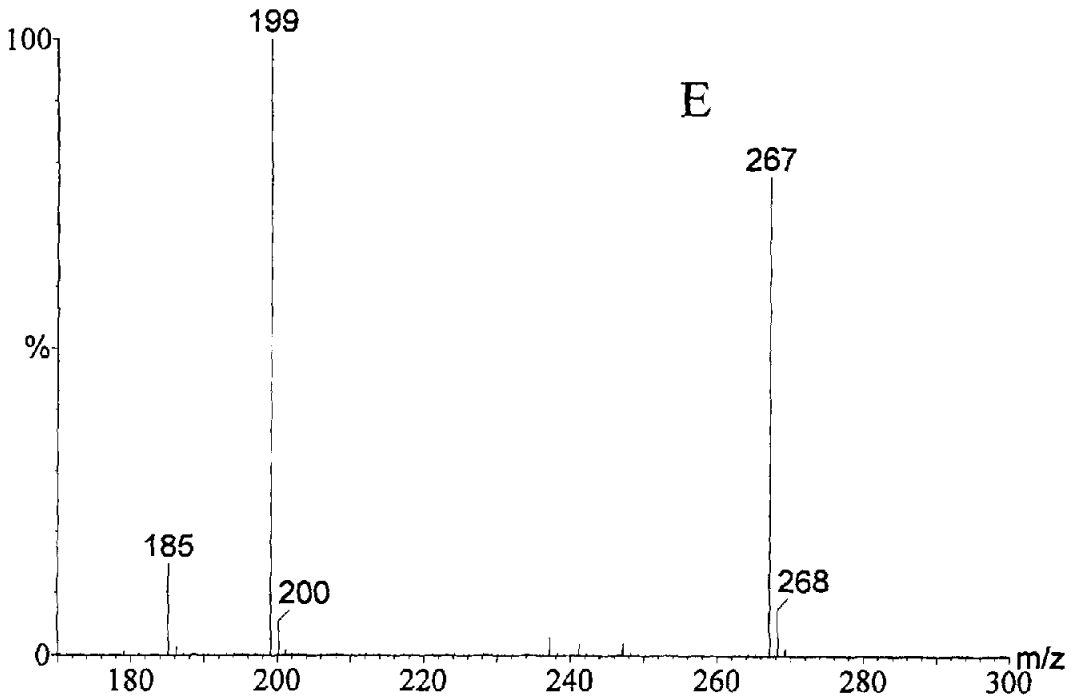


Fig. 2. Continued.



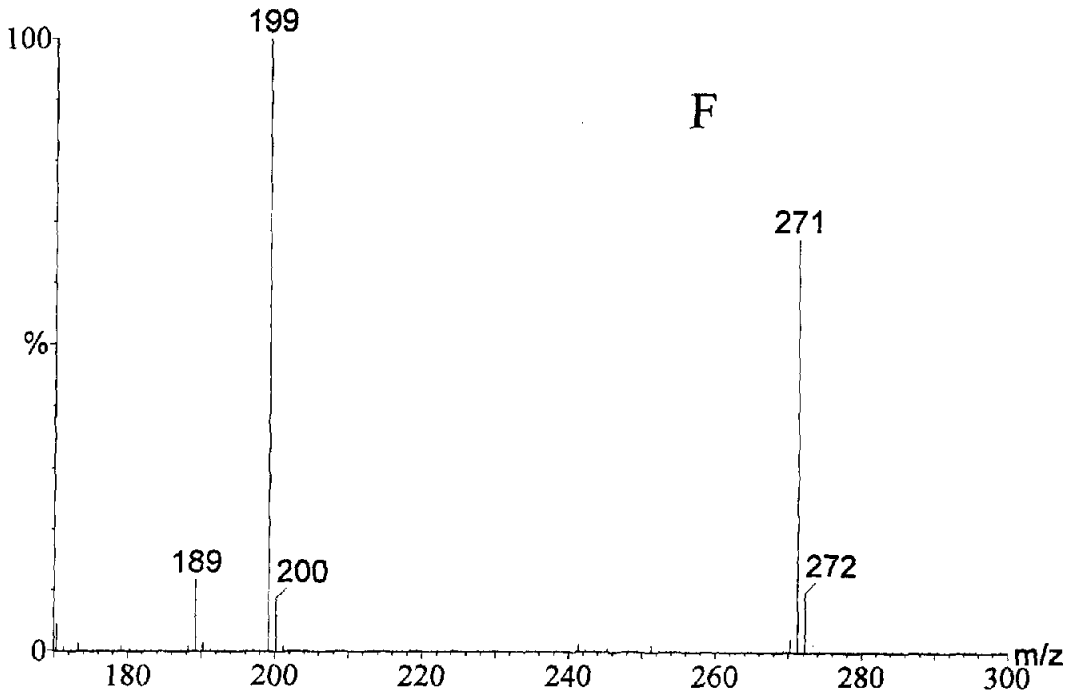


Fig. 2. Continued.

vestigated. The relative standard deviation for 10 urine samples spiked to a concentration of 5 μg/l was found to be 6.3%.

Detection limit

Using GC–TSD the response for PFPA, ethyl chloroformate and the TFECF derivatives of HDA were in the same range since the detector is essentially sensitive to the nitrogen content in HDA. In acidic aqueous air sampling solutions, where the chromatograms appear to be virtually free from interfering peaks, the detection limits were less than 10 pg injected amount. For biological fluids the detection limit was several orders of magnitude higher due to the complex matrix.

GC–SIM and chemical ionisation with ammonia showed the same detection limit for the monitoring of negative and positive ions. The detection limit defined as five times the noise was found to be *ca.* 0.5 μg/l. This corresponds to 10 pg HDA injected. The detection limit can possibly be lowered further by dissolving the dry residue in less volume, and an enrichment factor of 5 should be possible.

4. Conclusions

A method for the biological monitoring of exposure to HDI, at sub-TLV levels, by determination of HDA in hydrolysed urine is demonstrated. The two-phase derivatization with TFECF is simple as the work-up procedure is performed in one step. The method presented also offers enhanced possibility to determine endogenous and exogenous aliphatic di-amines at low concentrations (μg/l range) in biological fluids. The method is also applicable for the determination of HDI in air using impinger technique and acidic aqueous sampling solution.

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