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Determination of 1-(2-methoxyphenyl)piperazine derivatives of isocyanates at low concentrations by temperature-programmed miniaturized liquid chromatography

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Abstract

A temperature-programmed packed capillary LC method with large-volume injection on-column focusing has been developed for screening and determination of 1-(2-methoxyphenyl)piperazine derivatives of airborne toluene-2,4-diisocyanate, toluene-2,6-diisocyanate, hexamethylenediisocyanate and methylenebisphenyl-4,4-diisocyanate, based on sampling methods described in MDHS 25/3. Injection volumes up to 100 μl were successfully loaded onto the 250×0.32 mm I.D. capillary column packed with 3 μm Hypersil ODS particles. The isocyanate derivatives were loaded at 10°C and eluted by a three-step temperature program starting at 10°C for 10 min, followed by a temperature ramp of 2.5°C min^{-1} to 45°C and then 9.9°C min^{-1} to 90°C. The mobile phase consisted of acetonitrile–acetate buffer (3% triethylamine, pH 4.5) (45:55, v/v). The isocyanate derivatives were dissolved in acetonitrile–acetate buffer (3% triethylamine, pH 4.5) (30:70, v/v) to achieve sufficient focusing. The concentration limit of detection of the individual derivatives utilizing an ''U'' shaped flow cell with a 8.0 mm light path and an injection volume of 100 μl was 44, 87, 43 and 210 pg ml^{-1} for toluene-2,6-diisocyanate, hexamethylenediisocyanate, toluene-2,4-diisocyanate and methylenebisphenyl-4,4-diisocyanate, respectively. Within the investigated concentration range, 10–500 ng ml^{-1}, the linear calibration curves gave correlation coefficients ranging from 0.994 to 0.998. The repeatability of the method with regard to retention time and peak height ranged from 0.3 to 1.1% and 1.1 to 2.3% (n=9) relative standard deviation, respectively. The average recovery of the method, with regard to toluene-2,4-diisocyanate, was 97.7±1.6% (n=9).

Keywords: Air analysis; Temperature programming; Large-volume injections; Methoxyphenylpiperazine; Isocyanates

1. Introduction

Isocyanates are major industrial materials, mainly used in the production of polyurethane foams, paints, lacquers, inks and adhesives. Toluene-2,4-diisocyanate (2,4-TDI), toluene-2,6-diisocyanate (2,6-TDI), hexamethylenediisocyanate (HDI) and methylenebisphenyl-4,4-diisocyanate (MDI) (Fig. 1) are the most commonly used isocyanates in such products [1]. Unfortunately, isocyanates act as respiratory irritants and skin and respiratory sensitizers [2–5]. Thus, monitoring of isocyanates in workroom air is important to industrial hygiene.

Sampling methods for isocyanates in air are mainly based on a measured volume of air that is drawn through an impinger containing an amine

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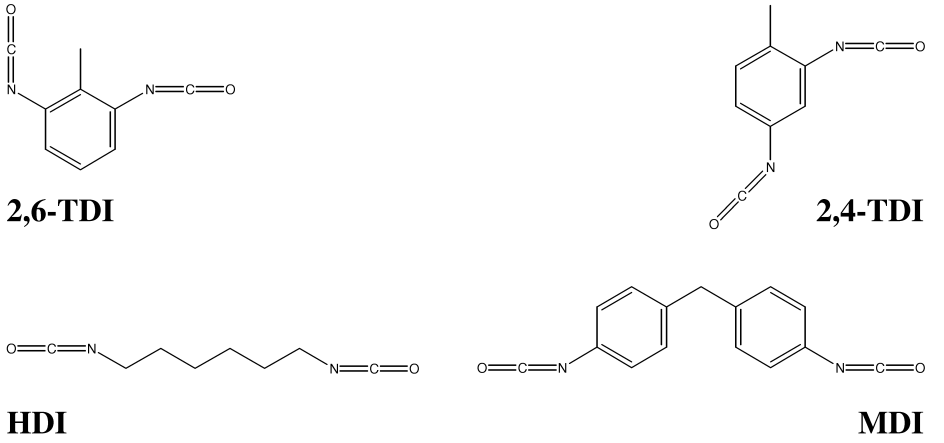

Fig. 1. Chemical structures of the diisocyanates.

reagent and/or a filter impregnated with the appropriate amine reagent, resulting in the formation of non-volatile urea derivatives from any organic isocyanate present [1]. A number of different methods using liquid chromatography (LC) with UV or electrochemical detection have been employed for the determination of diisocyanates [6–8]. However, the British standard LC–UV method for the determination of 2,4-TDI, 2,6-TDI, HDI and MDI; Methods for the Determination of Hazardous Substances (MDHS) 25/3, is widely used and has received broad acceptance in Europe [1]. The amine derivatization reagent employed in MDHS 25/3, 1-(2-methoxyphenyl)piperazine (1-2MP), provides rapid reaction rates with monomeric diisocyanates compared to other reagents [9].

There is a need for sensitive analytical methods for determination of lower concentrations of isocyanates in workroom air than provided by conventional LC methods. One approach to improve the detection limits of the existing LC methods is to utilize sensitive LC detection methods, like mass spectrometry (MS), and thereby achieve additional structural information [6,10]. A less expensive approach is to miniaturize the chromatographic system, as Brunmark et al. nicely exploited for sensitive determination of the 9-(*N*-methyl-aminomethyl)antracene derivatives of 2,4- and 2,6-TDI [11].

Packed capillary LC offers several advantages compared to conventional LC [12], and has received increased attention recently. Especially the increased mass sensitivity of packed capillary columns can be exploited when only limited sample volumes of low concentrations are accessible, if the sample is enriched upon injection [13–16]. This has traditionally been performed by dissolving the analytes in a solvent with non-eluting properties, allowing injection of volumes up to 200 μl on packed capillary columns [11].

Solvent gradient elution is the traditional way to adjust the elution strength of the mobile phase in LC during the separation run. However, this technique is not simple with columns of small inner diameters, due to instrumental limitations with regard to the low flow-rates required [17]. Packed capillary columns are recognized for their excellent suitability of using temperature programming for retention control, due to their low thermal mass [12]. Temperature-programmed packed capillary LC separations have still only been scarcely explored by a limited number of research groups [15,18–24]. Vissers claimed in a recent review on microcolumn LC that although presented results concerning test mixtures are in favor of conducting separation at high temperatures or with temperature gradients no workable applications of temperature-programmed LC have yet been demonstrated [25]. More recently however, Molander and co-workers have demonstrated the usefulness of performing temperature-programmed packed capillary LC for the separation of retinyl esters from polar seal liver [15], antioxidants extracted from polyolefins [26] and technical waxes [27]. However, there is still an absolute need for further performance evaluation of temperature-programmed packed capil-

lary LC separations of real samples, especially in combination with large-volume enrichment, if this technique is to be widely accepted.

The aim of this study was to explore the applicability of a temperature-programmed packed capillary LC screening method for the separation and determination of low amounts of derivatives of 2,4-TDI, 2,6-TDI, HDI and MDI in limited sample volumes, utilizing on-column enrichment of large sample volumes.

2. Experimental

2.1. Materials and reagents

Acetonitrile (ACN) of HPLC-grade and glass-distilled dimethylformamide (DMF) were obtained from Rathburn (Walkerburn, UK). Analytical-grade triethylamine (TEA) was purchased from Fluka (Buchs, Switzerland), while analytical-grade glacial acetic acid, sodium acetate, dichloromethane, toluene, acetic acid anhydride and hexane were purchased from Merck (Darmstadt, Germany). Water was deionized and glass distilled. 2,4-TDI, 2,6-TDI and HDI were obtained from Fluka. MDI and 1-2MP were obtained from Sigma–Aldrich (Steinheim, Germany).

All fused-silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA). Helium and nitrogen (99.998%) and carbon dioxide (99.99%) were purchased from AGA (Oslo, Norway).

2.2. Column preparation

The packed capillary columns were prepared according to a procedure previously described, using neat supercritical carbon dioxide as the slurry medium [18]. The stationary phase material was 3 μm Hypersil ODS 100 Å porous particles (HiChrom, Reading, UK). Valco ZU1C unions with 2-μm Valco 2SR1 steel screens served as column end fittings, and the columns were connected to the end fittings by Valco FS1.4 polyimide ferrules and steel nuts (Valco Instruments, Houston, TX, USA). The column body was fused-silica (320 μm I.D.×450 μm O.D.) with a polyimide protection layer. The columns were prepared in lengths of 25 cm.

2.3. Chromatographic instrumentation

A Merck–Hitachi L-7100 piston pump served as mobile phase deliverer (Tokyo, Japan). Manual injections were performed with either a Valco Model C4 injection valve with an internal loop of 0.05 μl or a Rheodyne Model 7010 sample injection valve (Cotati, CA, USA) with a 1/16 in. O.D.×0.5 mm I.D. external loop of 100 μl for large-volume injections (1 in.=2.54 cm). The sample was transferred from the loop during large-volume injection in opposite direction of that used during loading, making available time-limited injections of various volumes utilizing the same loop. The column was coupled to the injection valve by a 10-cm fused-silica capillary (50 μm I.D.×375 μm O.D.). A Mistral 880 served as column oven (Spark Holland, Emmen, The Netherlands). UV detection was performed using a Thermo Separation System UV 2000 (San Jose, CA, USA), operated at 235 nm, using either on-column detection with 100 μm optical light path or a capillary UZ-LI-CAP ''U'' shaped detector cell with 8 mm light path (LC Packings, Amsterdam, The Netherlands). A 10 cm×2.0 mm I.D. stainless steel cooling jacket with circulating fluid of 5°C encapsulating the capillary from the column to the detector was employed. To prevent the mobile phase from boiling when operating at elevated temperatures, a fused-silica linear restrictor of 15 cm×20 μm I.D.×375 μm O.D. was connected to the end of the detector capillary. A C-R5A integrator was used for data sampling (Shimadzu, Kyoto, Japan). The mobile phase consisted of ACN–acetate buffer (5 g sodium acetate l^{-1} water adjusted to pH 4.5 with glacial acetic acid and added 3% TEA). The mobile phase was helium degassed for 15 min each day, and the volumetric flow-rate was 5 μl min^{-1} throughout the study.

2.4. Standard solutions

Preparation of 1-2MP solutions (50 μg ml^{-1}) and synthesis of isocyanate 1-2MP derivatives were carried out according to MDHS 25/3 [1]. Stock solutions of the different diisocyanate derivatives in

DMF were prepared in concentrations of 10 mg ml^{-1} using an ultrasonic bath for 15 min and stored in a refrigerator at 4°C. Standard solutions in the range of 10–500 ng ml^{-1} were prepared daily from these stock solutions by appropriate dilution with ACN and a predetermined amount of the acetate buffer solution (3% TEA, pH 4.5), always keeping the DMF content below 2%.

Calibration solutions of all diisocyanate derivatives (10, 50, 100, 200, 300, 400 and 500 ng ml^{-1}) were prepared in ACN–acetate buffer (3% TEA, pH 4.5) (30:70, v/v).

2.5. Filter sample preparation

25-mm Gelman glass fiber filters (SKC, PA, USA) were impregnated and preconditioned according to MDHS 25/3 [1]. The filter was placed in a 4-ml glass vial containing 2 ml of the 1-2MP solution and spiked with 1.00 ml ACN containing 200 ng 2,4-TDI derivative. Subsequently, 100 μl acetic anhydride was added to the vials for acetylation of excess 1-2MP, prior to thorough mixing and evaporation under nitrogen to dryness. To the residue was added 2 ml ACN, it was placed in an ultrasonic bath for 5 min prior to filtration through a 0.22-μm Millex-GV syringe filter (Millipore, MA, USA) and evaporation under nitrogen to dryness. To this residue was added 0.60 ml ACN, it was placed in an ultrasonic bath for 5 min and subsequently diluted with 1.40 ml of the acetate buffer solution (3% TEA, pH 4.5) prior to packed capillary LC analysis. Three filter sample replicates were prepared.

3. Results and discussion

3.1. Chromatographic conditions

The MDHS 25/3 sampling method used in the present study is well characterized with regard to monomeric isocyanates, with sampling efficiencies normally close to 100% [1]. Consequently, the focus of the present paper was towards the determination of low concentrations of all the investigated isocyanates utilizing a single temperature-programmed packed capillary LC method.

In the standard MDHS 25/3 conventional LC method for the determination of isocyanates, a Hypersil ODS 5 μm stationary phase material was employed [1]. In the present study, packed capillary columns of 25 cm length were prepared with Hypersil ODS 3 μm packings, attempting to improve the separation efficiency. Initially, different mobile phase compositions of ACN and the sodium acetate buffer solution (3% TEA, pH 4.5) were investigated at ambient temperature. As in the standard reference method, resolution between the first eluting compounds, the 2,6-TDI and HDI derivatives, was not achieved within a reasonable time regardless the composition of the two mobile phase components. Furthermore, in order to achieve resolution between the 2,4- and 2,6-TDI derivatives, the elution time of the last eluting compound, the MDI derivative, increased substantially, resulting in a broad peak and reduced sensitivity, in accordance with results achieved using the reference method [28]. In addition, preliminary analysis of sampling solutions revealed the need for relatively large retention of the first eluting compounds to avoid coelution with compounds from the reagent matrix. Thus, temperature programming was explored, in order to separate the derivatives of the TDI isomers and HDI from the reagent matrix compounds and reduce the elution time of the MDI derivative. For this purpose a mobile phase composition of ACN–acetate buffer (3% TEA, pH 4.5) (45:55, v/v) was employed. Different temperature programs starting from 10°C were investigated. A sub-ambient initial temperature was chosen to expand the practical available temperature range, due to the possible risk of hydrolysis of the stationary phase at temperatures close to 100°C when aqueous mobile phases are used. It was necessary to maintain the initial temperature of 10°C for 10 min to separate HDI and 2,6-TDI from the reagent matrix compounds. A temperature gradient of 2.5°C min^{-1} to 45°C was used for elution of derivatives of HDI and 2,4- and 2,6-TDI. However, separation of the derivatives of 2,6-TDI and HDI was not achieved. Furthermore, an additional step of 9.9°C min^{-1} to 90°C was successively employed to elute MDI. When ending the temperature program at 45°C the MDI derivative did not elute within 2 h.

Renn and Synovec have earlier reported of rising baseline effects during temperature-programmed LC–UV, most probably due to changes in the

refractive index of the mobile phase with temperature [29]. Similar behavior was observed in this study, but was to some extent reduced by introducing a 10 cm×0.1 mm I.D. stainless steel cooling jacket with circulating fluid of 5°C encapsulating the capillary from the column to the detector. Thus the mobile phase was attempted cooled to a constant temperature prior to detection. The introduction of this post column dead volume did not influence significantly on the separation efficiency or peak heights. The reduced plate height of the 2,4-TDI derivative was determined to be 4.2 with a relative standard deviation (RSD) of 3.7% ($n=3$) at 25°C.

3.2. Large-volume injections

Different sample solvent compositions were investigated in order to focus large sample volumes onto the capillary columns. Initially an injection volume of 50 μl was explored, and the peak widths at half the peak heights of the first eluting peak 2,6-TDI derivative were measured as a function of the composition of ACN and acetate buffer (3% TEA, pH 4.5) at 10°C. The peak widths increased when the content of ACN exceeded 30% (v/v), indicating insufficient focusing. Consequently, a sample solvent composition of ACN–acetate buffer (3% TEA, pH 4.5) (30:70, v/v) was employed, to secure sufficient focusing and still adequate solubility of the isocyanate derivatives. Furthermore, various concentrations of the 2,6-TDI derivative in the final solvent composition were injected in various injection volumes ranging from 0.05 to 100 μl, always introducing an absolute mass of 10 ng, showing no significant variation in peak widths at half the peak heights ($P=0.05$).

In Fig. 2a and b the separation of the investigated isocyanate derivatives in the standard solutions using the established on-column large-volume injection temperature-programmed packed capillary LC method with an injection volume of 100 μl is shown. As illustrated in Fig. 2a and b, separation of 2,6-TDI and HDI was not achieved. In Fig. 2b, the temperature program was ended at 45°C, after the elution of HDI. When the final step of the program of 9.9°C min^{-1} to 90°C was employed to elute MDI, it resulted in a rising baseline (Fig. 2a). However, the baseline leveled out when the final temperature was reached, prior to elution of MDI.

Fig. 3 shows the analysis of the filter sample solution containing 100 ng ml^{-1} 2,4-TDI utilizing an injection volume of 100 μl. The temperature program ended at 45°C. As illustrated, it was necessary to maintain the initial temperature of 10°C for 10 min, in order to elute reagent matrix compounds prior to 35 min, which corresponds to the retention times of the 2,6-TDI and HDI derivatives.

3.3. Linearity

Calibration curves were established by injecting 100 μl of the calibration solutions ranging from 10 to 500 ng ml^{-1} of each isocyanate derivative ($n=1$). Peak height measurements were used. The correlation coefficients for the 2,6-TDI, HDI, 2,4-TDI and MDI derivatives were, 0.998, 0.998, 0.994 and 0.998, respectively.

3.4. Repeatability

The within-day repeatability and between-day variation of retention times and peak heights for the present LC method were determined by injecting 100 μl of a standard solution containing 100 ng ml^{-1} of each isocyanate derivative. The within-day repeatability of the retention time was 0.7, 0.3, 0.7 and 1.1% RSD with regard to the 2,6-TDI, HDI, 2,4-TDI and MDI derivatives, respectively ($n=3$). The within-day repeatability of peak heights displayed RSDs of 1.3, 1.1, 1.1 and 2.3% for the 2,6-TDI, HDI, 2,4-TDI and MDI derivatives, respectively ($n=3$). An analysis of variance (ANOVA) test showed no significant differences between the within- and between-day ($n=3$) measurements of retention times and peak heights ($P=0.05$).

3.5. Recovery

The sample preparation method used in this study was based on MDHS 25/3. However, an additional evaporation and dissolution step was added in order to obtain accurate sample volumes. The recovery of the 2,4-TDI derivative using the present sample preparation and chromatographic method was calculated. For this purpose 100 μl of the filter sample

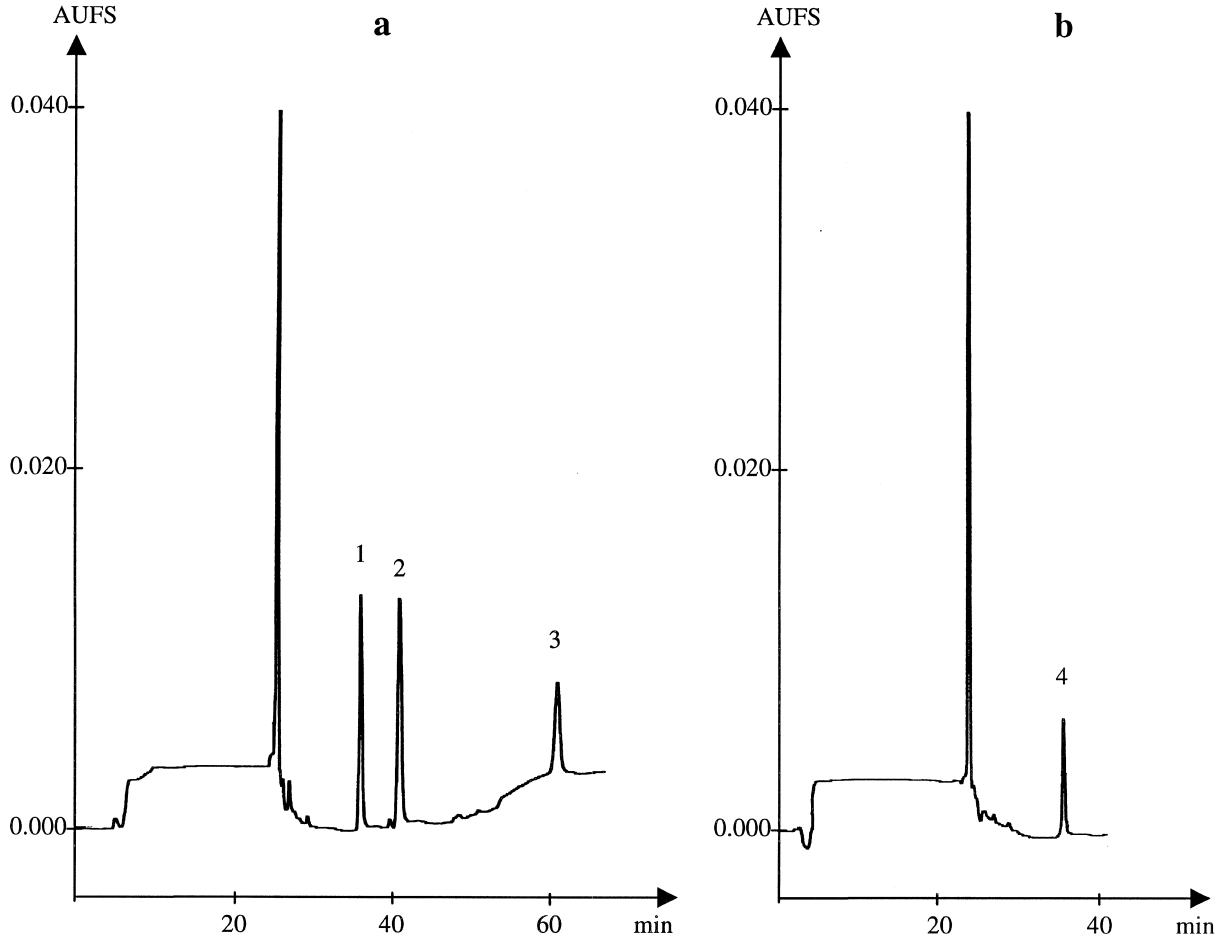

Fig. 2. Determination of the derivatives of (a) 65 ng 2,6-TDI (1), 65 ng 2,4-TDI (2) and 125 ng MDI (3) and (b) 65 ng HDI (4) dissolved in ACN–acetate buffer (3% TEA, pH 4.5) (30:70, v/v). A 25 cm×320 μm I.D. column packed with 3 μm Hypersil ODS particles was used with a mobile phase of ACN–acetate buffer (3% TEA, pH 4.5) (45:55, v/v). Injection of 100 μl was performed at 10°C. After completed loading, 10°C was held for 10 min, followed by a temperature program from 10 to 45°C, at 2.5°C min^{-1} (b), and then 9.9°C min^{-1} to 90°C (a). On-column detection with an optical light path of 100 μm was employed.

solutions containing 100 ng 2,4-TDI derivative ml^{-1} were injected and the peak heights were measured. The recovery was calculated using the established calibration curve. An ANOVA test ($P=0.05$) showed that there was no significant difference between sample replicates ($n=3$) and injection replicates ($n=3$). The average recovery of the 2,4-TDI derivative was 97.7±1.6% RSD (n=9). The high precision and accuracy of the method supported the use of external calibration.

3.6. Detection limits

Initially an on-column detector cell with an optical light path of 100 μm was employed. The concentration limit of detection (cLOD) when injecting 100 μl was determined to be 1.0, 2.0, 1.0 and 5.0 ng ml^{-1}, corresponding to mass limit of detection (mLOD) of 0.1, 0.2, 0.1 and 0.5 ng, of the 2,6-TDI, HDI, 2,4-TDI and MDI derivatives, respectively ($S/N=3$). Later an ''U'' shaped detector cell with an

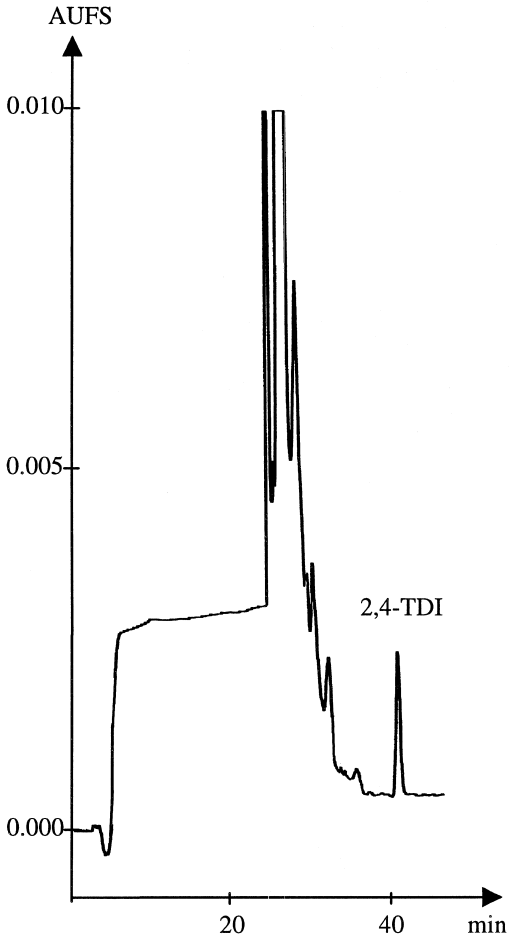

Fig. 3. Determination of a filter sample spiked with the 2,4-TDI derivative (10 ng). All other conditions as in Fig. 2.

optical light path of 8.0 mm was employed, resulting in substantially improved detection limits. The cLOD utilizing the ''U'' shaped flow cell was 44, 87, 43 and 210 pg ml^{-1}, corresponding to a mLOD of 4.4, 8.7, 4.3 and 21 pg of the 2,6-TDI, HDI, 2,4-TDI and MDI derivatives, respectively ($S/N=3$). With regard to 2,4- and 2,6-TDI, this is an improvement of a factor of 3 compared to the results presented by Brunmark et al. [11] and a factor of 30 compared to a conventional LC method [28]. The effect of rising baseline increased when utilizing the ''U'' shaped flow cell, but this effect did not influence on the quantitative measurements.

3.7. Robustness

The column efficiency was maintained and the column showed no sign of degradation during 2 months of extensive use. Furthermore, identical chromatographic performance was observed when the column was replaced with another column prepared identically. The stock solutions did not degrade throughout the whole study (2 months).

4. Conclusions

The present study has shown that temperature-programmed LC with large-volume injection on-column focusing is suitable for determination of low concentrations of diisocyanates prepared according to MDHS 25/3, and that the method is robust and accurate with regard to the samples investigated. Due to the excellent suitability of coupling packed capillary LC to MS, further emphasis should be directed towards this instrumental combination, to achieve structural information and improved detection limits of airborne isocyanates from workrooms.

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