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Determination of 1-(2-methoxyphenyl)-piperazine derivatives of airborne diisocyanates by packed capillary liquid chromatography with pre-column large-volume enrichment

A reliable, sensitive, and robust two-valve column-switching temperature-programmed packed capillary liquid chromatography method with on-column ultraviolet detection has been developed and validated for the simultaneous determination of 2,4-toluene-diisocyanate, 2,6-toluene-diisocyanate, hexamethylene-diisocyanate, and 4,4-methylene-bisphenyl-diisocyanate in workroom air, based on an established 1-(2-methoxyphenyl)-piperazine derivatization filter sampling method. The isocyanate derivatives were enriched on a 0.32 × 30 mm 5 μm Inertsil C_8 pre-column using a non-eluting solvent composition of acetonitrile-10 mM ammonium formate (pH 4.0) (4:96, *v/v*) at a flow rate of 50 μL/min, prior to back-flushing on a 0.32 × 250 mm 3-μm Hypersil ODS column, using a mobile phase composition of acetonitrile-10 mM ammonium formate (pH 6.0) (40:60, *v/v*) at a flow rate of 5 μL/min. Injection volumes up to 1.0 mL were loaded onto the pre-column. An initial temperature of 80°C provided beneficial selectivity effects as compared to ambient temperature, providing baseline separation of the 2,6-toluene-diisocyanate and hexamethylene-diisocyanate derivatives. Temperature programming from 80 to 95°C provided efficient elution of late eluting 4,4-methylene-bisphenyl-diisocyanate. The method was validated using spiked filters with 5 to 250 ng of the 2,4-toluene-diisocyanate derivative, yielding a coefficient of correlation of 0.997 when using an injection volume of 1.0 mL. The within-assay ($n = 4$) and between-assay ($n = 4$) precisions were in the range 2.7–29.0 and 2.0–18.0%, respectively, and the within- and between-assay recoveries of the isocyanate derivatives were 92.3–97.8 and 95.4–96.7% for all concentrations except for the lowest level. The mass limit of detection of the isocyanate derivatives for the LC method was in the range 0.12–0.25 ng, corresponding to a concentration limit of detection of 12–23 ng/m^3 total isocyanate groups in air using a 15-L air sampling volume, with 20% sample exploitation, 100% sampling efficiency, and 50% recovery at low concentrations.

Key Words: Isocyanates; Air monitoring; Liquid chromatography; Packed capillary columns; Column switching; On-column focusing; Temperature programming

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1 Introduction

Isocyanates are major industrial chemicals, and their widespread use is related to their important role as raw materials for the production of polyurethanes to form foams, paints, lacquers, inks, insulating materials, varnishes, rubber modifiers, and bonding- and vulcanizing agents [1]. 2,4-Toluene-diisocyanate (2,4-TDI), 2,6-toluene-diisocyanate (2,6-TDI), hexamethylene-diisocyanate (HDI), and 4,4-methylene-bisphenyl-diisocyanate (MDI) (**Figure 1**) are among the most frequently used isocyanates in such products [1]. Unfortunately, isocyanates are highly toxic substances that act as respiratory irritants and skin- and respiratory sensitizers, with the possibility of causing diseases like bronchitis, pulmonary emphysema, and asthma [2–4] in addition to allergic reactions [3]. Furthermore, isocyanates have a mutagenic potential through their ready reaction with proteins and DNA to form adducts [5]. Thus, the monitoring of isocyanates in workroom air is important to industrial hygiene.

Isocyanates are highly reactive, and HDI and the TDI isomers are volatile. Thus, sampling methods for isocyanates in workroom air usually include a derivatization step in which a volume of air is pumped through an impinger

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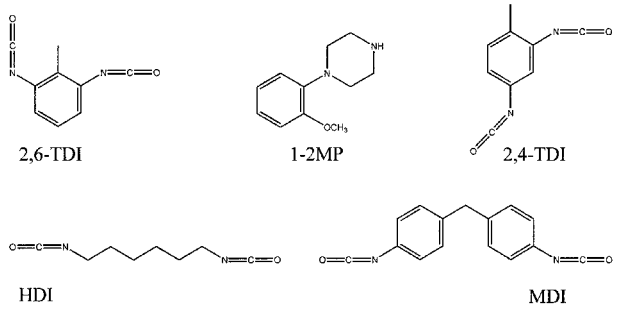

Figure 1. Structures of the isocyanates and the derivative reagent.

solution containing an amine reagent and/or a filter impregnated with the amine reagent, resulting in the formation of stable, non-volatile urea derivatives of any organic isocyanate present [6]. A number of different amine reagents have been explored for the sampling of isocyanates, including 1-(2-methoxyphenyl)-piperazine (1-2MP), dibutylamine (DBA), and 9-(*N*-methylaminomethyl)-anthracene (MAMA) [6–9], while numerous liquid chromatographic (LC) methods with electrochemical, ultraviolet (UV) or mass spectrometric (MS) detection have been presented for the determination of the isocyanate derivatives [7]. Nevertheless, the British standard "Methods for the Determination of Hazardous Substances (MDHS) 25/3" [6] is widely used in Europe for the measurement of isocyanates in workroom air, and is based on derivatization with 1-2MP prior to LC-UV quantification.

There is an increasing demand for analytical techniques that can measure components at low concentrations in often limited amounts of environmental and biological samples, e.g. air monitoring of isocyanates in occupational settings. Miniaturization is the key word when it comes to development of such new techniques, including the recent work on nano-particle technology [10] and chemistry-on-a-chip [11], in addition to the general trend of utilization of micro-scaled separation systems and detectors. Among these miniaturized techniques, packed capillary LC has shown considerable progress and improvements in its practical value [12].

The use of miniaturized columns in LC offers enhanced mass sensitivity due to reduced dilution of the chromatographic band as compared to the use of conventional columns [13]. Further improvements in concentration sensitivity are accessible if focusing techniques are employed upon sample introduction, permitting total sample exploitation. On-column focusing is traditionally performed by dissolving the sample in solvent compositions of non-eluting properties [4, 14–18], although Molander et al. have recently introduced the concept of sub-ambient temperature-assisted solute focusing in packed capillary LC, where the analytes are enriched at the column inlet at low temperatures where elution is suppressed [19–20]. Using focusing techniques, sample volumes up 200 μL have efficiently been loaded onto packed capillaries [4]. However, loading of such large injection volumes is a time consuming process considering the low flow rates used in packed capillary LC, typically in the range 1–10 μL/min. Hence, capillary scale pre-column switching systems have been explored, where large sample volumes are loaded onto shorter pre-columns generating low back pressures, allowing efficient sample loading at increased flow rates prior to column-switching back-flushed solute elution at linear velocities close to optimal [21–25]. Furthermore, column switching systems have potential for allowing elution from the analytical column simultaneously to pre-column loading of the next sample, in addition to the capability of on-line sample clean-up implementation.

Modern pressurized LC started as a complementary technique to gas chromatography (GC), replacing temperature with solvent strength as elution parameter. Thus, the need for temperature control in LC appeared to be less urgent, and solvent gradient elution has evolved as the conventional way to manipulate the elution strength in LC during the chromatographic run. However, this technique requires expensive dedicated micro flow pumping systems when using miniaturized columns, due to the low flow rates required. Packed capillary LC is, however, especially well suited for temperature programming, due to the low thermal mass of the small bore columns, and temperature-programmed packed capillary LC has in recent studies proven to be a feasible and elegant alternative to solvent gradients [17–20, 26–32]. Nonetheless, fears of decomposing analytes or the stationary phases, sometimes unwarranted, have added to the neglect of temperature as a variable in LC, although nearly all physical parameters of importance in LC are a function of temperature [33]. Thus, development of modern, more temperature stable stationary phase materials has to some extent inspired the use of elevated temperatures in LC, resulting in reduced column back pressures, and often enhanced efficiency [16, 33] and advantageous selectivity effects [17, 34].

The aim of this study was to develop and validate a highly sensitive large-volume packed capillary LC-UV column-switching screening method for the simultaneous determination of 2,4-TDI, 2,6-TDI, HDI, and MDI as their 1-2MP derivatives, based on the sampling methods described in MDHS 25/3 [6].

2 Experimental

2.1 Reagents and materials

Analytical grade ammonium formate was provided from Sigma (St. Louis, MO, USA), while analytical grade

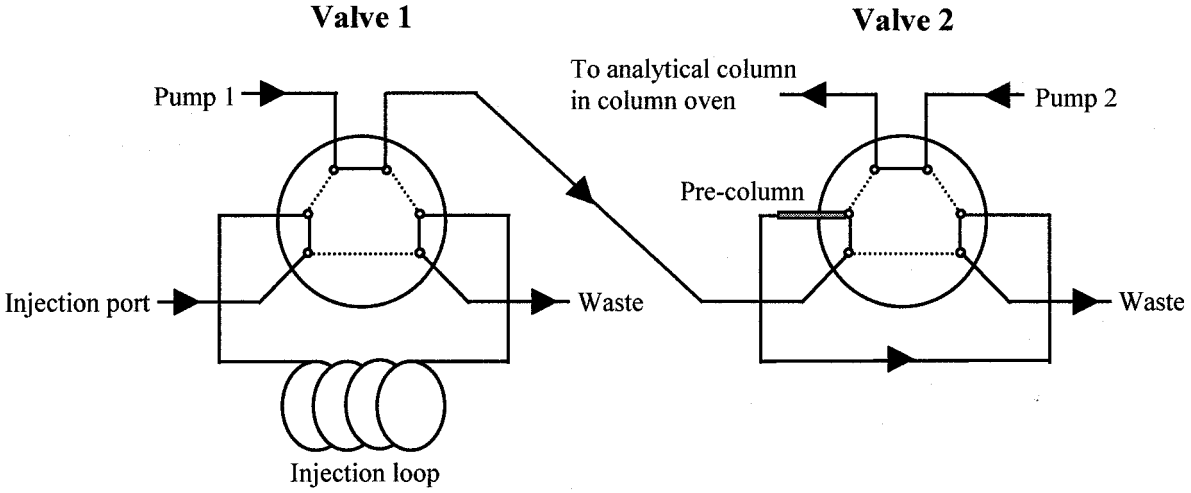

Figure 2. Schematic diagram of the column switching system.

sodium hydroxide, formic acid, toluene, acetic acid anhydride and dimethyl formamide were purchased from Merck (Darmstadt, Germany). HPLC grade acetonitrile and methanol were obtained from Rathburn (Walkerburn, UK) and Lab Scan (Dublin, Ireland), respectively. Analytical grade 2,4-TDI, 2,6-TDI, and HDI were obtained from Fluka (Buchs, Switzerland), while MDI and 1-2MP were obtained from Sigma. Deionized water was obtained from a Milli-Q station (Millipore, Bedford, MA, USA). Carbon dioxide (99.998%), helium (99.998%), and nitrogen (99.998%) were obtained from Aga (Oslo, Norway). 25 mm Gelman glass filters impregnated with 1-2MP were obtained from SKC (Pittsburgh, PA, USA) and 0.22-μm Millex-GV syringe filters were purchased from Millipore (Bedford, MA, USA). Fused silica capillaries with a protective polyimide layer were purchased from Polymicro Technologies INC (Phoenix, AZ, USA).

2.2 Chromatographic system

A schematic diagram of the manually operated column switching system is shown in **Figure 2**. The miniaturized LC column switching system consisted of two six-port switching valves and two pumps. The external 1-mL stainless steel sample loop was mounted in a Rheodyne model 7725 injection valve (Cotati, CA, USA) (valve 1), which was connected to an Upchurch Scientific M-435–1 low-dispersion micro switching valve (Oak Harbor, WA, USA) (valve 2) containing the enrichment column which was mounted between port 1 and 4. A Waters 590 pump (Milford, MA, USA) served as solvent deliverer for the transportation of the injected sample to the enrichment pre-column at a flow rate of 50 μL/min (pump 1). The 1 mL sample volumes were loaded onto the pre-column for a period of 21 minutes, simultaneously with the analytical column separation of compounds from the previous injection. A Hitachi L-7100 pump (Merck, Darmstadt, Germany) delivered a mobile phase flow rate of 5 μL/min for back-flushed desorption of the enriched analytes from the pre-column to the analytical column and subsequent temperature-programmed elution from the analytical column (pump 2). Valve 2 was back-switched after 2 minutes of sample back-flushing, followed by a pre-column washing procedure and loading of the subsequent sample.

The 0.32×250 mm packed capillary analytical columns were prepared according to a method previously described, using neat supercritical CO_2 as the slurry medium [31]. The stationary phase material was 3-μm Hypersil ODS (Hichrom, Reading, UK). Valco ZU1C unions with 2SR1 steel screens with 2-μm pores served as column end fittings for the analytical columns using Valco FS1.4 polyimide ferrules and steel nuts (Houston, TX, USA). The 0.32×30 mm enrichment pre-columns were prepared identically with a 5 μm Inertsil C_8 stationary phase material (Thermo Hypersil-Keystone, Cheshire, UK). A ceramic frit was prepared at the pre-column inlet according to a previously described method [31], while the column outlet was connected to a 0.1×100 mm fused silica open tubular capillary through a Valco ZU1C union with a 2SR1 screen. Thus, the pre-column inlet and the connected open tubular fused silica capillary could be mounted directly between port 1 and 4 in valve 2. The analytical column was coupled to injection valve 2 by a 0.05×100 mm fused silica capillary, while a 0.1×300 mm on-column detection capillary from which a short spot of the polyimide layer had been removed was connected to the column outlet. A 0.02×200 mm fused silica capillary was connected to the end of the detector capillary, to prevent the mobile phase from boiling at elevated temperatures.

A Hewlett-Packard 5790A gas chromatograph served as temperature programmable oven for the analytical column

(Wilmington, DE, USA), and a Spectrasystem UV-2000 detector was used for time-programmed wavelength on-column UV detection with 0.1-mm optical light path at 230 and 254 nm, respectively (Thermo Separation Products INC, San Jose, CA, USA). A Shimadzu C-R6A integrator was used for data sampling (Kyoto, Japan).

A non-eluting solvent composition of acetonitrile-10 mM ammonium formate (pH 4.0) (4 : 96, *v/v*) was used for pre-column analyte enrichment (pump 1), while the eluting mobile phase consisted of acetonitrile-10 mM ammonium formate (pH 6.0) (40 : 60, *v/v*) (pump 2). The pH was adjusted with formic acid and sodium hydroxide in the non-eluting solvent composition and in the mobile phase, respectively. A pre-column washing procedure with the injection of 1 mL acetonitrile-10 mM ammonium formate (pH 6.0) (20 : 80, *v/v*) was carried out between each run.

The analytes were enriched and desorbed from the pre-column at ambient temperature, while the analytical column was kept at 80°C for 39 minutes after the back-flushed column switching prior to temperature programming at 5°/min to 95°C which was maintained for 25 minutes.

3.3 Preparation of stock and standard solutions

Stock solutions of the 1-2MP derivatives of 2,6-TDI, 2,4-TDI, HDI, and MDI were prepared in dimethyl formamide in concentrations of 10 mg/mL. Acetonitrile-10 mM ammonium formate (pH 4.0) calibration solutions (4 : 96, *v/v*) of the derivatives were prepared from these stock solutions in concentrations of 1.0, 12.0, 25.0, 38.0, and 50.0 ng/mL. The 25.0 ng/mL calibration solution was used for robustness testing of the LC method. Solutions of 5, 60, 125, 190, and 250 ng 1-2MP-2,4-TDI/mL acetonitrile were prepared for spiking of sampling filters. All solutions were stored at 4°C.

3.4 Preparation of 1-2MP-2,4-TDI standard solutions from sampling filters

Non-exposed 1-2MP impregnated glass filters were spiked with 1.0 mL of the 5.00, 60.0, 125, 190, and 250 ng/mL of 1-2MP-2,4-TDI in 4-mL vials. 0.1 mL acetic anhydride was added to the vials for acetylation of excess 1-2MP reagent, prior to thorough mixing and evaporation under nitrogen to dryness. The residues were redissolved in 2 mL toluene and placed in an ultrasonic bath for 5 minutes prior to filtration through 0.22 μm syringe filters and evaporation under nitrogen to dryness. These residues were redissolved in 0.2 mL acetonitrile using ultrasonic treatment for 5 minutes, followed by dilution with 4.8 mL 10 mM ammonium formate (pH 4.0). The solutions were stored at 4°C.

Acetonitrile (1 mL) and acetic anhydride (0.1 mL) were added to filters used for air sampling prior to execution of the same procedure as described above.

3.5 Quantification

Quantification of the diisocyanate derivatives was performed utilizing peak height measurements. Calculation of recoveries of the diisocyanate derivatives from spiked filters was obtained from comparative LC calibration solutions.

4 Results and discussion

4.1 Pre-column focussing

Brunmark et al. have previously used large volume injection packed capillary LC for the determination of MAMA derivatives of the TDI isomers [8], while Molander et al. recently explored temperature-programmed large volume injection packed capillary LC for the determination of 1-2MP derivatives of HDI, MDI, and the TDI isomers [18]. The two studies have in common that the sample loading process of 100 μL sample was time-consuming, leading to practical limitations regarding the maximum injection volume applied. Column switching techniques, however, potentially offer fast and efficient loading of much larger injection volumes and were explored in the present study.

Several stationary phase materials were evaluated for pre-column sample enrichment in the present column switching system. A critical step was to establish a solvent composition that was strong enough to elute the heavily retained MDI derivative from the enrichment column while still being able to resolve the less retained 2,4-TDI, 2,6-TDI, and HDI derivatives on the analytical column. A 0.32×30 mm pre-column packed with 5-μm C_8 Inertsil particles provided both satisfactory enrichment and efficient desorption of all the analytes, and was subsequently employed as pre-column.

Various ratios of acetonitrile-10 mM ammonium formate (pH 4.0) were evaluated for establishment of a non-eluting solvent composition. An ammonium formate buffer pH of 4.0 in the non-eluting solvent composition provided reduced transfer of front-eluting reagent matrix components to the analytical column as compared to the use of a buffer of pH 6.0. The peak widths at half the peak height were measured as a function of the acetonitrile content in the non-eluting solution, always injecting 1.0 mL of a solution containing 10 ng/mL of the first eluting 2,6-TDI derivative at flow rate of 50 μL/min. The 2,6-TDI derivative was desorbed from the pre-column and chromatographed on the analytical column using pre-determined conditions (described in the next section). The peak widths at half the

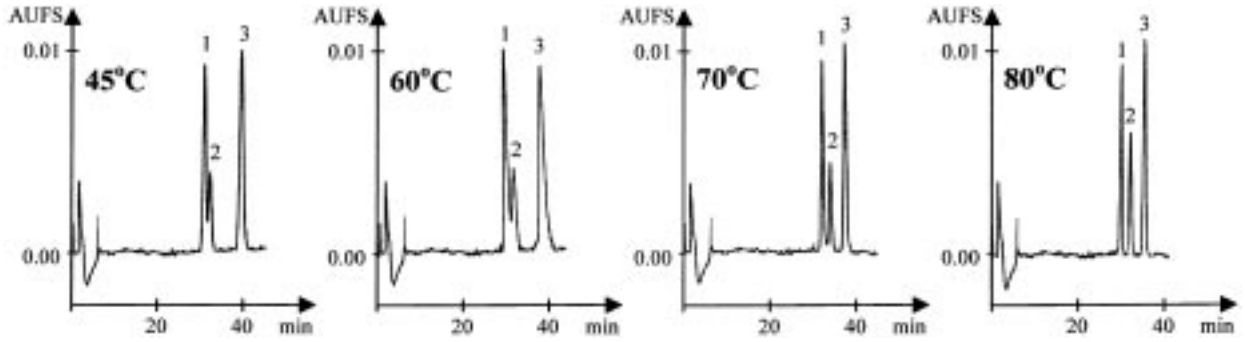

Figure 3. Influence of elevated temperatures upon the selectivity of the separation of the 2,6-TDI (1), HDI (2), and 2,6-TDI (3) 1-2MP derivatives using mobile phase pH 6.0 (10 ng of each).

peak height decreased linearly on reducing the acetonitrile content gradually from 15 to 4%, while they remained invariant on using acetonitrile contents below 4%, indicating sufficient focussing of 1-2MP-2,6-TDI using a non-eluting solvent composition of acetonitrile-10 mM ammonium formate (pH 4.0) (4:96, *v/v*). This solvent composition provided sufficient solubility of all the isocyanate derivatives in the investigated concentration range, and was subsequently used as non-eluting solvent composition throughout the study.

In order to determine the maximal tolerated pre-column sample introduction flow rate of the system, 1 mL of the 10 ng/mL 2,6-TDI derivative non-eluting sample solution was loaded at different flow rates between 30 and 100 μL/min, measuring the peak height as a function of flow rate. The 2,6-TDI derivative was desorbed from the pre-column and chromatographed on the analytical column using predetermined conditions (described in the next section). The peak height remained invariant for flow rates up to 60 μL/min, while it decreased linearly when loading at flow rates between 60 and 100 μL/min. The peak areas, however, remained invariant in the investigated flow rate region, supporting a theory that increased mass transfer band broadening effects caused the observed reduced peak heights at the highest flow rates, and that sample break-through did not appear. A pre-column sample loading flow rate of 50 μL/min was subsequently used throughout the study.

Furthermore, different sample loop volumes ranging from 50 nL to 1 mL were evaluated in order to confirm the efficacy of the sample focusing, using the optimized, predetermined conditions described above and injecting the 10 ng/mL 2,6-TDI derivative non-eluting sample solution. A plot of the peak heights vs. the injection loop volumes showed a linear relationship within the investigated loop volume range with a coefficient of correlation of 0.994, indicating efficient focusing independent of the applied injection volume.

4.2 Separation of the isocyanate derivatives

4.2.1 Temperature effects

A 3.0-μm Hypersil ODS material was employed as stationary phase, based on our previous experiments on separation of 1-2MP derivatives of isocyanates [18]. As compared to a 3.5-μm Kromasil ODS material, the former phase provided improved peak shapes for all analytes. A mobile phase composition of acetonitrile-10 mM ammonium formate (pH 6.0) (40:60, *v/v*) provided fast and efficient desorption of the derivatives from the pre-column and reasonable resolution and retention on the 0.32×250 mm analytical column, and was subsequently used as mobile phase in the final method. Unfortunately, the 2,6-TDI and HDI derivatives were not resolved at ambient temperature under these conditions, in accordance with previous reversed phase LC studies using different mobile and stationary phases [6, 18]. However, the column temperature can have a pronounced effect on the selectivity of a separation [33]. Accordingly, the separation of the 2,6-TDI and HDI derivatives was evaluated at different temperatures. Elevated temperatures usually provide reduced retention and enhanced efficiency in LC [33]. In the present study, however, the analytes responded differently to the elevated temperatures, resulting in favorable selectivity effects. The retention of the TDI isomer derivatives decreased with temperature as distinct from the slightly increased retention of the HDI derivative, resulting in baseline separation and improved peak shapes of the three analytes at 80°C (**Figure 3**). The resolution between the 2,6-TDI and HDI derivatives increased linearly between 45 and 80°C. The TDI isomer derivatives were labile when operating at 90°C. Although the pre-column was operated at ambient temperature and the separation column at 80°C, a phase-focussing mechanism combined with diminished extra-column volumes in switching valve 2 and the connecting capillaries, granted low dispersion when transferring the components from the pre-column to the analytical column.

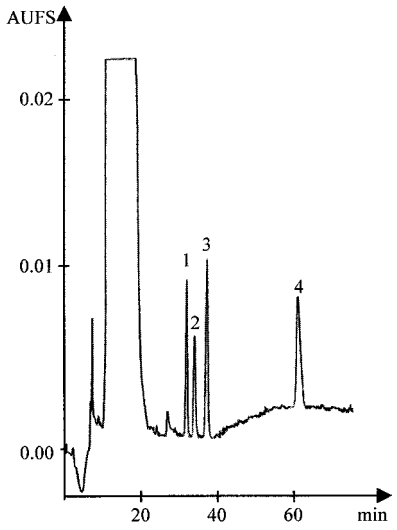

Figure 4. Packed capillary column-switching LC separation of 12 ng of each the 1-2MP derivatives of 2,6-TDI (1), HDI (2), 2,4-TDI (3), and MDI (4) from a spiked air sampling filter. The injection volume was 1.0 mL, the mobile phase pH was 6.0 and a temperature program from 80 (37 min) to 95°C at 5°C/min was used.

Shorter column lengths than 250 mm did not provide baseline separation of the 2,6-TDI and HDI derivatives.

The MDI derivative was heavily retained at 80°C and eluted after 82 minutes as a relatively broad peak, illustrating the need for gradient action. Isothermal analyses revealed that the MDI derivative responded favorably to elevated temperatures in means of reduced retention, and that it was stabile up to at least 95°C. In addition, the stationary phase showed no signs of degradation by operation at these temperatures in aqueous environment. Thus, temperature programming from 80 to 95°C was explored. The initial temperature was held for 37 minutes after column switching, in order to completely elute the TDI isomer derivatives at 80°C where they were stable. Furthermore, the prolonged initial conditions of 80°C offered sufficient separation the first eluting isocyanate derivatives from the matrix front. A subsequent temperature ramp of 5°/min to 95°C provided elution of the MDI derivative after 63 minutes, which is a 23% reduction as compared to the isothermal run at 80°C. The peak shape was also remarkably sharpened, resulting in an increased MDI derivative peak height of 152%. This temperature program was subsequently used in the final method. The detector wavelength was automatically changed from 230 to 254 nm between the elution of the 2,4-TDI and the MDI derivatives, in order to operate at maximum absorbancy for each individual derivative. **Figure 4** shows the temperature-programmed column-switching packed capillary LC separation of 12 ng of the respective isocyanate derivatives prepared from a spiked sampling filter, using an injection volume of 1.0 mL.

4.2.2 Mobile phase pH effects

The pH of the acetonitrile-10 mM ammonium formate (40:60, v/v) mobile phase at 80°C had a pronounced effect on the peak shapes of the 2,4-TDI, 2,6-TDI, and HDI derivatives. By increasing the pH from 4.0 to 6.0, significantly reduced peak tailing and subsequent improved peak resolution were observed due to suppressed ionization of the derivatives resulting in reduced secondary interactions with residual silanols. The pH was subsequently adjusted to 6.0 in the 10 mM ammonium formate mobile phase buffer in the final method.

4.3 Method validation

4.3.1 Linearity

The method was validated in the concentration range 5 to 250 ng of isocyanate derivative, which corresponds to the concentration range 0.050–2.5, 0.050–2.5, and 0.040–2.2 $\mu g/m^3$ of total isocyanate groups (NCO) for the TDI isomers, HDI and MDI, respectively, using a 15-L air sampling volume.

LC calibration curves were established by injecting 1.0 mL sample volumes of the calibration solutions ranging from 1.0 to 50 ng/mL of each isocyanate derivative. All the LC calibration curves were linear with coefficients of correlation >0.999. Only the 2,4-TDI isomer was subjected to method validation, as the remaining isocyanates are expected to behave similarly to the 2,4-TDI derivative during extraction from air filters [6, 18]. The calibration curve for 2,4-TDI was established by spiking filters with absolute amounts of 1–2MP-2,4-TDI in the range 5 to 250 ng. 1.0 mL of the prepared total sample volumes of 5.0 mL was injected onto the enrichment column. The method was linear within the investigated concentration range with a coefficient of correlation of 0.997.

4.3.2 Precision

The within- and between-assay precision was established by injecting four sets of samples at three spike concentration levels (1.0, 25.0, and 50.0 ng/mL) within one day and within two weeks, respectively, by the same analyst. The within-assay ($n = 4$) and between-assay ($n = 4$) precision were in the range 2.7–29.0 and 2.0–18.0%, respectively, as summarized in **Table 1**. The relative standard deviations of the within- and between-assay precision were comparatively higher at the lowest validated concentration level as compared to at higher concentrations.

4.3.3 Recovery

The within- and between-assay recoveries of the isocyanate derivatives were 92.3–97.8 and 95.4–96.7%, respectively, except at the lowest concentration, where the

Table 1. Within- and between assay precision and recovery of the method.

Analyte	Injected mass (from filter) (ng)	Within assay precision (% RSD, $n = 4$)	Between assay precision (% RSD, $n = 4$)	Within assay recovery (%)	Between assay recovery (%)
1-2MP-2,4-TDI	1.00	29	18	48.8	54.3
	12.0			92.3	
	25.0	4.5	2.5	97.8	96.7
	38.0			94.1	
	50.0	2.7	2.0	93.1	95.4

Table 2. Detection limits of the isocyanate derivatives.

Analyte	mLOD derivative LC method (ng)	mLOD derivative method (ng)	mLOD NCO (ng)	cLOD NCO 15-L air sample (ng/m^3)
1-2MP-2,4-TDI	0.12	1.2	0.18	12
1-2MP-2,6-TDI	0.15	1.5	0.23	15
1-2MP-HDI	0.23	2.3	0.35	23
1-2MP-MDI	0.25	2.5	0.33	22

within- and between-assay recoveries were 48.8 and 53.3%, respectively, as summarized in Table 1. The observed low recoveries at low analyte concentrations are most probably related to losses during the sample preparation.

4.3.4 Detection limits

The LC method mass limits of detection (mLOD) of the isocyanate derivatives were established using a signal-to-noise ratio of 3:1. Only 1.0 mL of the total sample volume of 5.0 mL was injected in the present study, resulting in increased mLOD of the method with a factor of five as compared to the LC method mLOD. The mLOD of the LC method were in the range 0.12–0.25 ng. Furthermore, the recovery of the method was approximately 50% for masses close to the mLOD, resulting in a method mLOD in the range 1.2–2.5 ng for the derivatives, corresponding to a mLOD of 0.18–0.35 ng NCO. The concentration LOD (cLOD) of the method was thus between 12–23 ng/m^3 NCO in air for the derivatives, using a 15-L air sampling volume and assuming 100% sampling efficiency. The LODs are summarized in **Table 2**.

Although the present method provided sufficient efficiency, the method LODs have potential for improvement by several orders of magnitude by using a detector flow cell with a longer optical light path than 100 μm or by using e.g. MS or electrochemical detection, or simply by dissolving the derivatives in a smaller sample volume or by injecting larger sample volumes.

4.3.5 Robustness

The within- and between-assay precision of retention times using temperature-programmed elution was below 6%, and the column efficiency remained invariant throughout the study. Identical chromatographic performance was observed when the pre-column and the analytical column were replaced with columns prepared identically. All calibration solutions and real sample solutions were stable for at least two months when stored at 4°C.

4.3.6 Application

The validated method was used for analyses of isocyanate samples collected at a shipbuilding yard during a spray painting process, using a sampling volume of 20 L (**Figure 5**). It was calculated that the 5.0-mL sample contained 160 and 38.0 ng of 1-2MP-2,4-TDI and 1-2MP-MDI, respectively, which corresponds to a total NCO concentration in the workroom atmosphere of 1.46 μg/m^3.

5 Conclusion

A reliable and robust miniaturized temperature-programmed column-switching LC method for determination of isocyanates in workroom air has been developed and validated, based on the 1-2MP filter sampling methods described in MDHS 25/3 [6]. The packed capillary column-switching LC method provided simultaneous separation of the isocyanate derivatives and high sensitivity, and can easily be automated. Thus, the method is suitable for routine determination of isocyanates in workroom air within the validated concentration range.

Due to the excellent capability of coupling miniaturized LC to electrospray (ESI) MS for sensitive and selective determination of isocyanates, our future work will be directed to

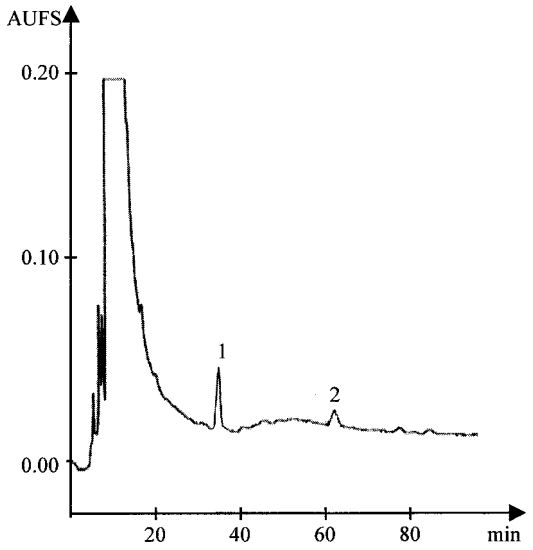

Figure 5. Packed capillary column-switching LC separation of the 1-2MP derivatives of 2,4-TDI (1) and MDI (2) from a 20 L air sample collected at a shipbuilding yard during a spray painting process. All other conditions as in Figure 4.

towards this instrumental combination, making the need for large sample volumes even less redundant. Thus, further emphasis will also be directed towards development of miniaturized personal sampling instrumentation, in order to develop devices that do not obstruct the working procedures during sampling in occupational settings.

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