Journal of Chromatography, 134 (1977) 451–458

CHROM. 9764

SIMPLE UREAS DERIVED FROM DIISOCYANATES AND THEIR LIQUID CHROMATOGRAPHY ON A 5-cm COLUMN

CORAZON R. HASTINGS VOGT*, CHAN YAN KO and TIMOTHY R. RYAN
Environmental Trace Substances Research Center, University of Missouri, Columbia, Mo. 65201 (U.S.A.)
(Received September 7th, 1976)

SUMMARY

Aliphatic and aromatic diisocyanates were converted to ureas by a chromophoric derivatizing reagent. The ureas were isolated in the pure form and characterized by various methods including nuclear magnetic resonance and mass spectrometry. A chromatographic analytical separation of the urea test solutions was established. The method was used to demonstrate the derivatization of the isocyanates and direct determination of the produced ureas from the reaction mixture.

INTRODUCTION

The isocyanates are widely used in the urethane foam industry. These compounds are known to cause skin and respiratory tract irritations. Methods of sampling and analysis of airborne isocyanates include colorimetry[1–3], thin-layer chromatography (TLC)[4] and high-performance liquid chromatography (HPLC)[5].

In the development and evaluation of a sampling technique for isocyanates, it became necessary to procure (1) standards for effective analysis, and (2) liquid chromatographic (LC) columns that could be repeatedly replaced. While the former are not commercially available, basic reaction procedures for their preparation have been reported[5]. The high cost of pre-packed columns made their use impractical, and led to our own column preparation. This paper reports the preparation, isolation and characterization of ureas that serve as reference standards in the analysis. It also reports a highly efficient, inexpensive column capable of separating ureas derived from common diisocyanates, either in their isolated forms or direct from reaction mixtures.

EXPERIMENTAL

Apparatus

A Model 202 liquid chromatograph (Waters Assoc., Milford, Mass., U.S.A.)

* To whom reprint requests should be addressed.

was equipped with a Model U6K Universal LC Injector, a Model 660 Solvent Programmer, and two Model 6000 pumps needed for gradient elution. A Schoeffel Model 770 spectroflow monitor (Schoeffel, Westwood, N.J., U.S.A.) or Waters Assoc. Model 440 absorbance detector, set at 254 nm, was used.

Chemicals

The 1,6-diisocyanatohexane (98%), toluene 2,4-diisocyanate (99%), *p*-tolylisocyanate (99%), *n*-propylamine (98%) and 4-nitrobenzyl chloride (99%) were obtained from Aldrich (Milwaukee, Wisc., U.S.A.). The 4,4'-diphenylmethane diisocyanate, Mondur TD (mixture of 65% toluene-2,4-diisocyanate and 35% toluene-2,6-diisocyanate) and Desmodur N-100 (a high-molecular-weight biuret of 1,6-diisocyanatohexane) were obtained from Mobay (Pittsburgh, Pa., U.S.A.).

Preparation of derivatizing reagent

Fifty grams (0.29 moles) of 4-nitrobenzyl chloride were dissolved in 240 ml of benzene. The solution was brought to reflux, then 36 g (0.61 moles) of *n*-propylamine were added dropwise over a 15-min period and refluxing continued for 5 h. The solvent was stripped off in a rotary evaporator (Büchi Rotavapor-R, Switzerland; distributed by Fisher Scientific, St. Louis, Mo., U.S.A.) at 50°. The residue, dissolved in 80 ml of double distilled water, was treated with 30 ml of a 45% NaOH solution. Then 100 ml of benzene were added and the benzene layer was separated after stirring for 5 min. Benzene and the excess *n*-propylamine were stripped off in a rotary evaporator. The product (N-4-nitrobenzyl-N-*n*-propylamine) was dissolved in 50 ml of acetone and its salt was formed by the addition of 34 g of conc. HCl. The mixture was evaporated to dryness at 50° in a rotary evaporator, and the salt, washed three times with acetone–benzene (1:1) under suction filtration, was dried overnight in a vacuum oven at 50° [m.p., 230°–232°, IR (KBr) 1340, 1520 cm^{-1} (C–NO_2); in addition to the 2 IR bands of the salt, the free amine showed a band at 3320 cm^{-1} (N–H)]. From here on the N-4-nitrobenzyl-N-*n*-propylamine is referred to as nitro reagent. This derivatizing reagent, introduced by Keller *et al.* for TLC[4] and Dunlap *et al.* for HPLC[5], is now available from Regis (Morton Grove, Ill., U.S.A.)[6].

Preparation of derivatives

The ureas of interest have been prepared before by Dunlap *et al.*[5], who analyzed the products from the reaction mixture by HPLC. The procedures were adopted in the present work, which include the additional isolation, purification and characterization of the reaction products. The disappearance of the IR (KBr) band at 2275 cm^{-1} (N=C=O) of the isocyanates served to monitor the extent of reaction during the preparation of the ureas.

In each of the preparations, a minimum of 1:2 molar ratio of diisocyanate to nitro reagent was maintained. Since the primary objective of the preparation was to acquire the solid ureas needed as primary standards for isocyanate analysis, no attempts were made to optimize yield.

The 4,4'-diphenylmethane-di[3-n-propyl-3(4-nitrobenzyl)] urea (4,4'-MDIU). A 2.43-g portion of the hydrochloride of nitro reagent was weighed and dissolved in 25-ml distilled water; 15 ml of 1 *N* NaOH was added to precipitate the free amine, which was then extracted into 50 ml *n*-heptane (toluene and benzene can also be used).

A 1.30-g sample of 4,4'-diphenylmethane diisocyanate (4,4'-MDI), purified, and dissolved in 25 ml CH_2Cl_2, was poured with stirring into the nitro reagent heptane solution. The urea of MDI was precipitated and was subsequently filtered and dried. It was purified further by precipitation with *n*-heptane from the CH_2Cl_2 solution (about 1.1 g). 4,4'-MDIU: m.p., 151°–153°; IR (KBr), 1340, 1500–1520, 1630–1650 and 3330 cm^{-1}; UV (CH_2Cl_2) ε_{254}, 4.76×10^4 and ε_{270}, 2.44×10^4; (70 eV) *m/e* (relative intensity): 638 (3.5) $C_{35}H_{38}N_6O_6$, 502 (0.7) $C_{28}H_{32}N_5O_4$, 444 (7.0) $C_{25}H_{24}N_4O_4$, 194 (100) $C_{10}H_{14}N_2O_2$; NMR ($CDCl_3$) δ 0.90–1.15 (*t*, 3H, $J_{1,2} = 3.5$ Hz, γ-CH_3), δ 1.50–1.90 (*d* of *q*, 2H, $J_{1,2} = 3.5$ Hz, $J_{2,3} = 3.8$ Hz, β-CH_2), δ 2.25 (*s*, 2H, phenyl–CH_2–phenyl), δ 3.23–3.50 (*t*, 2H, $J_{2,3} = 3.8$ Hz, α-CH_2), δ 4.00 (*s*, 1H, N–H), δ 4.90 (*s*, 2H, phenyl–CH_2–N), δ 7.06–7.40 (*m*, 4H, phenyl H's), δ 7.50–7.66 (*d*, 2H, $J_{o,m} = 4.0$ Hz, phenyl H's *m* to nitro), δ 8.20–8.38 (*d*, 2H, $J_{o,m} = 4.0$ Hz, phenyl H's *o* to nitro); CHN analysis, calculated for $C_{35}H_{38}N_6O_6$: C 65.83, H 5.96, N 13.7; found, C 65.57, H 6.37, N 13.62.

The 2,4-(1-tolyl)-di[3-n-propyl-3-(4-nitrobenzyl)] urea (2,4-TDIU). The hydrochloride of nitro reagent (1.03 g) was extracted into 50 ml of toluene as described earlier. A 0.32-g portion of toluene 2,4-diisocyanate (2,4-TDI) dissolved in 30 ml toluene was slowly mixed with the nitro reagent. The precipitate was filtered and purified by reprecipitation with hexane from the minimal amount of CH_2Cl_2 (about 0.15 g). 2,4-TDIU: m.p., 131°–134°; IR (KBr), 1340, 1520, 1630, and 3280 cm^{-1}; UV (CH_2Cl_2) ε_{254}, 2.23×10^4 and ε_{270}, 1.89×10^4; (70 eV) *m/e* 562 (0.8) $C_{29}H_{34}N_6O_6$, 368 (100) $C_{19}H_{20}N_4O_4$, 194 (61) $C_{10}H_{14}N_2O_2$; NMR ($CDCl_3$) δ 0.97–1.23 (*t*, 3H, $J_{1,2} = 3.5$ Hz, γ-CH_3), δ 1.30–1.90 (*d* of *q*, 2H, $J_{1,2} = 3.5$ Hz, $J_{2,3} = 4.0$ Hz, β-CH_2), δ 2.14 (*s*, 3H, phenyl–CH_3), δ 3.10–3.50 (*t*, 2H, $J_{2,3} = 4.0$ Hz, α-CH_2), δ 4.75 (*s*, 2H, phenyl–CH_2–N), δ 6.28–6.45 (*d*, 1H, $J = 5.5$ Hz, N–H), δ 7.18–7.50 (*t*, 3H, $J = 5.0$ Hz, phenyl H's), δ 7.63–7.78 (*d*, 2H, $J_{o,m} = 4.0$ Hz phenyl H's *m* to nitro), δ 8.22–8.37 (*d*, 2H, $J_{o,m} = 4.0$ Hz, phenyl H's *o* to nitro); CHN analysis, calculated for $C_{29}H_{34}N_6O_6$: C 61.92, H 6.05, N 14.95; found, C 62.06, H 6.11, N 14.70.

The 2,6-(1-tolyl)-di[3-n-propyl-3-(4-nitrobenzyl)] urea (2,6-TDIU). A solution of 0.13 g Mondur TD in 25 ml toluene was slowly added with stirring to the 50 ml toluene solution of the nitro reagent. It was left standing for 30 min. The precipitate was filtered and dried under vacuum. The 2,6-TDIU was recovered by dissolving the precipitate into minimum amount (3–5 ml) of CH_2Cl_2 and adding just enough toluene to initiate precipitation. (Note that it was proven in an earlier testing that in a solution of 2,4-TDI, 2,6-TDI and nitro reagent, the 2,6-TDIU precipitates first from CH_2Cl_2 by the addition of toluene). The precipitate was filtered, washed with a small amount of toluene and dried under vacuum (about 0.05 g). 2,6-TDIU: m.p., 185°–187°; IR (KBr), 1340, 1480–1500, 1580–1630 and 3360 cm^{-1}; UV (CH_2Cl_2) ε_{254} 2.89×10^4 and ε_{270} 2.67×10^4; (70 eV) *m/e* 562 (3.5) $C_{29}H_{34}N_6O_6$, 368 (55) $C_{19}H_{20}N_4O_4$, 194 (100) $C_{10}H_{14}N_2O_2$, 174 (73) $C_9H_6N_2O_2$; NMR ($CDCl_3$) δ 0.85–1.10 (*t*, 3H, $J_{1,2} = 3.5$ Hz, γ-CH_3), δ 1.53 (*s*, 2H, β-CH_2), δ 1.95 (*s*, 3H, phenyl–CH_3), δ 3.22–3.45 (*t*, 2H, $J_{2,3} = 4.0$ Hz, α-CH_2), δ 4.66 (*s*, 2H, phenyl–CH_2–N), δ 6.15 (*s*, 1H, N–H), δ 7.23 (*s*, 3H, phenyl H's), δ 7.40–7.55 (*d*, 2H, $J_{o,m} = 4.5$ Hz, phenyl H's *m* to nitro), δ 8.18–8.33 (*d*, 2H, $J_{o,m} = 4.5$ Hz, phenyl H's *m* to nitro). CHN analysis, calculated for $C_{29}H_{34}N_6O_6$: C 61.92, H 6.05, N 14.95; found, C 61.57, H 6.24, N 15.10.

The 1,6-Hexane-di[3-n-propyl-3(4-nitrobenzyl)] urea (1,6-HDIU). .1 g of the hydrochloride of nitro reagent was extracted into 25 ml of benzene as the free amine

and 25 ml of acetone was added followed by 0.168 g 1,6-diisocyanatohexane. After standing for several minutes, most of the solvents were stripped off by rotary evaporation. Hexane was added to precipitate the product (about 0.25 g). 1,6-HDIU: m.p., 131°–133°; IR (KBr), 1340, 1500–1540 and 1620 cm^{-1}; UV (CH_2Cl_2) ε_{254} 1.04×10^4 and ε_{270} 1.56×10^4; (70 eV) *m/e* 556 (0.5) $C_{28}H_{40}N_6O_6$, 362 (30) $C_{18}H_{26}N_4O_4$, 194 (100) $C_{10}H_{14}N_2O_2$; NMR ($CDCl_3$) δ 0.90–1.13 (*t*, 3H, $J_{1,2} = 3.5$ Hz, γ-CH_3), δ 1.27–1.60 (*d* of *t*, 2H, $J_{1,2} = 3.5$ Hz, $J_{2,3} = 3.8$ Hz, β-CH_2), δ 1.77 (*s*, 3H, phenyl–CH_3), δ 3.18–3.42 (*t*, 2H, $J_{2,3} = 3.8$ Hz, α-CH_2), δ 4.75 (*s*, 2H, phenyl–CH_2–N), δ 7.40 (*s*, 1H, N–H), δ 7.48–7.65 (*d*, 2H, $J_{o,m} = 4.0$ Hz, phenyl H's *m* to nitro), δ 8.25–8.43 (*d*, 2H, $J_{o,m} = 4.0$ Hz, phenyl H's *o* to nitro); CHN analysis, calculated for $C_{28}H_{40}N_6O_6$: C 60.4, H 7.19, N 15.11; found, C 60.50, H 7.48, N 14.65.

Preparation of LC column

A 5-cm column was evenly cut from a 6-ft. precision-bore stainless-steel tube (Analabs, North Haven, Conn., U.S.A.). The tube (1/4 in. O.D., 4.5 mm I.D.) was cleaned with soap solution, water, methanol, chloroform and acetone. No attempt was made to smooth the inside of the tube further.

The column packing used was Partisil™ 5 (5 μm; Whatman, Clifton, N.J., U.S.A.), a microparticulate silica packing with a surface area of about 350 m^2/g and pore size of about 50 Å. The particles have a narrow size range.

A 1/4–1/16 in. Swagelok stainless-steel reducing union with a 2-μm porous disc (St. Louis Valve & Fitting Co., Ferguson, Mo., U.S.A.) at the outlet, and 5-μm disc at the inlet, were used as retainers at the ends of the column. These fittings have low dead volume (*i.e.*, column end drilled). The packing was done by a balanced-density slurry method (tetrabromoethane–carbon tetrachloride mixed in proper ratios to match the density of the microparticulate packing). Chloroform hydraulic pressure of 5500 p.s.i. was applied using an air-driven constant-pressure liquid pump (Haskel, Burbank, Calif., U.S.A.). Chloroform was pumped through the packed column till it smelled free of the packing liquids.

LC operating conditions

Results presented were obtained using a linear gradient of B–A (1:10) to 100% B, completed in 10 min at a rate of 2 ml/min, where solvent B is 9.1% isopropanol and A is dichloromethane. The analytical column was easily regenerated by a continuous flush with isopropanol followed by dichloromethane, each for 30 min or longer, depending on the day's work load of the column.

RESULTS AND DISCUSSION

The calibration curves shown in Fig. 1 were obtained with solutions of the crystallized ureas. The minimum amounts detected were 1.2 ng for 4,4'-MDIU, 2,4-TDIU and 2,6-TDIU and 6.2 ng for 1,6-HDIU injected.

A mixture of four diisocyanates plus a polyfunctional aliphatic isocyanate, Desmodur N-100, was reacted with nitro reagent solution. The diisocyanate–nitro reagent mixture was allowed to react overnight and dilutions were made from this reaction mixture. Then the solvent was evaporated in a rotary evaporator and the residue re-dissolved in 1 ml dichloromethane. Aliquots were injected. Fig. 2 shows the

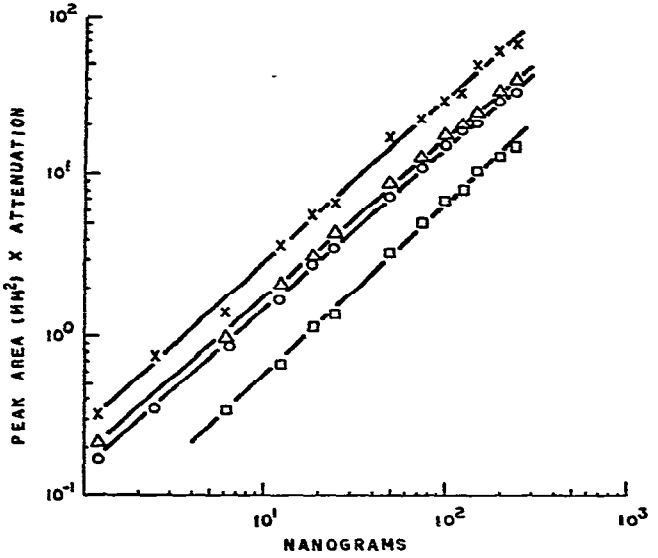

Fig. 1. Urea calibration curves on Partisil 5. × = 4,4′-MDIU; △ = 2,4-TDIU; ○ = 2,6-TDIU; □ = 1,6-HDIU.

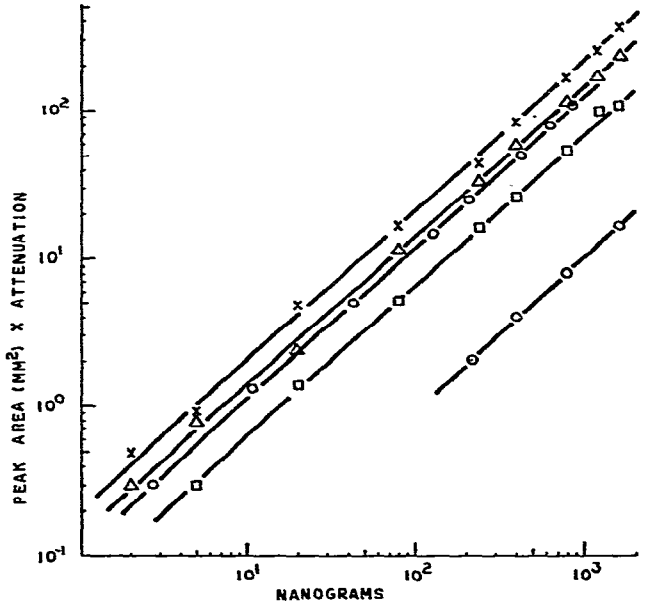

Fig. 2. Calibration curves of isocyanate–nitro reagent reaction mixture on Partisil 5. × = 4,4′-MDI; △ = 2,4-TDI; ○ = 2,6-TDI; □ = 1,6-HDI; ⊙ = Desmodur N-100.

calibration curves of peak area *versus* amount of diisocyanates injected. The minimum quantities detected were 2 ng each of 4,4′-MDI and 2,4-TDI; 2.7 ng of 2,6-TDI; 5 ng of 1,6-HDI and 240 ng of Desmodur N-100.

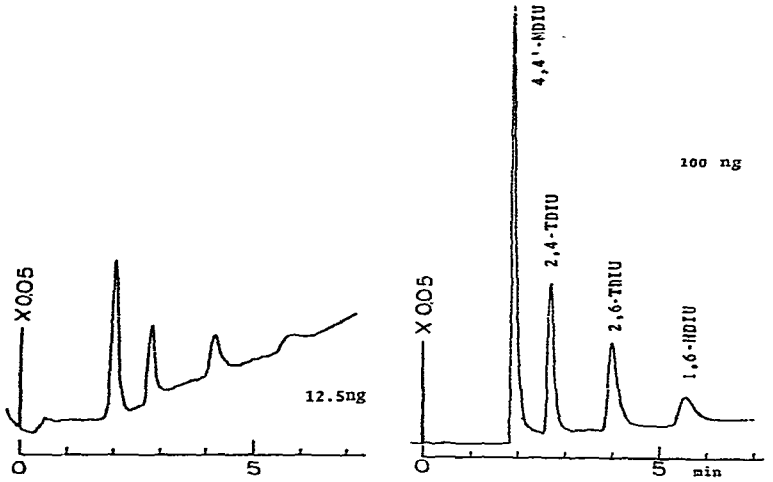

Fig. 3. Typical chromatograms of urea standard solutions at the lower nanogram range. Partisil 5 (5 cm × 4.5 mm I.D.) linear gradient from B–A (1:10) to 100% B (see text), in 10 min (2 ml/min), Waters Assoc. Model 440 absorbance detector (254 nm). Amount injected for each compound as shown.

Representative liquid chromatograms of the urea and the isocyanate–nitro reagent mixtures are shown in Figs. 3 and 4, respectively. In the case of toluene diisocyanates, 2,6-TDI is 53.8% of 2,4-TDI (*i.e.*, composition of Mondur TD).

Linearity was obtained up to 1,600 ng in the isocyanate–nitro reagent mixture, with unchanged peak symmetry, peak width and retention time. Higher solute concentrations were not investigated. In the present work, the injected volumes varied from 1 to 10 μl, even though a larger injection volume of 50 μl could also be used. In some cases, a monoisocyanate (*p*-tolylisocyanate) has been used to remove excess nitro reagent and/or serve as internal standard.

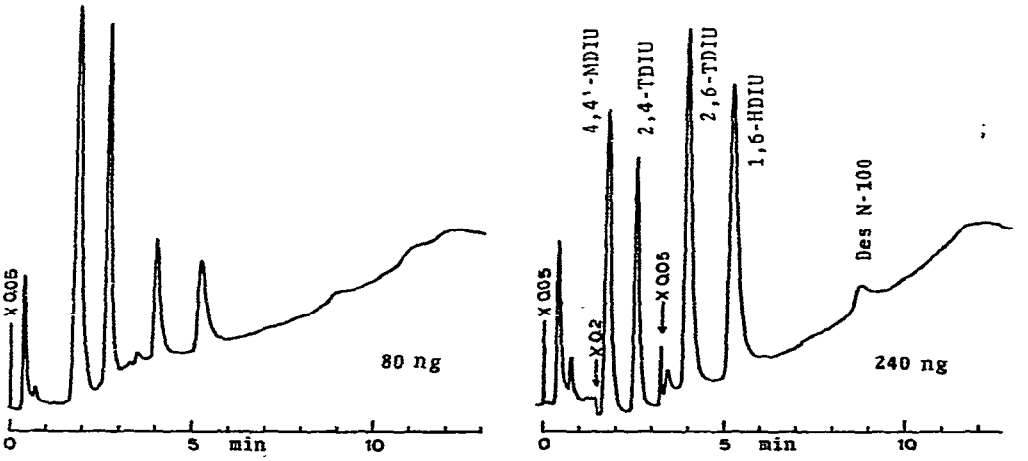

Fig. 4. Chromatograms of the isocyanate–nitro reagent reaction mixtures on Partisil 5. LC conditions are the same as Fig. 3. The nanogram amounts shown refer to the amount injected of each except for 2,6-TDI which is 53.8% of 2,4-TDI.

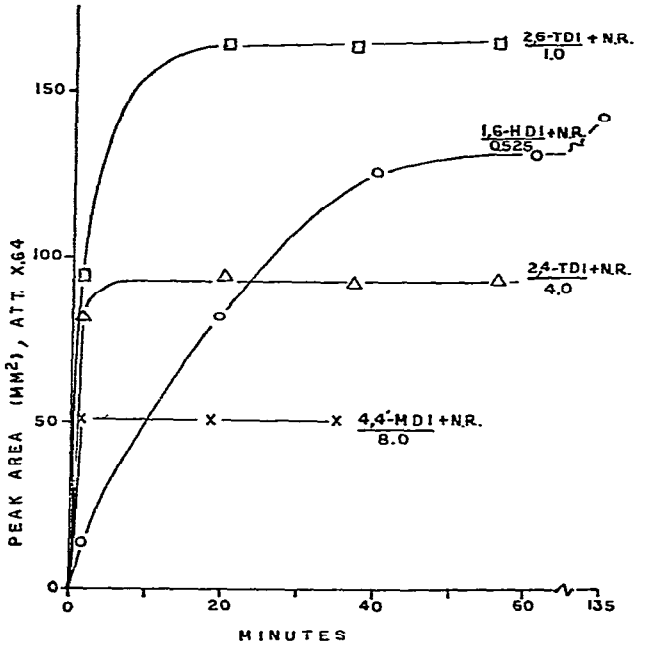

Fig. 5. The apparent reaction time of the isocyanate–nitro reagent solutions. LC on Corasil II, 37–50 μm, 40 cm × 2.1 mm I.D., linear gradient from acetonitrile–dichloromethane (1:20) to 100% acetonitrile in 10 min (2.0 ml/min), Schoeffel Spectroflow Monitor at 254 nm.

Of particular interest is the speed of the isocyanate–nitro reagent reactions. Therefore, a simple experiment was devised to determine the time dependence of the isocyanate reaction. One milliliter of 1.0 mg/ml nitro reagent in hexane was added to individual solutions containing the following diisocyanates in 1 ml dichloromethane: 44 μg 4,4'-MDI, 15.6 μg 2,4-TDI, 8.4 μg 2,6-TDI and 3.0 μg 1,6-HDI. Aliquots of each solution were then injected at certain time invervals. The column used was Corasil II and the LC conditions were those shown in Fig. 5. Rapid reaction is, of course, the basis for the impinger/reaction method of collecting organic vapors in air. Fig. 5 shows that the apparent reaction time is quite rapid. 4,4'-MDI and 2,4-TDI show instant complete reaction, 2,6-TDI took about 20 min and 1,6-HDI more than 1 h.

CONCLUSIONS

Derivatives of common industrial isocyanates intended for use as analytical standards were isolated and characterized. A reproducible liquid chromatographic method is demonstrated to allow detection at the low nanogram levels.

ACKNOWLEDGEMENT

This work was supported by the National Institute for Occupational Safety and Health, contract no. CDC-210-75-0052.

REFERENCES

1 K. Marcali, *Anal. Chem.*, 29 (1957) 552.
2 K. E. Grim and A. L. Linch, *Amer. Ind. Hyg. Ass., J.*, 25 (1964) 285.
3 R. L. Larkin and R. E. Kupel, *Amer. Ind. Hyg. Ass., J.*, 30 (1969) 640.
4 J. Keller, K. L. Dunlap and R. L. Sandridge, *Anal. Chem.*, 46 (1974) 1845.
5 K. L. Dunlap, R. L. Sandridge and J. Keller, *Anal. Chem.*, 48 (1976) 497.
6 Regis Lab Notes, Regis Chemical Co., Morton Grove, Ill., No. 20, July 1976.