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DETERMINATION OF TRACE ATMOSPHERIC ISOCYANATE CONCENTRATIONS BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING 1-(2-PYRIDYL)PIPERAZINE REAGENT

P. A. GOLDBERG*, R. F. WALKER*, P. A. ELLWOOD and H. L. HARDY
Occupational Medicine and Hygiene Laboratories, Health and Safety Executive, 403–405 Edgware Road, Cricklewood, London NW2 6LN (Great Britain)
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SUMMARY

A reversed-phase high-performance liquid chromatographic method is described for the determination of both aliphatic and aromatic isocyanates in air. The test atmosphere is drawn through a $2 \cdot 10^{-4}$ *M* solution of 1-(2-pyridyl)piperazine in toluene at a sampling rate of 1 l min^{-1} for 5–20 min. The sample solution is evaporated to dryness and the residue dissolved in 100 μl of acetonitrile. A 10-μl aliquot of the resulting solution is chromatographed using a 25 cm × 4.6 mm I.D. stainless-steel column packed with ODS-Hypersil 5 μm silica gel and eluted, isocratically, with acetonitrile–0.1 *M* ammonium acetate mobile phase at a flow-rate of 2.0 ml/min. The ammonium acetate solution is adjusted to pH 6.2 with acetic acid.

INTRODUCTION

Diisocyanates and their high-molecular-weight oligomers are extensively used in the production of polyurethane materials ranging from flexible foams to enamel wire coatings, so that a large number of workers are potentially at risk of isocyanate exposure. Adverse physiological effects of inhalation of isocyanate vapour or aerosol extend from mucous membrane irritation to respiratory sensitization[1,2]. The latter effect may possibly be due to combination reactions of isocyanate groups with body protein[3,4]. A progressive diminution in lung function has been reported to occur when workers are regularly exposed to sub-threshold limit value[5] isocyanate concentrations[6,7]. Although conflicting evidence has been put forward on this point[8,9], the current trend suggests that lower threshold limit values may be considered in the future[10]. Consequently, sensitive analytical methods for the determination of isocyanates and their oligomers are of increasing importance.

In order to achieve adequate sensitivity and resolution, the majority of recent analytical methods for the determination of isocyanates employ either high-perform-

* Present address: Imperial Chemical Industries Ltd., Organics Division, Hexagon House, Blackley, Manchester M9 3DA, Great Britain.

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ance thin-layer chromatography (HPTLC) or high-performance liquid chromatography (HPLC). Previous colorimetric-based methods are generally incapable of resolving individual isocyanate component concentrations and are susceptible to interferences in many cases[11–18]. Although several HPLC methods have been reported for the determination of free isocyanates in their bulk prepolymers[19–22], the first generally applicable atmospheric isocyanate determination employed a double derivative thin-layer chromatographic technique[23]. The method, based on the reaction of isocyanates with N-4-nitrobenzyl-N-*n*-propylamine (nitro reagent) to form urea derivatives, has a lower detection limit of 80 $\mu g\ m^{-3}$, and an improved sampling technique[24] has recently increased its sensitivity to 2 $\mu g\ m^{-3}$ (for toluene diisocyanate, 4,4′-methylene bisphenyl isocyanate and 1,6-hexamethylene diisocyanate) for a 1-l air sample.

The determination of atmospheric isocyanates, using nitro reagent as the isocyanate-reactive entity, was later extended to gradient-elution HPLC as the method of analysis[25]. Further studies[26–28] indicate, however, that the presence of excess nitro reagent in the sample solution seriously reduces the life of the pellicular-silica column specified due to adsorption onto the column packing. As a result of such reagent adsorption the urea derivatives have a tendency to elute with sloping baselines, especially in the case of higher retention time peaks, causing a reduction in precision. The need to use gradient elution leading to significant column recovery times is a further limitation to be considered. Sangö[29] has recently examined the application of a bonded octadecylsilyl phase using isocratic elution with acetonitrile–water (75:25) to minimise the disadvantages associated with the nitro reagent. Nevertheless, decomposition of the nitro reagent, both during and after sampling, may occur in reducing or oxidizing atmospheres[25,26], possibly due to the presence of the aromatic nitro group in this secondary amine.

The reversed-phase HPLC method reported here is based on the reaction of isocyanates with 1-(2-pyridyl)piperazine to form stable urea derivatives. 1-(2-Pyridyl)piperazine has negligible steric hindrance at the –NH position and reaction with both aliphatic and aromatic isocyanates is rapid and exothermic. In addition, the substituted ureas formed possess significantly higher molar absorptivities in the ultraviolet region than those derived from the nitro reagent[30]. The method is capable of measuring 500 pg of methylene bisphenyl isocyanate, equivalent to 48 ppt (10^{12}) in a 10-l air sample.

EXPERIMENTAL

Chromatographic apparatus

The liquid chromatograph comprised an Altex Model 110A constant-flow reciprocating diaphragm pump, a Rheodyne Model 7120 syringe loading sample injector with a 10-μl loop and a Pye-Unicam LC–UV detector set at 254 nm. The column consisted of 25 cm × 4.6 mm I.D. Apollo stainless-steel tubing, slurry packed at 6000 p.s.i. with ODS-Hypersil (5 μm diameter mean particle size, Shandon Southern Products, Runcorn, Great Britain). The mobile phase was deaerated using helium and pumped at ambient temperature through the column at a flow-rate of 2.0 ml/min.

Mobile phase

Three mobile phases, consisting of acetonitrile in 0.1 *M* aqueous ammonium acetate solution were used in the isocratic mode. The ammonium acetate solution was adjusted to pH 6.2 with acetic acid. Mobile phase A (acetonitrile–ammonium acetate solution, 31:69) was used in the determination of 2,4- and 2,6-toluene diisocyanate (TDI), 1,6-hexamethylene diisocyanate (HMDI), phenyl isocyanate (PhI) and naphthalene diisocyanate (NDI) urea derivatives. Mobile phase B (acetonitrile–ammonium acetate solution, 37:63) was used in the determination of the isophorone diisocyanate (IPDI) urea derivatives, while mobile phase C (acetonitrile–ammonium acetate solution, 40:60) was used in the determination of the methylene bisphenyl isocyanate (MDI) and HMDI oligomer (Desmodur N; Bayer, Richmond-upon-Thames, Great Britain) urea derivatives.

Isocyanates

The following isocyanates were used: HMDI (Desmodur H, Bayer); IPDI (Veba-Chemie, Gelsenkirchvuer, G.F.R.); 4,4′-MDI (ICI, Macclesfield, Great Britain); 1,5-NDI (Desmodur 15, Bayer); PhI (Eastman-Kodak, Rochester, NY, U.S.A.); 2,4-TDI (Fluorochem, Glossop, Great Britain); 2,6-TDI (ICI, Blackley, Manchester, Great Britain) and HMDI oligomer (Desmodur N, Bayer).

Standard urea derivatives

A freshly prepared solution of pure, monomeric isocyanate (0.8 mmole) in 3 ml of dimethyl sulphoxide (DMSO), for example, is added to a stirred solution of 1-(2-pyridyl)piperazine (280 μl, 1.8 mmole) in DMSO (3 ml). The mixture is stirred for 15 min at 60°C and then 200 ml of distilled water is slowly added. A white precipitate forms which is filtered off and washed with distilled water. The white solid is dried at 50°C and recrystallised to produce approximately 500 mg of the urea derivative. The aromatic isocyanate urea derivatives are recrystallised from a mixture of toluene and DMSO, except for the phenyl isocyanate urea derivative which is recrystallised from a mixture of toluene and *n*-hexane. The aliphatic isocyanate urea derivatives are recrystallised from a mixture of water and dimethylformamide.

To prepare an HMDI oligomer urea derivative, it is necessary to use a three-fold molar excess of 1-(2-pyridyl)piperazine with dry acetonitrile as the solvent. The reaction mixture is stirred at 60°C for 30 min, after which it is set aside for several days, during which time the urea derivative forms as a gummy off-white deposit. Excess distilled water is then added at ambient temperature, the solution is decanted off, the residue is dried at 50°C and eventually solidifies to a white solid.

Alternatively, standard urea solutions can be produced for all the isocyanates and their oligomers by adding a suitable quantity of the isocyanate under investigation, dissolved in dichloromethane, to a $2 \cdot 10^{-2}$ *M* solution of 1-(2-pyridyl)piperazine in dichloromethane. The resulting solution is diluted to give standards in the required range.

Absorbing solution

A 320-μl volume (326 mg) of 1-(2-pyridyl)piperazine is pipetted into a 100-ml volumetric flask and made up to volume with toluene. A 5-ml aliquot of this solution is made up to 500 ml with toluene to give a $2 \cdot 10^{-4}$ *M* absorbing solution. This

solution should be stored in a dark glass-stoppered bottle. Experience indicates that the stored solution remains stable for several months.

Air sampling and sample preparation

A sample of the test atmosphere is drawn through 8 ml of the absorbing solution in a Greenburg–Smith type midget impinger at a flow-rate of 1 l min^{-1} for at least 5 min. Alternatively, for time-weighted-average sampling purposes, a lower flow-rate (50 ml min^{-1}) can be used. After sampling, the impinger and its contents are removed to an uncontaminated atmosphere and any liquid is expelled from the inlet tube with a blow-ball. Suitable amounts of the absorbing solution are successively transferred into a 2-ml micro-reaction vessel and evaporated to dryness with nitrogen. Finally, the impinger receiver is washed with 0.5 ml of toluene and the washings also transferred to the micro-reaction vessel. The residue, after evaporation, is redissolved in 100 μl of dry acetonitrile and a 10-μl aliquot is injected into the liquid chromatograph.

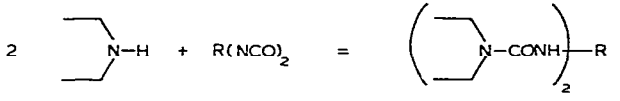
Determination of collection efficiency

A dynamic standard atmosphere apparatus[31], modified to include double dilution, was used to generate known concentrations of HMDI in a constant-flow stream of dried air. These atmospheres were sampled at 1 l min^{-1} through three midget impingers connected in series, each containing 8 ml of the absorbing solution. The absorbing solutions were treated as described above and the extent of breakthrough determined.

RESULTS AND DISCUSSION

Selection of derivatising reagent

The choice of 1-(2-pyridyl)piperazine as a suitable derivatising reagent for isocyanates was made after a systematic study of several related compounds with the required characteristics, that is (i) a secondary aliphatic amine group as part of a ring structure with negligible steric hindrance and (ii) an aromatic substituent. The one-step addition reaction of isocyanates with the –NH group eliminates the possibility of further reaction, as may occur with 1-naphthyl methylamine[32], which has recently been used in the HPLC analysis of isocyanates using fluorescence detection. The presence of an aromatic substituent on the derivatising molecule ensures that the urea derivatives will have sufficiently high molar absorptivities even when derived from aliphatic isocyanates, and the presence of the pyridyl group confers solubility on the urea derivatives in dilute acids, from which they may be recovered unchanged by alkali precipitation. Crystalline urea derivatives of the general formula



were prepared from 2,4-TDI and HMDI using the range of secondary aliphatic amines shown below. The molar absorptivities and wavelengths of maximum absorbance, using dioxan as reference solvent, are shown in Table I.

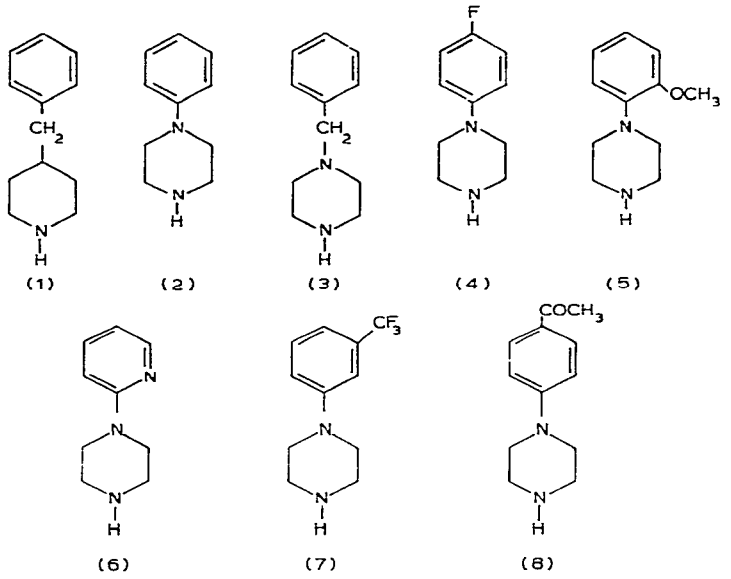


TABLE I

MOLAR ABSORPTIVITIES AND WAVELENGTHS OF MAXIMUM ABSORBANCE FOR ISOCYANATE UREA DERIVATIVES USING DIOXAN AS A REFERENCE SOLVENT

Reagent	*2,4-TDI*		*HMDI*	
	$\lambda_{max.}$ *(nm)*	ε $(l \cdot mol^{-1} \cdot cm^{-1})$	$\lambda_{max.}$ *(nm)*	ε $(l \cdot mol^{-1} \cdot cm^{-1})$
1 4-Benzylpiperidine	247	14900	260	763
2 1-Phenylpiperazine	249	52400	250	24800
3 1-Benzylpiperazine	248	20300	245	1100
4 1-(4-Fluorophenyl)piperazine	250	49500	249	17300
5 1-(2-Methoxyphenyl)piperazine	247	42100	255	20000
6 1-(2-Pyridyl)piperazine	252	60500	252	34700
7 N-(α,α,α-Trifluoro-*m*-tolyl)-piperazine	255	51800	258	26300
8 4-Piperazinoacetophenone	314	45900	317	35900

It can be seen from Table I that 1-(2-pyridyl)piperazine-based urea derivatives are the most suitable for use in the determination of isocyanates by HPLC with UV detection. 1-(2-Pyridyl)piperazine also has high solubility in water, which makes the preparation of the urea standards easier, and the pyridyl ring enables visualisation of the urea derivatives, using HPTLC, with, for example, iodoplatinate reagent. Such a method has recently been developed[33] and provides the occupational hygienist with the facility to determine isocyanate in air concentrations on-site; HPLC analysis in the laboratory can be used to achieve greater precision and further qualitative confirmation when necessary.

Collection efficiency of sampling method

The efficiency of the sampling procedure was examined for a range of standard atmosphere concentrations using three impingers in series in the sampling train. HMDI was selected for this experiment because of its relatively low boiling point compared with those of the other aliphatic isocyanates studied. The results, shown in Table II, indicate that the collection efficiency of one impinger is greater than 90% for HMDI levels as high as 1600 $\mu g\ m^{-3}$. Similar experiments with TDI, reported earlier[33], showed the collection efficiency of one impinger to be greater than 97% for TDI levels up to ten times the current threshold limit value ($10 \times 140\ \mu g\ m^{-3}$).

TABLE II

EFFICIENCY OF THE SAMPLING PROCEDURE FOR THE COLLECTION OF HMDI IN $2 \cdot 10^{-4}$ *M* 1-(2-PYRIDYL)PIPERAZINE

*Calculated concentration of atmosphere at 20°C**		*Mean concentration of isocyanate collected ($\mu g\ m^{-3}$)*			*Efficiency of trap 1 (%)*
$\mu g\ m^{-3}$	*ppm*	*Trap 1*	*Trap 2*	*Trap 3*	
146	0.021	143	3	<2	98
170	0.024	150	20	<2	88
173	0.025	170	3	<2	98
173	0.025	170	3	<2	98
198	0.028	190	8	<2	96
233	0.033	221	8	4	95
242	0.035	237	5	<2	98
269	0.038	259	10	<2	96
271	0.039	255	16	<2	94
309	0.044	288	14	7	93
324	0.046	305	19	<2	94
425	0.061	389	36	<2	92
460	0.066	435	18	7	95
492	0.070	462	20	10	94
524	0.075	491	33	<2	94
579	0.083	507	72	<2	88
672	0.096	629	43	<2	94
685	0.098	626	48	11	91
722	0.103	646	60	16	89
761	0.109	667	60	34	88
783	0.112	653	82	48	83
823	0.118	748	48	27	91
979	0.140	938	41	<2	96
1122	0.160	1040	61	21	93
1366	0.195	1278	88	<2	94
1661	0.237	1530	131	<2	92

* Assuming a collection efficiency of 100% in a 3-impinger sampling system.

Chromatographic conditions

Previous workers have found that the presence of a basic reagent in the reaction mixture to be chromatographed presents certain problems when silica is used as the column packing[34]. The use of nitro reagent, for example, in normal-phase HPLC studies, was reported to produce significant peak tailing and progressive column

degradation, possibly because of the secondary amino group on the nitro reagent, which interacts with the silanol groups of the silica column to produce retardation of the reagent on the column[36]. The presence of excess reagent[27] requires the addition of a suitable reagent scrubber such as *p*-tolyl isocyanate[27] or acetic anhydride[35] to the reaction mixture. In order to overcome this problem several workers[29,37] have successfully investigated the use of reversed-phase HPLC using a bonded octadecylsilyl stationary phase with isocratic pH-buffered elution.

ODS-Hypersil was selected as the most suitable reversed-phase column packing in the present investigation. For all three mobile phases used in the separation of the various urea derivatives excess 1-(2-pyridyl)piperazine is eluted first, and since the elution mode is isocratic, the next analysis can, if required, be started immediately. The reagent as commercially supplied contains the disubstituted piperazine, 1,4-(2,2′-dipyridyl)piperazine, as an impurity. This can be removed by vacuum distillation if desired (boiling point of the reagent is 114°C at 3 Torr), but this was not found to be necessary in this study as the impurity did not interfere with the resolution of the urea derivative peaks.

Fig. 1 shows a chromatogram of the standard urea derivatives of a mixture of the two TDI isomers and PhI with unreacted reagent and the disubstituted piperazine impurity present. Fig. 2 shows a typical chromatogram of a polyurethane foam (TDI-oligomer based) hot-wire cutting process air sample. TDI monomer is a degradation

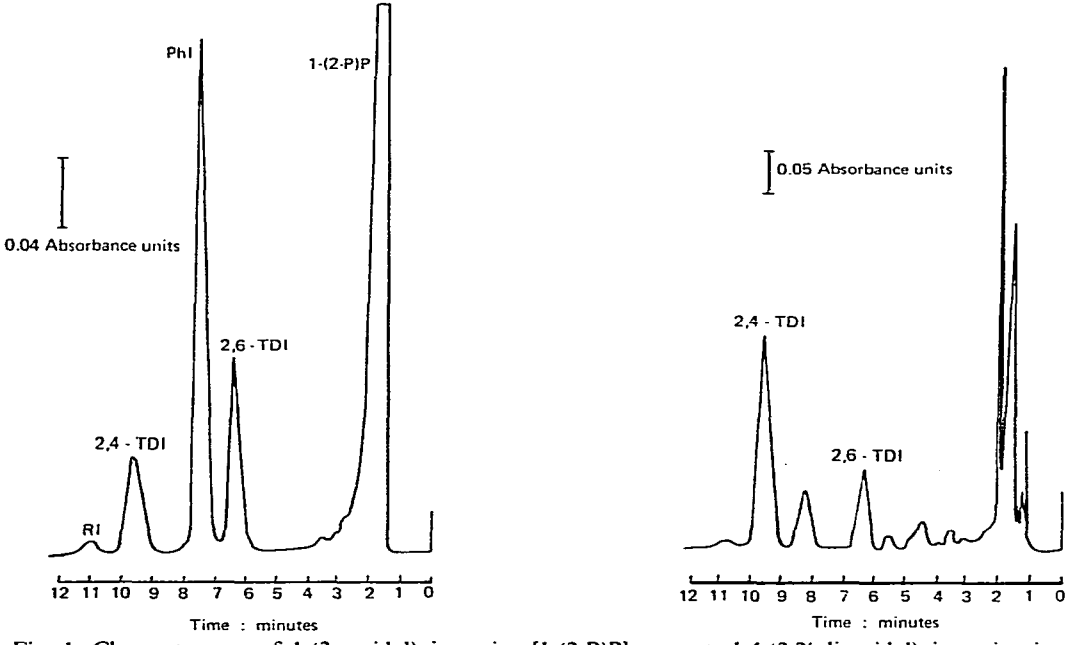
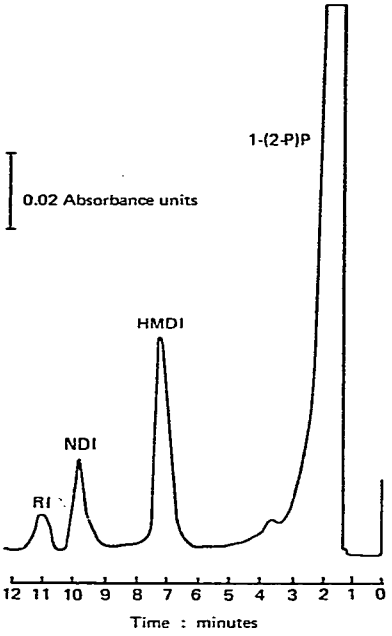


Fig. 1. Chromatogram of 1-(2-pyridyl)piperazine [1-(2-P)P] reagent, 1,4-(2,2′-dipyridyl)piperazine impurity and the PhI and TDI urea derivatives. Conditions: column, 250 × 4.6 mm I.D., ODS-Hypersil; mobile phase, acetonitrile–0.1 *M* aqueous ammonium acetate (31:69), the water phase adjusted to pH 6.2 with acetic acid; temperature, ambient; flow-rate, 2.0 ml/min; sample volume, 10 μl; detection, UV at 254 nm and 0.08 a.u.f.s.

Fig. 2. Chromatogram of a TDI in air sample taken above the hot-wire cutting of a flexible polyurethane foam based on a TDI oligomer. Conditions as in Fig. 1, except: sensitivity, 0.64 a.u.f.s.

product of such a process, and as can be seen from the chromatogram, is well resolved from the other breakdown products. Fig. 3 shows a chromatogram of the HMDI and NDI standard urea derivatives.

By increasing the concentration of acetonitrile in the mobile phase it is possible to elute the MDI and HMDI oligomer urea derivatives within 11 min as shown in Figs. 4 and 5, respectively indicating that the chromatographic system can be used in the analysis of the higher molecular weight isocyanate prepolymers frequently used by industry. The retention times and wavelengths of maximum absorbance for the urea derivatives studied are listed in Table III.



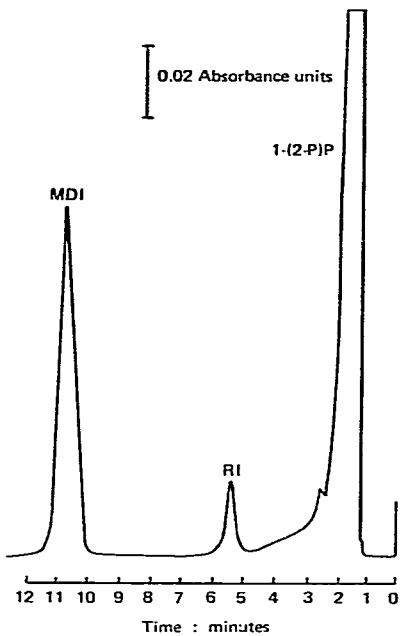


Fig. 3. Chromatogram of 1-(2-pyridyl)piperazine reagent, 1,4-(2,2′-dipyridyl)piperazine impurity and the HMDI and NDI urea derivatives. Conditions as in Fig. 1, except: sensitivity, 0.16 a.u.f.s.

Fig. 4. Chromatogram of the MDI urea derivative together with excess reagent and its impurity. Conditions as in Fig. 1, except: mobile phase, acetonitrile–0.1 *M* aqueous ammonium acetate (40:60), the water phase adjusted to pH 6.2 with acetic acid; sensitivity, 0.16 a.u.f.s.

It can be seen from Table III that the separation of isocyanate mixtures that commonly occur together in certain industrial atmospheres is easily achieved. For example, HMDI oligomer, commonly used in two-pack polyol-cure paint systems, generally contains approximately 0.7% of HMDI monomer. In many cases mixed isocyanates do not occur and it is relatively easy to modify the elution conditions to reduce the retention times; this will additionally give sharper peaks.

Calibration graphs, over the concentration 0–100 ng, were prepared for the isocyanate urea derivatives studied. A linear response was exhibited in each case between the concentration of the urea and its absorbance. A typical calibration graph, for the 2,6-TDI urea, is shown in Fig. 6. Detection limits, based on a signal-to-

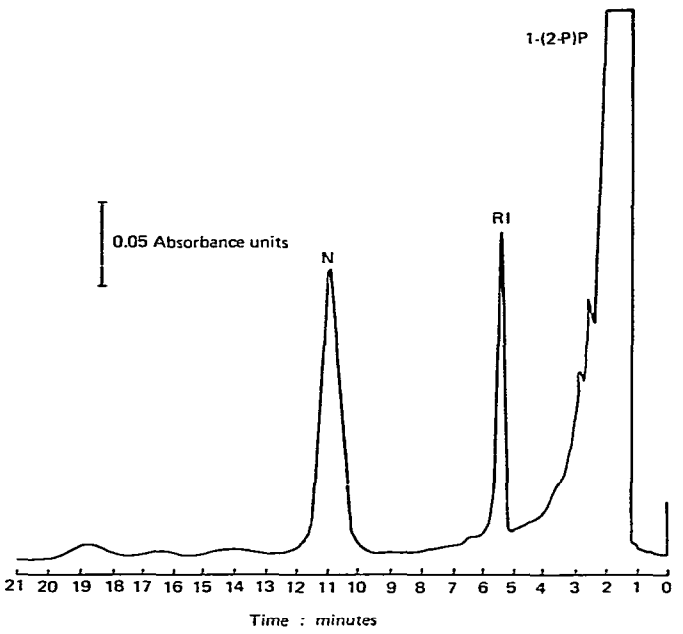


Fig. 5. Chromatogram of the HMDI oligomer (Desmodur N) urea derivative together with excess reagent and its impurity. Conditions as in Fig. 1, except: sensitivity, 0.32 a.u.f.s.

TABLE III

RETENTION TIME DATA AND WAVELENGTHS OF MAXIMUM ABSORBANCE FOR ISOCYANATE UREA DERIVATIVES
See text for description of mobile phases.

Compound	*Mobile phase*	*Retention time (min)*	$\lambda_{max.}$ *(nm)*
1-(2-Pyridyl)piperazine	A	1.3	255
2,6-TDI-urea	A	6.3	252
HMDI-urea	A	7.2	255
PhI-urea	A	7.3	251
2,4-TDI-urea	A	9.5	252
NDI-urea	A	9.7	253
1,4-(2,2-Dipyridyl)piperazine	A	10.9	–
1-(2-Pyridyl)piperazine	B	1.3	255
1,4-(2,2-Dipyridyl)piperazine	B	8.6	–
IPDI-urea	B	12.9 and 23.2	254
1-(2-Pyridyl)piperazine	C	1.3	255
1,4-(2,2-Dipyridyl)piperazine	C	5.5	–
MDI-urea	C	10.9	256
HMDI oligomer urea	C	11.0	253

noise ratio of 3 with the detector set at 0.005 a.u.f.s., are given in terms of free isocyanate in Table IV.

The lower limit may be decreased by taking a larger air sample, and the upper limit increased by using a more concentrated absorbing solution or smaller air sample.

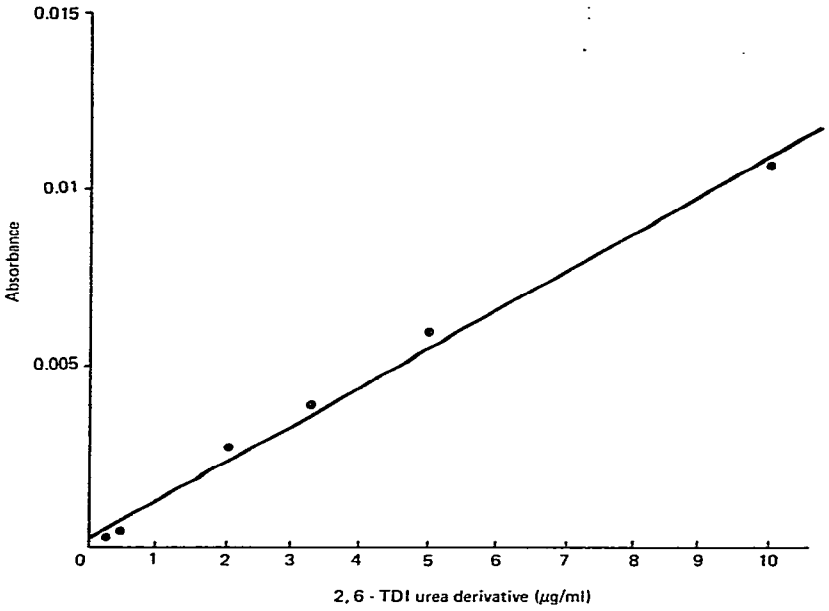


Fig. 6. Calibration curve for the 2,6-TDI urea derivative. Conditions as in Fig. 1.

TABLE IV

ISOCYANATE CONCENTRATION LIMITS

Concentration limits are based on a 10-litre air sample.

Isocyanate	*Lower limit ($\mu g\ m^{-3}$)*	*Upper limit ($mg\ m^{-3}$)*
2,4-TDI	0.5	17
2,6-TDI	0.5	17
MDI	0.5	25
PhI	0.5	21
HMDI	0.7	17
NDI	1.0	21
IPDI	1.5	22
HMDI oligomer	0.75	32

CONCLUSION

A reversed-phase HPLC method has been developed for the determination of atmospheric isocyanate concentrations using 1-(2-pyridyl)piperazine to form urea derivatives which exhibit very high molar absorptivities in the ultraviolet region. The sensitivity of the method is such that as little as 48 ppt of MDI or TDI may be determined in a 10-l air sample, equivalent to one four-hundreth of the current threshold limit values for these compounds[5]. This means that shorter sampling periods can be used giving rise to better hygiene control. In theory, the sensitivity of the method is sufficiently high to omit the concentration step prior to HPLC analysis, and to inject a 100-μl aliquot directly into the chromatograph. However, toluene is not suitable as an

injection solvent and there are problems associated with the use of acetonitrile as the sample absorbing solvent due to its toxic and volatile nature. In addition, we have evidence that the concentration step may be of importance in ensuring that urea formation goes to completion. It has also been shown that impurities in acetonitrile may react with isocyanates.

Unlike the nitro reagent and 9-(N-methylaminomethyl)anthracene, a reagent recently suggested for the HPLC-fluorescence determination of isocyanates[38], 1-(2-pyridyl)piperazine is a stable, high-boiling liquid (boiling point 283°C) requiring no preparation before use. In addition, HPTLC analysis[33] may be carried out on-site prior to HPLC analysis, using 1-(2-pyridyl)piperazine reagent, enabling the occupational hygienist to make an immediate assessment of the extent of any isocyanate hazard that may be present.

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