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FOREWORD

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SUMMARY

The analysis of the industrial isocyanates using high speed (HSLC) liquid chromatography is shown and the advantages of HSLC over that of the TLC counterpart are demonstrated. Minimum detectable limit of 2 ng was observed. Linearity up to 1,600 ng has been shown on the 25 cm Partisil 10 and 5 cm Partisil 5 columns. Reproducibility of repeated injections are within experimental errors. Samples are stable for at least 10 days. Column life and efficiency can be preserved by flushing the column daily and minimizing the amount of unreacted nitro reagent injected.

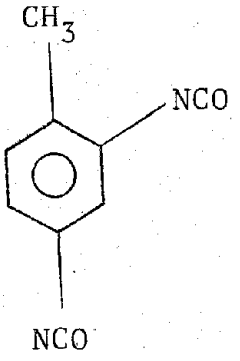
Ureas that may serve as "primary standards" can be synthesized, isolated, purified and characterized.

INTRODUCTION

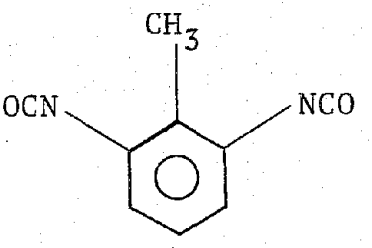
While conventional liquid chromatography has its own meritorious place in analytical chemistry, this technique can be more effectively applied by adopting state-of-the-art technology. Developments in high performance liquid chromatography include instrumentation, more sensitive detectors, numerous packing supports, packing technology, and, of course, better and better understanding of the separation power of liquid chromatography, LC.

The purpose of this investigation is to modify the existing analytical procedure of determining organic isocyanates that employs thin layer chromatography, TLC, as published by Keller, et. al.[1] to one using high speed liquid chromatography, HSLC. Specific compounds that are qualitatively and quantitatively analyzable by the modified method are those generally found in working environments, i.e., 2,4-toluene diisocyanate; 2,6-toluene diisocyanate, 4,4'-methylenebis(phenylisocyanate), 1,6-hexane diisocyanate, and 1,3,5-tris(6-isocyanatohexyl) biuret. The chemical structures of these compounds are given on the next page. The workers' exposure to diisocyanates must be controlled to prevent adverse effects of the compounds to their health and safety. A fast, accurate, reproducible and readily available method that has low detection limit (approx. 1 ng/µl based on 40 liters of workplace air) must exist to monitor exposure of the workers to these compounds.

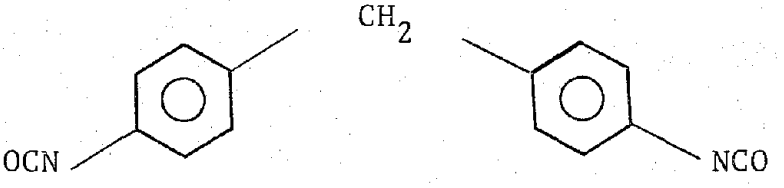
Some methods developed, but specifically that of Mercali[2] cum modification by Grim and Linch[3] and Larkin and Kupel[4] have been used to determine toluene diisocyanate (TDI) in work air. Though the Mercali procedure has been the recommended method by the National Institute for Occupational Safety and Health (U.S. Dept. of Health, Education and Welfare) in formulating the criteria document for occupational exposure to TDI, the method suffers interferences, particularly from aromatic amines.



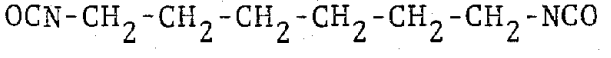
1) 2,4-Toluene Diisocyanate; TDI. CAS #589-84-9*



2) 2,6-Toluene Diisocyanate; TDI. CAS #91-08-7*

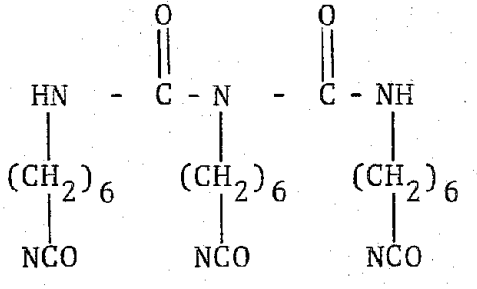


3) 4,4'-Methylenebis(phenyl isocyanate); MDI. CAS #101-68-8*



4) 1,6-Hexane Diisocyanate; HDI; CAS #822-06-0*

5) 1,3,5-Tris (6-isocyanatohexyl) biuret; HDI biuret; CAS #4035-89-6.*



* CAS # = Chemical Abstracts Registry Number

Both the Mercali and Keller et. al. procedures are applicable to monitor working atmospheres. Yet, each has its own drawback--Mercali, that of aromatic amine interferences; Keller et. al. that which is inherent to TLC, i.e., slow, tedious and subject to individual color biases. Another drawback of the Keller et. al. method is the possible reduction of the derivatizing agent, i.e., the N-4-nitrobenzyl-N-n-propylamine. In actual air sampling, where contaminants are diversified and plentiful, this may prove to be a problem requiring innovative solution.

The specific purpose of this investigation, therefore, is to modify the TLC method to an analytical method which uses HSLC. The modified procedure is able to separate and detect the urea derivatives of the five isocyanates mentioned above. This was achieved by using a highly efficient LC column and a uv detector at wavelength showing maximum absorbance for these compounds.

While this report was in the final stages of manuscript preparation, a related work appeared in the literature.[5]

EXPERIMENTAL SECTION

INSTRUMENTS

The Waters Associates Model 202 liquid chromatograph (Waters Associates, Inc., Milford, Mass. 01757, U.S.A.) was equipped with a Universal LC Injector, Model U6K (Waters Associates, Inc.). The liquid chromatograph was also equipped with a Waters Associates Model 660 Solvent Programmer and two Waters Associates Model 6000 pumps which were needed for gradient elution. A Schoeffel Model 770 spectroflow monitor (Schoeffel Instruments Corp., Westwood, N.J.) set at 254 nm, was used in the LC in the Chromatography and Isocyanate Reaction Time sections. Starting from the Calibration Curves section, a Waters Associates Model 440 absorbance detector, set at 254 nm, was used.

CHEMICALS

N-4-nitrobenzyl-N-n-propylamine: Fifty g (0.29 moles) of 4-nitrobenzyl chloride (99% pure, Aldrich Chemical Co., Inc., Milwaukee, Wis. 53233) was dissolved in 240 ml of benzene. The solution was brought to boiling under reflux conditions. Then 36 g (0.61 moles) of n-propylamine (98% pure, Aldrich Chemical Co., Inc.) was added dropwise to the refluxing solution over a 15 minute period. It was refluxed for five hours. The solvent was stripped off in a rotary evaporator (Büchi Rotavapor-R, distributed by Fisher Scientific Co., Fairlawn, N.J. 07410) at 50°C. The residue was dissolved in 80 ml of double distilled water, and 30 ml of a 45% NaOH solution was slowly added. Then 100 ml of benzene was added and the mixture was stirred for five minutes. The benzene layer was separated. The 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 34 g of concentrated HCL was added to form its salt. The mixture was evaporated to dryness at 50°C in a rotary evaporator. The salt was washed with a 1:1 mixture of

acetone:benzene followed by suction filtration. The washing step was repeated three times. The solid salt (about 25 g) was dried in a vacuum oven at 50°C. mp 230-232°C, ir (KBr) 1340, 1520cm^{-1} (C-NO_2). In addition to the 2 ir bands of the salt, the free amine (see below) showed a band at 3320cm^{-1} (N-H). From here on the N-4-nitrobenzyl-N-n-propylamine is referred to as "nitro reagent" or "N.R.".

Preparation of Nitro Reagent Solution: A typical procedure for the routine preparation of the N.R. solution is as follows:

About 120 mg (5.2×10^{-4} moles) of the hydrochloride of nitro reagent was dissolved in 25 ml of distilled water. Thirteen ml of 1 N NaOH was added to precipitate the free amine. The free amine was extracted with 50 ml of toluene. The toluene layer was dried over anhydrous $CaSO_4$ (Drierite, W.A. Hammond Drierite Co., Xenia, Ohio) and the resulting solution was diluted to 250 ml to prepare the 2×10^{-3} M solution. The nitro reagent solution was stored in the refrigerator. The solution was not used after five days of storage.

During the course of the study, various concentrations of N.R. solutions were used, therefore, the procedure described above was changed proportionately.

Purification of 4,4'-Diphenylmethane diisocyanate (MDI): At the start of the study, 4,4'-diphenylmethane diisocyanate, less than 85% pure, was obtained (Pfaltz and Bauer, Inc., 126-04 Northern Blvd., Flushing, New York 11368). This material was white and only sparingly soluble in CH_2Cl_2. In the meantime, another source of MDI was found (Mobay Chemical Corp., Pittsburgh, Pa. 15205). The registered trade name is Multrathane M. This time the material was yellow and more soluble in CH_2Cl_2.

The MDI from Mobay Chemical Corp. was purified by partly dissolving 5 g of MDI in 30 ml of CH_2Cl_2 (reagent grade, Fisher Scientific Co.). The residue was filtered off and discarded. The CH_2Cl_2 solution was then rotary evaporated to

about 5-10 ml. A small amount of reagent grade n-heptane was added to the concentrated solution to start precipitation. The MDI precipitate was filtered using water aspirator and dried under vacuum. This product was purified MDI (about 2.7 g). MDI: mp, 38°-39°C, ir (KBr) 2275 cm^{-1} (N=C=O).

Isocyanates: The 1,6-Diisocyanatohexane (98%), toluene 2,4-diisocyanate (97%), and p-tolylisocyanate (99%) were obtained from Aldrich Chemical Corp., 4,4'-Diphenylmethane diisocyanate (purified as described in previous paragraphs), Mondur TD, (65% of toluene-2,4-diisocyanate and 35% of toluene-2,6-diisocyanate, respectively) and Desmodur N-100 (a high molecular weight biuret of 1,6-diisocyanatohexane) were obtained from Mobay Chemical Corporation.

The disappearance of the ir (KBr) band at 2275 cm^{-1} (N=C=O) of the isocyanates served as a preliminary characterization during the preparation of the ureas.

The Ureas: Each urea was prepared by reacting the corresponding diisocyanate with the excess nitro reagent solution.

In each of the preparations, a minimum of 1:2 molar ratio of diisocyanate to N.R. was maintained. Details are given below.

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. Fifteen ml of 1 N NaOH was added to precipitate the free amine. The free amine was extracted into 50 ml n-heptane. A 1.3 g sample of MDI, purified, was dissolved in 25 ml CH_2Cl_2. This MDI solution was poured slowly with stirring into the nitro reagent heptane solution. (In this solution, the MDI/NR mole ratio is 1:2.1). The urea of MDI precipitated out. It was filtered and dried. It was purified again by reprecipitation with n-heptane from the CH_2Cl_2 solution, filtered, and dried (about 1.1 g). 4,4'-MDIU; mp 151-153°C, ir (KBr) 1340, 1500-1520, 1630-1650, 3330 cm^{-1}; uv

(CH_2Cl_2) ε_{254} 4.76 x 10^4, ε_{270} 2.44 x 10^4; mass spectrum (70 ev) m/e 638 (M^+), 444 (M^+-NR), 194 (NR), all very intense; nmr ($CDCl_3$) δ 0.90-1.15 (t,3H, $J_{1,2}$ = 7.0 hz, 1-CH_3), δ 1.50-1.90 (d of q, 2H, $J_{1,2}$ = 7.0 hz, $J_{2,3}$ = 7.5 hz, 2-CH_2), δ 2.25 (s, 2H, phenyl-CH_2-phenyl), δ 3.23-3.50 (t, 2H, $J_{2,3}$ = 7.5 hz, 3-CH_2), δ 4.00 (s, 1H, >N-H), δ 4.90 (s, 2H, >N-H), δ 7.06-7.40 (m, 4H, phenyl-H's), δ 7.50-7.66 (d, 2H, $J_{o,m}$ = 8.0 hz, phenyl-o-H and phenyl-o'-H of N.R.), δ 8.20-8.38 (d, 2H, $J_{o,m}$ = 8.0 hz, phenyl-m-H and phenyl-m'-H of N.R.); C, H, N, analysis: calculated for $C_{25}H_{38}N_6O_6$: 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 urea of tolylene-2,4-diisocyanate (2,4-TDI) was prepared from 99% pure 2,4-TDI. The hydrochloride of nitro reagent (1.0294 g) was extracted into 50 ml of toluene as described earlier. A solution of 2,4-TDI (0.3156 g/30 ml toluene) was slowly mixed with the nitro reagent (2,4-TDI/N.R. mole ratio = 1:2.5). The precipitate was filtered. It was then dissolved in minimal amount of CH_2Cl_2 and hexane was added to the solution to initiate precipitation of 2,4-TDIU (about 0.15 g). 2,4-TDIU: mp 131-134°C; ir (KBr) 1340, 1520, 1630, 3280 cm^{-1}; uv (CH_2Cl_2) ε_{254} 2.23 x 10^4, ε_{270} 1.89 x 10^4; mass spectrum (70 ev) m/e 444 (weak, M^+-NR), 194 (NR intense); nmr ($CDCl_3$) δ 0.97-1.23 (t, 3H, $J_{1,2}$ = 7.0 hz, 1-CH_3), δ 1.30-1.90 (d of q, 2H, $J_{1,2}$ = 7.0 hz, $J_{2,3}$ = 8.0 hz, 2-CH_2), δ 2.14 (s, 3H, phenyl-CH_3), δ 3.10-3.50 (t, 2H, $J_{2,3}$ = 8.0 hz, 3-CH_2), δ 4.75 (s, 2H, phenyl-CH_2-N<), δ 6.28-6.45 (d, 1H, J = 11.0 hz, >N-H), δ 7.18-7.50 (t, 3H, J = 10.0 hz, phenyl-H's), δ 7.63-7.78 (d, 2H, $J_{o,m}$ = 8.0 hz phenyl-o-H and phenyl-o'-H of the N.R.), δ 8.22-8.37 (d, 2H, $J_{o,m}$ = 8.0 hz, phenyl-m-H and phenyl-m'-H of the N.R.); C, H, N 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): The urea of tolylene-2,6-diisocyanate (2,6-TDI) was prepared from the Mondur TD as described below. A 0.57 g portion of the hydrochloride of nitro reagent was extracted as described earlier, into 50 ml of toluene. A solution of 0.13 g Mondur TD in 25 ml toluene was slowly added to the 50 ml of the nitro reagent solution with stirring. (TDI/N.R. mole ratio is 1:3.3). It was then left standing for 30 min. The precipitate was filtered and dried under vacuum. The 2,6-TDIU was recovered from the precipitate by dissolving into minimal (about 3-5 ml) amount of CH_2Cl_2. Toluene was slowly added to the CH_2Cl_2 solution just enough to initiate precipitation. (Note: It was proven in an earlier testing that in a solution of 2,4-TDIU and 2,6-TDIU, the 2,6-TDIU will precipitate first from CH_2Cl_2 by the addition of toluene). It was then filtered, washed with minimal amount of toluene several times, and dried under vacuum. This precipitate was 2,6-TDIU (about 0.05 g). 2,6-TDIU: mp 185-187°C, ir (KBr) 1340, 1480-1500, 1580-1630, 3360 cm^{-1}; uv (CH_2Cl_2) ε_{254} 2.89 x 10^4, ε_{270} 2.67 x 10^4; mass spectrum (70 ev) m/e 562 (M^+), 368 (M^+-NR), 194 (NR), all very intense. nmr ($CDCl_3$) δ 0.85-1.10 (t, 3H, $J_{1,2}$ = 7.0 hz, 1-CH_3), δ 1.53 (s, 2H, 2-CH_2), δ 1.95 (s, 3H, phenyl-CH_3), δ 3.22-3.45 (t, 2H, $J_{2,3}$ = 8.0 hz, 3-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, $J_{o,m}$ = 9.0 hz, phenyl-o-H and phenyl-o'-H of N.R.), δ 8.18-8.33 (d, 2H, $J_{o,m}$ = 9.0 hz, phenyl-m-H and m'-H of N.R.). C, H, N analysis: calculation 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): The urea of 1,6 diisocyanatohexane (1,6-HDI) was prepared by the reaction of excess N.R. with 1,6-HDI as follows: Exactly 1 g of the hydrochloride of nitro reagent was extracted into 25 ml of benzene as the free amine. Added 25 ml of acetone to this solution to keep the urea in solution. Added 0.168 g 1,6-HDI and let stand several minutes. (1,6 HDI/N.R. mole ratio =

1:3.9). Solvents were stripped off until precipitate was in a slurry with remaining solvent. Hexane was added to precipitate the white solid which was filtered and dried under vacuum (about 0.25 g). 1,6-HDIU: mp 131-133°C; ir (KBr) 1340, 1500-1540, 1620 cm^{-1}; uv (CH_2Cl_2) ε_{254} 1.04 x 10^4, ε_{270} 1.56 x 10^4; mass spectrum (70 ev) 362 (M-NR) weak, 194 (NR) intense. nmr ($CDCl_3$) δ 0.90-1.13 (t, 3H, $J_{1,2}$ = 7.0 hz, 1-CH_3), δ 1.27-1.60 (d of t, 2H, $J_{1,2}$ = 7.0 hz, $J_{2,3}$ = 7.5 hz, 2-CH_2), δ 1.77 (s, 3H, phenyl-CH_3), δ 3.18-3.42 (t, 2H, $J_{2,3}$ = 7.5 hz, 3-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, $J_{o,m}$ = 8.0 hz, phenyl-o-H and phenyl-o'-H of N.R.), δ 8.25-8.43 (d, 2H, $J_{o,m}$ = 8.0 hz, phenyl-m-H and phenyl-m'-H of N.R.). C, H, N analysis: calculation 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.

The urea of Desmodur N-100 stayed in solution in either hexane or toluene, and no further attempt was made to isolate the Desmodur N-100 urea.

1,6-Hexane diisocyanate was reacted with dried formic acid at various temperatures (100°-150°C) to form 1,6-HDI biuret. It was very difficult to control the polymerization reaction, and, therefore, 1,6-HDI biuret was not obtained. More refined experimental conditions were not further investigated.

CHROMATOGRAPHY

Test Mixtures: To test the capability of potential LC columns to separate the ultimate compounds of interest which are the 5 ureas of the isocyanates in the presence of N.R., a semi-quantitative mixture was made. Weighed amounts of the synthesized ureas were dissolved in CH_2Cl_2. Known amount of nitro reagent was added. Fresh test mixture was made every 10-14 days. This was then used to test promising LC columns.

Selection of LC Columns: Five columns were tested, these were Vydac Reverse Phase 30/44 μ; Durapak OPN/Porasil C, 37/75 μ; Corasil II, 37/50 μ; Partisil 5, 5 μ; and Partisil 10, 10 μ.

While the first two columns did not show obvious promise during the preliminary testing, the last three did. Therefore, efforts were concentrated on them.

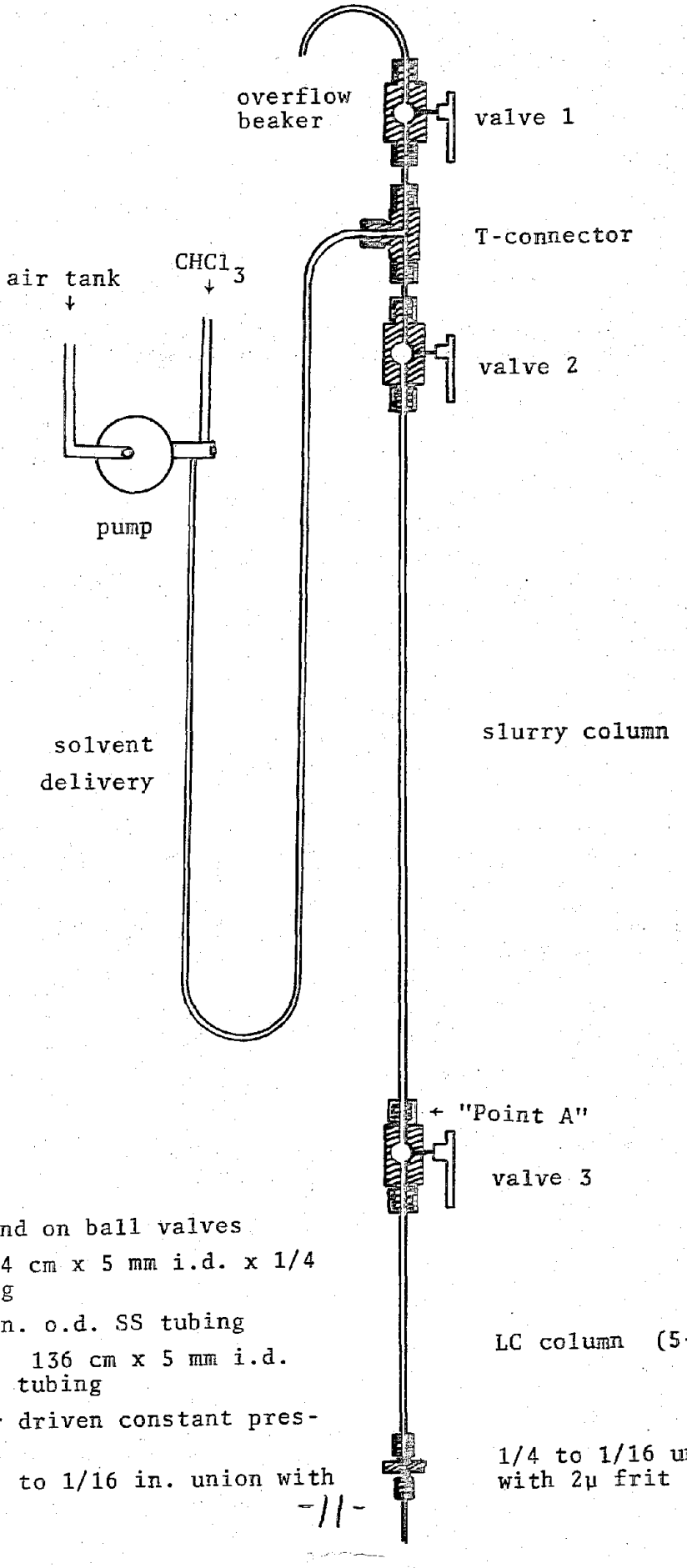
The isocyanate derivatives were successfully separated on columns of Corasil II, Partisil 5, and Partisil 10. They are described in detail in the following paragraphs.

Corasil II: Column - A 40 cm x 2.1 mm, i.d., 1/4" o.d. precision bore stainless steel column was cleaned with soap solution, water, methanol, chloroform, and acetone. A 1/16" to 1/4" SS union with a 2 μ SS frit was placed at one end of the empty column. A small amount (0.1 ml) of Corasil II, 37-50 μ, (Waters Associates, Inc.) was poured into the column at the top. The column was tapped on the floor 45 times in 45 seconds. This procedure was repeated until the column was filled with Corasil II. A 1/16" to 1/4" SS union with a 10 μ SS frit was placed at the inlet of the column. Then it was connected to the liquid chromatograph.

A linear gradient elution of 5% CH_3CN/CH_2Cl_2 (both reagent grades, Fisher Scientific Co.) to 100% CH_3CN was achieved in 10 minutes at 2.0 ml/min. On the liquid chromatograph, pump B was connected to reservoir A filled with CH_2Cl_2. The programmer was set at: curve select no. 6 i.e., linear gradient, 5% B initial conditions, program time 10 minutes, 100% B final conditions, and flow rate 2.0 ml/min.

Partisil™ 5: A 5 cm x 4.5 mm, i.d., 1/4" o.d. stainless steel column was cleaned with soap solution, water, methanol, chloroform, and acetone. Partisil™ 5 (Whatman, Inc., 9 Bridewell Place, Clifton, New Jersey 07014) has a nominal particle size of 5 μ. It is a silica gel adsorbent. It was slurried packed into the SS column by the balanced density method. The apparatus used to pack the column is given on Figure 1. A balance density slurry of Partisil 5 and tetrabromoethane (carbon tetrachloride can be used if needed) was made.

FIGURE 1. SETUP FOR SLURRY PACKING



Valves: SS off and on ball valves

Slurry column: 64 cm x 5 mm i.d. x 1/4 in. o.d. SS tubing

LC column: 1/4 in. o.d. SS tubing

Solvent delivery: 136 cm x 5 mm i.d. x 1/4 in. o.d. SS tubing

Pump: Haskel air driven constant pressure liquid pump

Snubber: 1/4 in. to 1/16 in. union with 2 or 5μ SS frit

The stepwise technique for slurry packing used to pack the Partisil 5 is as follows: The set-up was disconnected at Point A. The LC column (in this case one 5 cm and 10 cm and valve 3 were filled with 1,1,2,2-tetrabromoethane (TBE); valve 3 was closed and TBE was removed from top of valve 3. Then the slurry column and valve 2 were connected to valve 3. The balanced density slurry of Partisil 5 was introduced, using a 50 cc syringe and 70 cm piece of teflon tubing, to the slurry column through valve 2. The filling started at the bottom of the column and gradually moved upward. The column was vibrated as the packing was being introduced and the teflon tubing slowly withdrawn, to avoid trapping air bubbles. Valve 2 was closed. The T-connector with valve 1 was hooked up to a Haskel air driven constant pressure liquid pump. The latter is connected to $CHCl_3$ reservoir. Valve 1 was opened. A slight pressure was applied from the pump so the air could be bled off at valve 1. Valve 1 was closed. The system was then pressurized up to valve 2 with air pressure of 120 psi giving hydraulic pressure of about 5500 psi. Valve 2 was opened and again pressurized to 5500 psi. Then--the most important step--valve 3 was opened in a fraction of a second. The balance density slurry of Partisil 5 was pushed uniformly into the column in a single slug, and an excellent column was obtained. The packed column was flushed with $CHCl_3$ until no more trace of the TBE was detected. The column was gently disconnected and a snubber was carefully assembled at the inlet.

The above procedure was used to pack a 10 cm and a 5 cm SS column. The performance of the two columns was compared at identical LC conditions--i.e., linear gradient from 20% CH_3CN/CH_2Cl_2 to 50% CH_3CN/CH_2Cl_2 in 10 minutes at 2 ml/min.

The LC of the 5 cm Partisil 5 column was optimized at a different mobile phase composition. This was a linear gradient of 5% B/A → 100% B, completed in 10 minutes 2 ml/min., where B = 9.1% $i\text{-}C_3H_7OH/CH_2Cl_2$ and A = CH_2Cl_2. At these chromatographic conditions, different urea test mixtures were used--

(1) the ureas; (2) the ureas in the presence of an added amount of N.R.; (3) the ureas, the N.R. plus the Desmodur N-75 isocyanate; (4) the ureas, the N.R. plus the Desmodur N-100 isocyanate.

Partisil™ 10: At this period of the study, two factors pertinent to the eventual application of the method being developed were considered. These are, first, using a pre-packed, commercially available column in consideration of those laboratories who do not have the capability to pack small size particles, and, second, the direct reaction of the nitro reagent with the isocyanates as would be the case in an impinger collection, followed by the analyses of the ureas in the solution without isolating the analytes prior to LC. Heretofore, this type of approach is referred to as "Nitro Reagent-Plus-Isocyanates."

A commercially prepacked column, Partisil 10, 25 cm x 4.5 mm, i.d., (Whatman, Inc. Type Partisil 10 D010D, 0652511) was tested. Chromatography on this column was done in exactly the same (optimized) mobile phase composition as the 5 cm Partisil 5 column.

THE NITRO REAGENT-PLUS-ISOCYANATE REACTION TIME

During the course of the investigation, it became obvious that the rate of reaction of the isocyanates with the nitro reagent is of importance especially when projecting the use of the reagent in impingers during isocyanate sampling. Therefore, an apparent reaction time study was conducted on 2,4- and 2,6-TDI, MDI, and HDI. Each individual isocyanate was reacted with excess nitro reagent as follows: One ml of 1.0 mg/ml N.R. in hexane (5.2×10^{-6} moles) reacted with 1 ml each of the following disocyanates in CH_2Cl_2: 4,4'-MDI, 44 µg/ml; 2,4-TDI, 15.6 µg/ml (8.96×10^{-9} moles); 2,6-TDI, 8.4 µg/ml (4.83×10^{-8} moles); 1,6-HDI, 3.0 µg/ml (1.79×10^{-8} moles). Aliquots of each solution were then injected at known time intervals. The column used was Corasil II and the LC conditions were those described above for Corasil II column.

CALIBRATION CURVE, LINEAR DYNAMIC RANGE AND DETECTION LIMIT

The linearity of the calibration curves and the detection limit were determined and compared in several ways. First, the synthesized ureas were chromatographed on the 5 cm Partisil 5 column, second, the nitro reagent-plus-isocyanate mixture was chromatographed on the same column, and finally, the nitro reagent-plus-isocyanate was chromatographed on 25 cm Partisil 10 column. These experiments were performed under optimum conditions of instruments as well as columns. Waters Associates Model 440 absorbance detector, set at 254 nm, was used for these and all subsequent experiments.

The Urea Stock Solutions: Stock solutions of each of the ureas were prepared in CH_2Cl_2: MDIU-4.09 mg/4 ml; 2,4 TDIU-4.03 mg/4 ml; 2,6 TDIU-4.04 mg/4 ml and HDIU-4.05 mg/4 ml. Dilutions were prepared from stock solutions. These were used on 5 cm Partisil 5 column (see below).

The Isocyanate Stock Solutions and the Nitro Reagent-Plus-Isocyanate Mixtures: Individual isocyanate standard solutions were prepared. The following weights of the isocyanates were dissolved in 4.0 ml portions of CH_2Cl_2: 2.12 mg MDI; 29.60 of TDI (i.e., 19.30 mg of 2,4 TDI and 10.30 mg of 2,6 TDI), 21.14 mg of HDI and 22.78 mg of Desmodur N-100.

Then 775 µl of MDI, 83.1 µl of TDI, 75.5 µl of HDI, and 70.1 µl of Desmodur N-100, were mixed and 1.017 ml CH_2Cl_2, was added to make a total volume of 2.00 ml (200 ng/µl of each). Then 1.0 ml nitro reagent (2.06 mg/ml or 8.9×10^{-3} M in hexane) was added to 1.0 ml of the isocyanate mixture. The total NCO/N.R. mole ratio in this solution is 1:1. The reaction mixture was stored overnight. Dilutions were made from this solution. The solvent was evaporated in a rotary evaporator and the residue redissolved in 1 ml CH_2Cl_2. These solutions were used to establish the calibration curves, linear dynamic range and minimum detectable amount in both the 5 cm Partisil 5 and 25 cm Partisil 10 columns (see below).

The Ureas on the 5 cm Partisil 5 Column: Aliquots of the urea solutions prepared above were injected to the Partisil 5 LC column. The chromatographic conditions were linear gradient from 10% B/A → 100% B, in 10 minutes at a flow rate of 2 ml/min. where B = 9.1% i-C_3H_7OH/CH_2Cl_2 and A = CH_2Cl_2.

The Nitro Reagent-Plus-Isocyanate on the 5 cm Partisil 5 Column: Aliquot portions of the series of the nitro reagent-plus-isocyanate mixtures prepared above were injected to Partisil 5. The LC conditions were the same as in the preceeding paragraph.

The Nitro Reagent-Plus-Isocyanate on the 25 cm Partisil 10 Column: Likewise, aliquot portions of the nitro reagent-plus-isocyanate mixtures were injected to the 25 cm Partisil 10 column under the same LC conditions as the Partisil 5 column.

THE STABILITY OF THE NITRO REAGENT-PLUS-ISOCYANATE MIXTURE

Three ml of N.R. (2.06 mg/ml, 8.9×10^{-3} M) and 1 ml of the isocyanate solution prepared above (200 ng/µl each isocyanate) and 1 ml CH_2Cl_2 were mixed to give each isocyanate concentration of 40 ng/µl. The molar ratio was about 1:3 (8.54×10^{-6} moles isocyanate: 2.7×10^{-5} moles N.R.). Daily injections to the 25 cm Partisil 10 column were done over a period of 18 days. LC conditions were the same as above.

INTERNAL STANDARDS

A stock solution of 3,5-dimethylphenol (Aldrich Chemical Co., Inc., Milwaukee, Wis. 53233 U.S.A.) in CH_2Cl_2 was prepared. One ml of this solution was used to redissolve the dried residue for LC injection. The concentration of 3,5-dimethylphenol in the CH_2Cl_2 is adjusted according to the concentrations of the isocyanates in a particular test mixture so that its response would be on scale at the attenuation that the isocyanate mixture was run. Typical concentration was 1.0 mg/ml. This internal standard was used for Set 2 in the reproducibility test.

A second internal standard was sought. This time the criterion was to have one whose structure is close to that of the compounds of interest. To this end, the urea of a mono-isocyanate was prepared as follows:

A weighed portion, 0.5 g (3.8×10^{-3} moles) of p-tolylisocyanate, p-TI, was dissolved in 25 ml CH_2Cl_2, and reacted with 50 ml N.R. (7.4×10^{-3} moles N.R.) in hexane. A white precipitate appeared after 2-3 minutes, was filtered, washed with hexane, and reprecipitated from CH_2Cl_2 once with hexane. The white solid, 4-(1-tolyl)-3-n-propyl-3-(4-nitrobenzyl) urea, was dried under vacuum (0.9 g). ir (KBr) 1340, 1500-1530, 1630-1650, 3320 cm^{-1}; uv (CH_2Cl_2) ε_{254} 4.16×10^{-4}, ε_{270} 3.48×10^{-4}. The product was characterized by LC retention time.

REPRODUCIBILITY OF 3 SETS OF SAMPLES

The reproducibility tests were carried out at the suggested concentrations indicated below.

	Set 1	Set 2	Set 3
Mixture of TDI's	2 µg	20	200
MDI	2 µg	20	200
HDI	6 µg	60	600
Desmodur N-100	50 µg	200	500
Nitro reagent	9000 µg	6500	9000

There were 10 samples in a set and 3 sets at different concentrations ranging from 2 µg to 600 µg for the isocyanates. The isocyanates were dissolved in 15 ml of toluene which contained an excess of nitro reagent. To accommodate Set 3, N.R. solutions with concentrations greater than 2×10^{-4} M were used. Details are given below. Samples were allowed to sit overnight. They were then dried and 1 ml of CH_2Cl_2 containing an internal standard or p-TI was added to the residue. Aliquots of these solutions were then analyzed by LC on the 25 cm Partisil 10 column. The LC conditions were gradient from 10% B/A → 100% B in 10 min., flow

rate of 2 ml/min., where B = 9.1% i-C_3H_7OH/CH_2Cl_2 and A = CH_2Cl_2, chart speed of 0.5"/min., and attenuation varied according to injected amount.

Preparation of the Set Solutions and Mixtures:

Set #1 Stock Solutions: A 0.3061 g (1.3×10^{-3} moles) sample of nitro reagent hydrochloride was dissolved in 30 ml of distilled water and 15 ml of 1 N NaOH was added. It was extracted with 40 ml of toluene, dried over anhydrous $CaSO_4$ and diluted to 50 ml with dry toluene. Assuming 100% extraction (which is not likely), the concentration of nitro reagent was 6.1×10^{-3} g/ml (2.66×10^{-2} M). Twenty-five ml of this was diluted to 50 ml with dry toluene to give a 3×10^{-3} g/ml (1.3×10^{-2} M) N.R. solution.

A new portion of the yellowish MDI was recrystallized twice. Then 10.19 mg of MDI was dissolved in 50 ml CH_2Cl_2 (204 µg/ml). A 1:100 dilution gave a 2.04 µg/ml solution (8.2×10^{-6} M).

A stock solution of TDI was made by dissolving 21.24 mg of TDI (65:35 ratio of 2,4- and 2,6-TDI) in 50 ml of CH_2Cl_2 (425 µg/ml). A 1:100 dilution gave a 4.25 µg/ml solution (2.4×10^{-5} M).

A stock solution of HDI was made by dissolving 30.3 mg portion of HDI in 50 ml CH_2Cl_2 (606 µg/ml). A 1:100 dilution gave a 6.06 µg/ml solution (3.6×10^{-5} M).

A stock solution of Desmodur N-100 was made by dissolving 25.17 mg Des N-100 in 50 ml CH_2Cl_2 (503 µg/ml). A 1:10 dilution gave a 50.3 µg/ml solution (mol. wt. unknown). The molarity of this solution is calculated as follows:

$$\frac{50.3\ \mu g\ \text{Des-N-100}}{ml} = \frac{5.03 \times 10^{-5}\ g\ \text{Des-N-100}}{ml}$$

$$\frac{5.03 \times 10^{-5}\ g\ \text{Des-N-100}}{ml} \times \left(\frac{1\ \text{mole NCO}}{195\ g\ \text{Des-N-100}}\right)^{*} =$$

$$\frac{2.6 \times 10^{-7}\ \text{moles NCO}}{ml} \text{ or } 2.6 \times 10^{-4}\ M_{NCO}$$

* (From Mobay Chemical Corp. promotional brochures for chemical products)

The p-tolylisocyanate, 28.42 mg, was dissolved in 50 ml of 1:10 mixture $n\text{-}C_6H_{14}:CH_2Cl_2$ to give 5.7×10^{-4} g/ml (4.3×10^{-3} M). This solution was used to redissolve the urea residues.

Set #1 Mixtures: One ml each of the isocyanate solutions prepared above was placed in a 50 ml volumetric flask (4 ml total). Three ml of 1.3×10^{-2} M N.R. and 8.0 ml of dry toluene was added to make up a 15 ml solution. The ratio of (NCO:N.R.) is calculated as follows:

$$1\ ml \times \frac{8.2 \times 10^{-6}\ M}{1}\ \text{MDI} = 8.2 \times 10^{-9}\ \text{moles MDI} \times \frac{2\ \text{moles NCO}}{\text{mole MDI}} = 1.6 \times 10^{-8}\ \text{moles NCO}$$

$$1\ ml \times \frac{2.4 \times 10^{-5}\ M}{1}\ \text{TDI's} = 2.4 \times 10^{-8}\ \text{moles TDI's} \times \frac{2\ \text{moles NCO}}{\text{mole TDI}} = 4.8 \times 10^{-8}\ \text{moles NCO}$$

$$1\ ml \times \frac{3.6 \times 10^{-5}\ M}{1}\ \text{HDI} = 3.6 \times 10^{-8}\ \text{moles HDI} \times \frac{2\ \text{moles NCO}}{\text{mole HDI}} = 7.2 \times 10^{-8}\ \text{moles NCO}$$

$$1\ ml \times \frac{2.6 \times 10^{-4}\ M_{NCO}^{\text{DES-N-100}}}{1} = 26 \times 10^{-8}\ \text{moles NCO}$$

$$\sum \text{NCO} = 3.9 \times 10^{-7}\ \text{mole NCO}$$

$$3 \text{ ml} \times \frac{1.3 \times 10^{-2} \text{ M}}{1} \text{ NR} = 3.9 \times 10^{-5} \text{ moles NR}$$

moles NCO:moles N.R. = 1:100

Ten solutions were prepared at the same time. They were reacted overnight. Then they were rotary evaporated at 60°C. One ml of p-tolylisocyanate (5.7×10^{-4} g/ml in $n\text{-}C_6H_{14}$:CH_2Cl_2, 1:10 ratio) was used to redissolve the residue. Aliquots (10 µl) were injected into LC.

Set #2 Stock Solutions: A 0.9403 g (4.1×10^{-3} moles) sample of nitro reagent hydrochloride was dissolved in 30 ml of distilled water. It was then extracted, as described in Set #1 to give a 1.88×10^{-2} g/ml (8.15×10^{-2} M) N.R. solution. Then 4.90 ml of this was diluted to 50 ml to give a 1.84×10^{-3} g/ml (8.0×10^{-3} M) solution.

A weighed portion of purified MDI, 10.05 mg, was dissolved in 50 ml CH_2Cl_2. A 1:10 dilution gave a solution of 20.1 µg/ml (8.0×10^{-5} M).

The 42.4 µg/ml solution of TDI and 60.6 µg/ml of HDI were prepared from a 1:10 dilution of the stock solutions prepared in Set #1. A 201 µg/ml Desmodur N-100 solution was prepared from a 2:5 dilution of the stock solution.

The diluent containing the internal standard was prepared by dissolving 49.45 mg of 3,5-dimethylphenol into 50 ml of CH_2Cl_2 to give 0.989 mg/ml (8.1×10^{-3} M).

Set #2 Mixtures: One ml of each of the isocyanate solutions (4 ml total) were placed in a 50 ml volumetric flask. Three ml of 8.15×10^{-3} M N.R. and 8.0 ml of dry toluene were added to make up a 15 ml solution.

In a manner similar to Set #1, the total moles of NCO are calculated to be:

MDI - 1.6×10^{-7} moles NCO
TDI's - 4.8×10^{-7} moles NCO
HDI - 7.2×10^{-7} moles NCO
DES-N - 10.3×10^{-7} moles NCO

$\sum_{NCO} = 2.4 \times 10^{-6}$ moles NCO

$$3 \text{ ml} \times \frac{8.15 \times 10^{-3} \text{ M}}{1} \text{ NR} = 2.4 \times 10^{-5} \text{ mole NR}$$

moles NCO:moles NR = 1:10

Ten solutions were prepared and reacted overnight. The solutions were rotary evaporated to dryness at 60°C. The residue was redissolved with 1.0 ml CH_2Cl_2 containing 3,5-dimethylphenol, 0.989 mg/ml. Aliquots (5 µl) were injected into LC.

Set #3 Stock Solutions: The solutions used in this set were the stock solutions described earlier in Set #1. Twenty-five ml of 6.0×10^{-3} g/ml N.R. solution was diluted to 50 ml dry toluene to give 3×10^{-3} g/ml (1.3×10^{-2} M). These solutions were: TDI, 424 µg/ml; HDI, 606 µg/ml; Desmodur N-100, 503 µg/ml; MDI, 204 µg/ml; the p-tolylisocyanate, (p-TI 3.32×10^{-3} g/ml, was prepared by dissolving 0.1656 g p-TI in 50 ml $n\text{-}C_6H_{12}$:CH_2Cl_2, 1:10.

Set #3 Mixtures: One ml of each of the isocyanate solutions (4 ml total) were placed in a 50 ml volumetric flask. Three ml of 1.3×10^{-2} M N.R. and 8.0 ml of dry toluene were added to make up a 15 ml solution.

In a manner similar to Set #1, the total moles of NCO are calculated to be:

$$\text{MDI} - 1.6 \times 10^{-6} \text{ moles NCO}$$
$$\text{TDI's} - 4.8 \times 10^{-6} \text{ moles NCO}$$
$$\text{HDI} - 7.2 \times 10^{-6} \text{ moles NCO}$$
$$\underline{\text{DES-N} - 2.6 \times 10^{-6} \text{ moles NCO}}$$
$$\sum_{\text{NCO}} = 1.6 \times 10^{-5} \text{ moles NCO}$$

$$3 \text{ ml} \times \frac{1.3 \times 10^{-2} \text{ M}}{1} \text{ NR} = 3.9 \times 10^{-5} \text{ moles NR}$$

moles NCO:moles NR = 1:2.4

Ten solutions were prepared and reacted overnight. The solutions were rotary evaporated to dryness at 60°C. The residue was redissolved into solution with 1.0 ml of p-tolylisocyanate solution (3.32×10^{-3} g/ml of $n\text{-}C_6H_{12}$:CH_2Cl_2, 1:10). Aliquots (2 µl) were injected into LC.

RESULTS AND DISCUSSIONS

THE UREAS

The ureas prepared in our laboratory were characterized by the melting points, ir, uv, ms, nmr and C, H, N elemental analyses. The electron impact (ei) ms and nmr were run by Shrader Analytical and Consulting Laboratories, Inc., 3450 Lovett Ave., Detroit, Michigan 48210. The elemental analyses were done by PCR, Inc., P.O. Box 1466, Gainesville, Florida 32602. Our laboratory also ran ms for all the ureas except the 2,4-TDIU. Our runs were likewise done using the ei mode except 1,6-HDIU which was by chemical ionization. While the Shrader Analytical Lab. used a probe temperature of 150-250°C and source temperature of 200°C, we used 130-150°C and 180-185°C of probe and source temperatures, respectively.

Except for the molecular ions which were observed in our runs for 2,6-TDIU and 4,4'-MDIU, the mass spectra obtained by the two laboratories generally agreed. The M-$C_{10}H_{14}O_2N_2$ fragments in the two runs indicated the R groups to be correct, and that two nitrophenyl tails were attached. The mass ions given in the text were taken from our runs. The interpretations of the nmr, the ms, and the suggested ms fragmentations (Tables 1-8) were done by one of us (Dr. C. Y. Ko). The ir and nmr spectra are included in the Appendix.

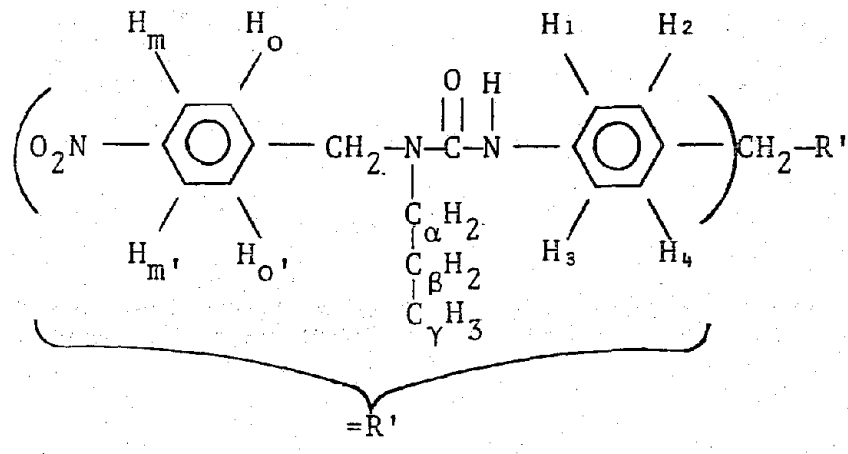
THE LIQUID CHROMATOGRAPHY

The separations of the ureas in the presence of small amounts of added N.R. were achieved on 40 cm Corasil II, 5 cm Partisil 5 and 25 cm Partisil 10 columns, the last two giving baseline separations.

While Figure 2 shows the separations on Corasil II, Figure 3 compares the same separations on 5 cm and 10 cm Partisil 5 columns. The chromatographic separations are baseline. However, the peaks exhibit some tailing. It is known

Table 1

NMR of 4,4'-MDIU



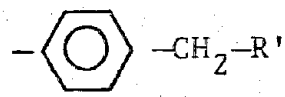
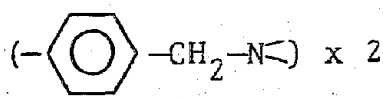
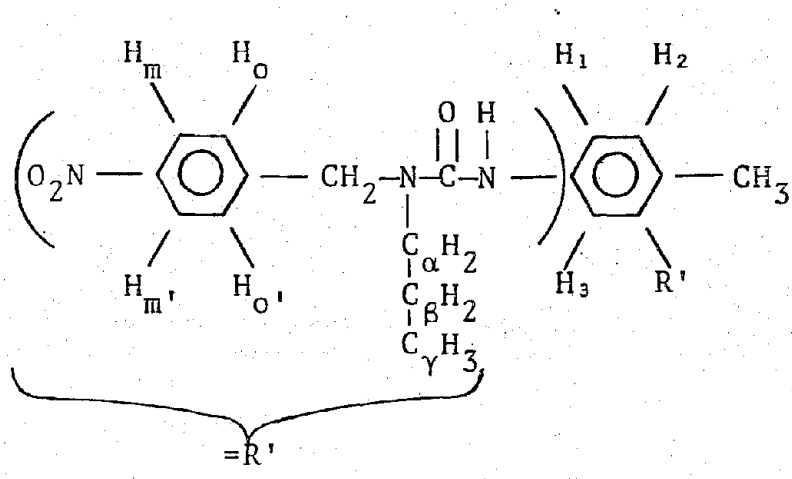
δ	State	J hz	Description
0.90-1.15	Triplet	7.0	$(-C_\gamma H_3)$ x 2
1.50-1.90	Multiplet	-	$(\succ C_\beta H_2)$ x 2
2.25	Singlet	-	
3.23-3.50	Triplet	7.5	$(\succ C_\alpha H_2)$ x 2
4.00	Singlet	-	$(\succ N-H)$ x 2
4.90	Singlet	-	 x 2
7.06-7.40	Multiplet	-	(H_1, H_2, H_3, H_4) x 2
7.50-7.66	Doublet	8.0	$(H_o$ & $H_o,)$ x 2
8.20-8.38	Doublet	8.0	$(H_m$ & $H_m,)$ x 2

Table 2

NMR of 2,4-TDIU



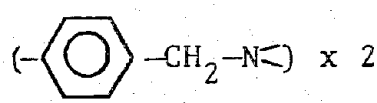
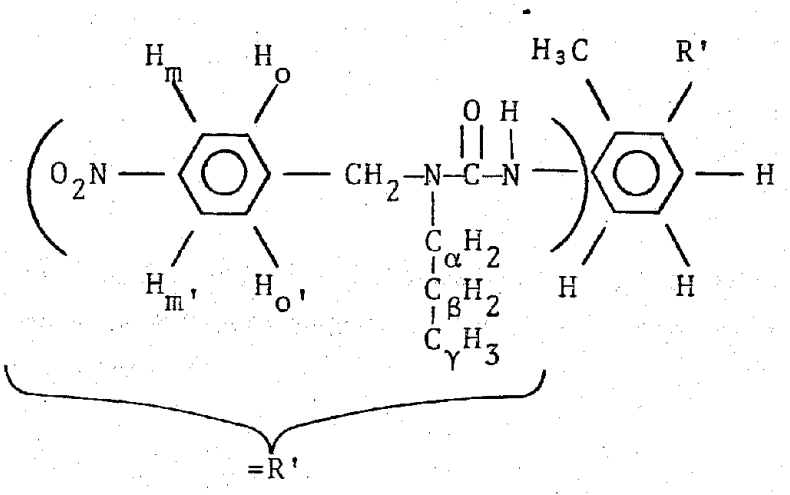
δ	State	J hz	Description
0.87-1.10	Triplet	7.0	$(-C_\gamma H_3) \times 2$
1.30-1.90	Doublet of Triplet	-	$(\supset C_\beta H_2) \times 2$
2.14	Singlet	-	$(-CH_3) \times 1$
3.10-3.50	Multiplet	-	$(\supset C_\alpha H_2) \times 2$
4.75	Singlet	-	 $\times 2$
6.28-6.45	Doublet	11.0	$(\supset N-H) \times 2$
7.18-7.50	Triplet or Multiplet	10.0	$(H_1, H_2, H_3) \times 2$
7.63-7.78	Doublet	8.0	$(H_o \text{ \& } H_{o'}) \times 2$
8.22-8.37	Doublet	8.0	$(H_m \text{ \& } H_{m'}) \times 2$

Table 3

NMR of 2,6-TDIU



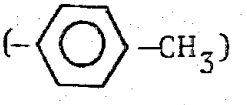
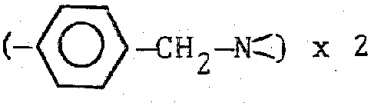
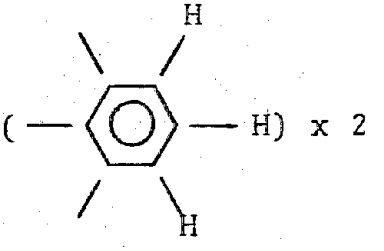
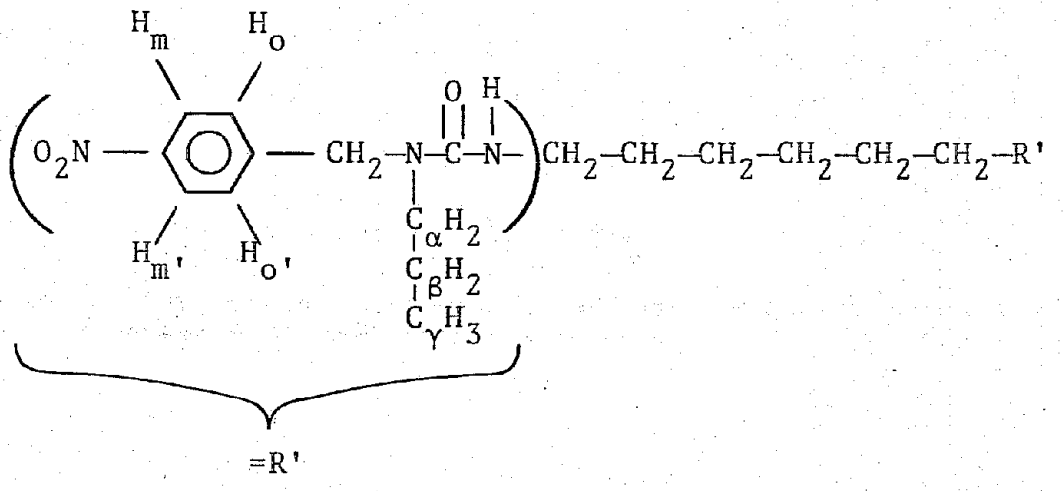
δ	State	J hz	Description
0.85-1.10	Triplet	7.0	$(-C_\gamma H_3) \times 2$
1.53	Singlet	-	Should be a multiplet but showed up as singlet. $(\supset C_\beta H_2) \times 2$
1.95	Singlet	-	
3.22-3.45	Triplet	8.0	$(\supset C_\alpha H_2) \times 2$
4.66	Singlet	-	
6.15	Singlet	-	$(\supset N-H) \times 2$
7.23	Singlet	-	
7.40-7.55	Doublet	9.0	$(H_o \text{ \& } H_{o'}) \times 2$
8.18-8.33	Doublet	9.0	$(H_m \text{ \& } H_{m'}) \times 2$

Table 4

NMR of 1,6-HDIU



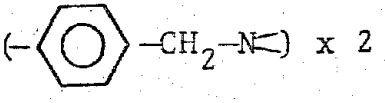
δ	State	J hz	Description
0.90-1.13	Triplet	7.0	$(-C_\gamma H_3)$ x 2
1.27-1.60	Multiplet	-	$-(CH_2)_6-$
1.77	Singlet	-	$(\succ C_\beta H_2)$ x 2
3.18-3.42	Triplet	7.5	$(\succ C_\alpha H_2)$ x 2
4.75	Singlet	-	 x 2
7.40	Singlet	-	(>N—H) x 2
7.48-7.65	Doublet	8.0	$(H_o$ & $H_{o'})$ x 2
8.25-8.43	Doublet	8.0	$(H_m$ & $H_{m'})$ x 2

Table 5

EI-MS of 4,4'-MDIU
probe: 130°; source: 185°
theor. mol. weight 638

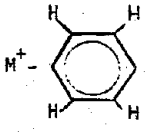
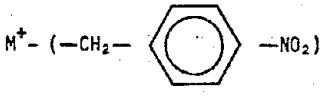
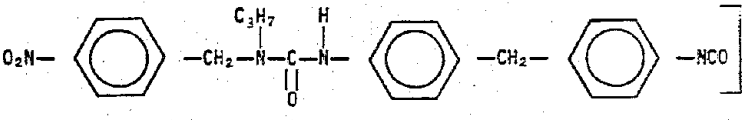
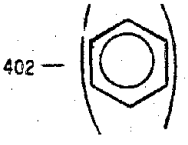
m/e	Relative Intensity	Characteristic Ions	Descriptions
638	7	M^+	molecular ion, see "A"
580	2	$M^+-(2C_2H_5^+)$	see "B"
562	3		see "C"
523	4.7		
502	1.5		see "D"
445	10.2		
444	13.5	$M^+-(NR)$	
402	6.0	$M^+-(NR-NCO)$	
389	30.0		
388	140.0		
369	4.5		
367	5.7		
359	2.9		
358	4.8		
330	82.0		
329	>300.0		
327	20.0		
326	90.0		see "E"

Table 5 EI-MS of 4,4'-MDIU (cont)

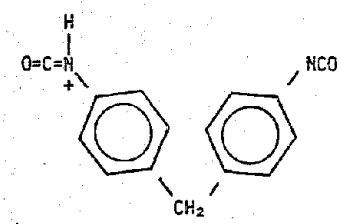
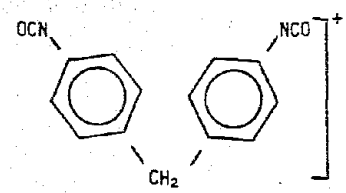
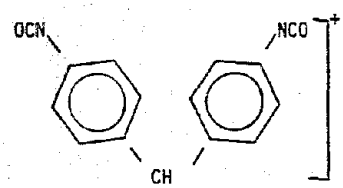
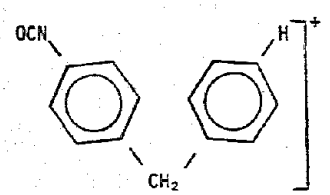
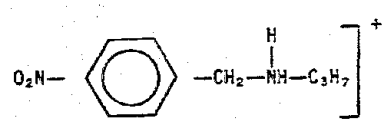
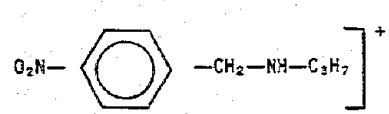
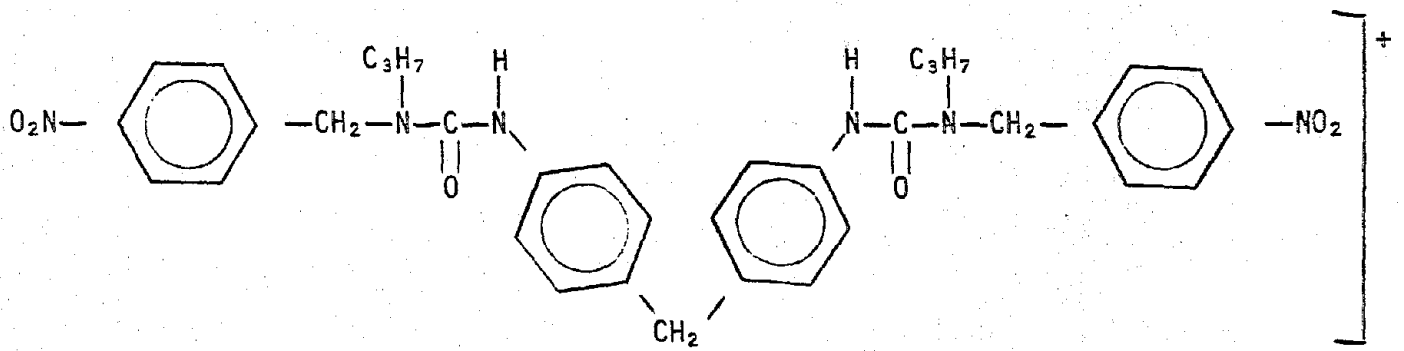
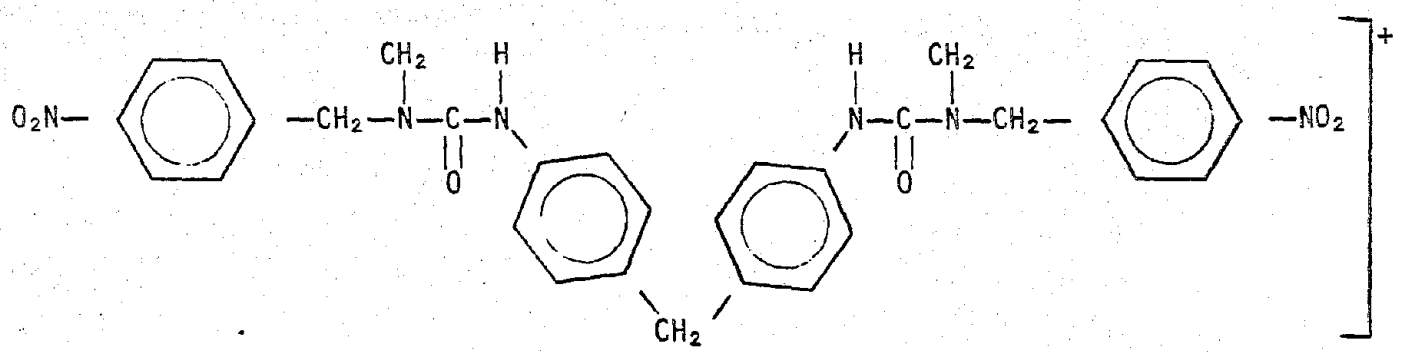
m/e	Relative Intensity	Characteristic Ions	Descriptions
251	150.0	$MDI+H^+$	
250	300.0	MDI	
249	150.0	$MDI-H^+$	
209	>400.0	$MDI+H^+-(NCO)$	
196	>300.0		
195	>400.0	$NR+H^+$	
194	>200.0	NR	

Table 5 EI-MS of MDIU (cont.)

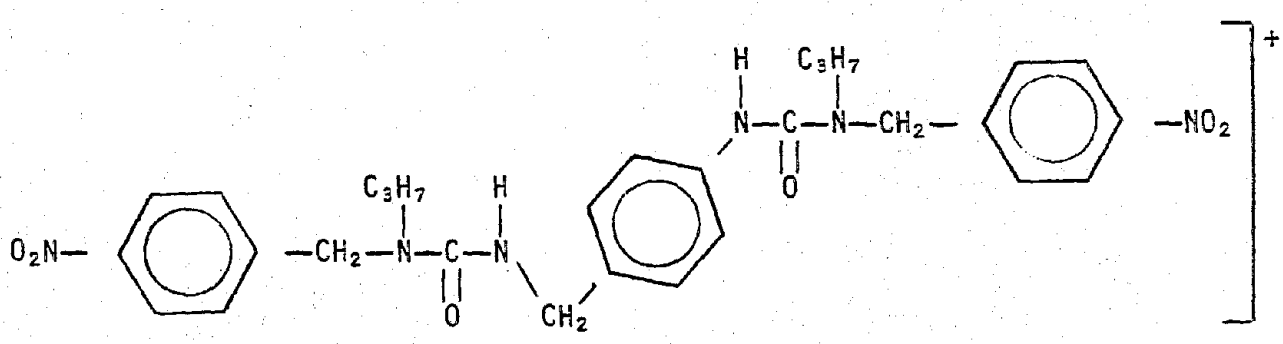
A.



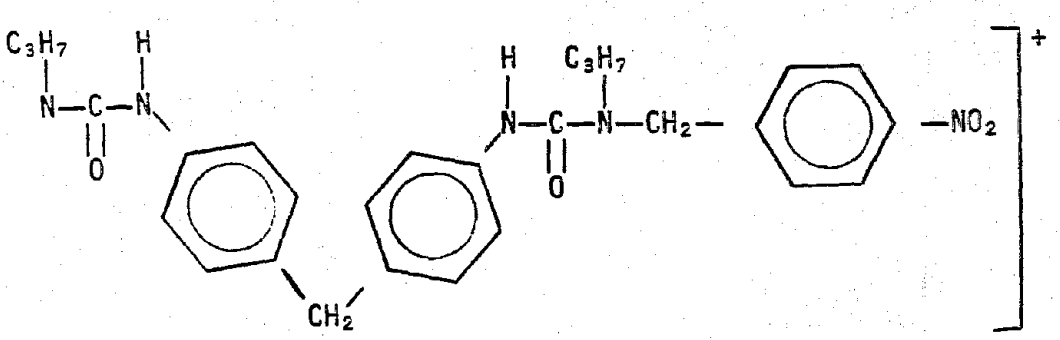
B.



C.



D.



E.

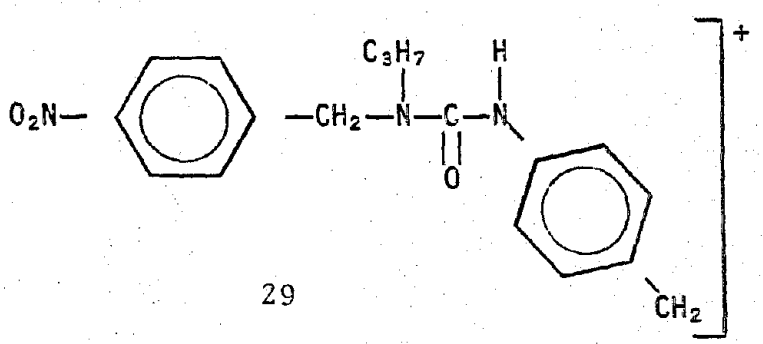


Table 6

EI-MS of 2,6TDIU
probe: 150°; source: 185°
theor. mol. weight: 562

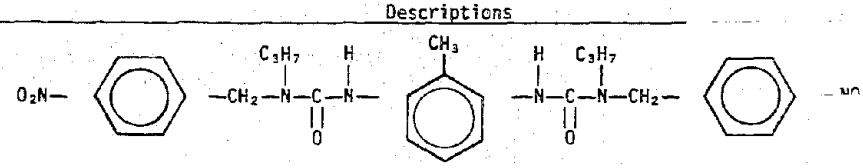
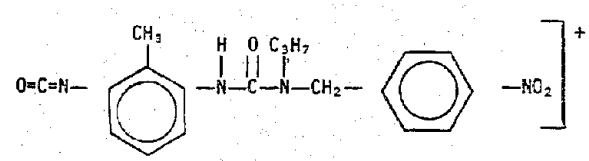
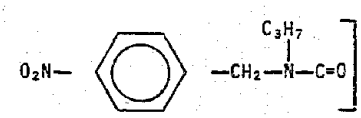
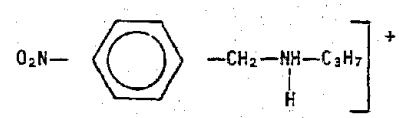
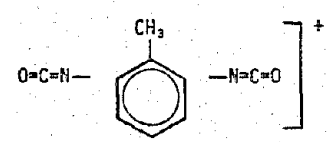
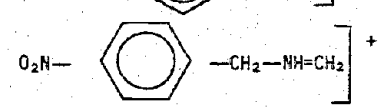
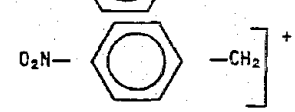
m/e	Intensity	Characteristic Ions	Descriptions
562	3.5	M^+	
502	14.0		(molecular ion)
393	3.0		
368	55.0	M^+-NR	
367	55.0	$M^+-(NR+H)^+$	
352	4.0		
330	43.0		
283	2.0		
221	11.5		
195	>100	$NR+H^+$	
194	>100	NR	
174	73	$M^+-2NR(=TDI)$	
165	>100		
136	>100		
78	55		

Table 7

CI-MS of 1,6-HDIU
probe: 150°; source: 180°
theor. mol. weight: 556
Reagent gas: Isobutane

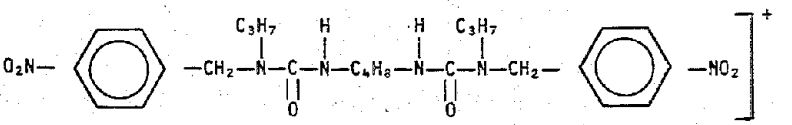
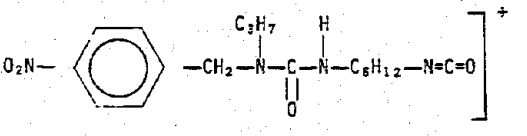
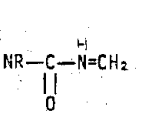
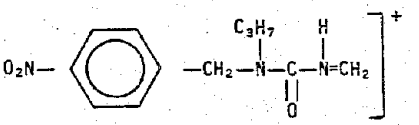
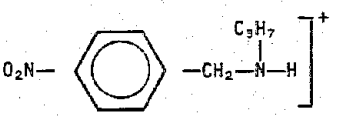
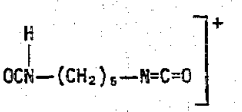
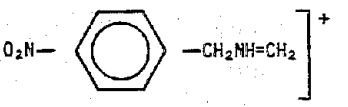
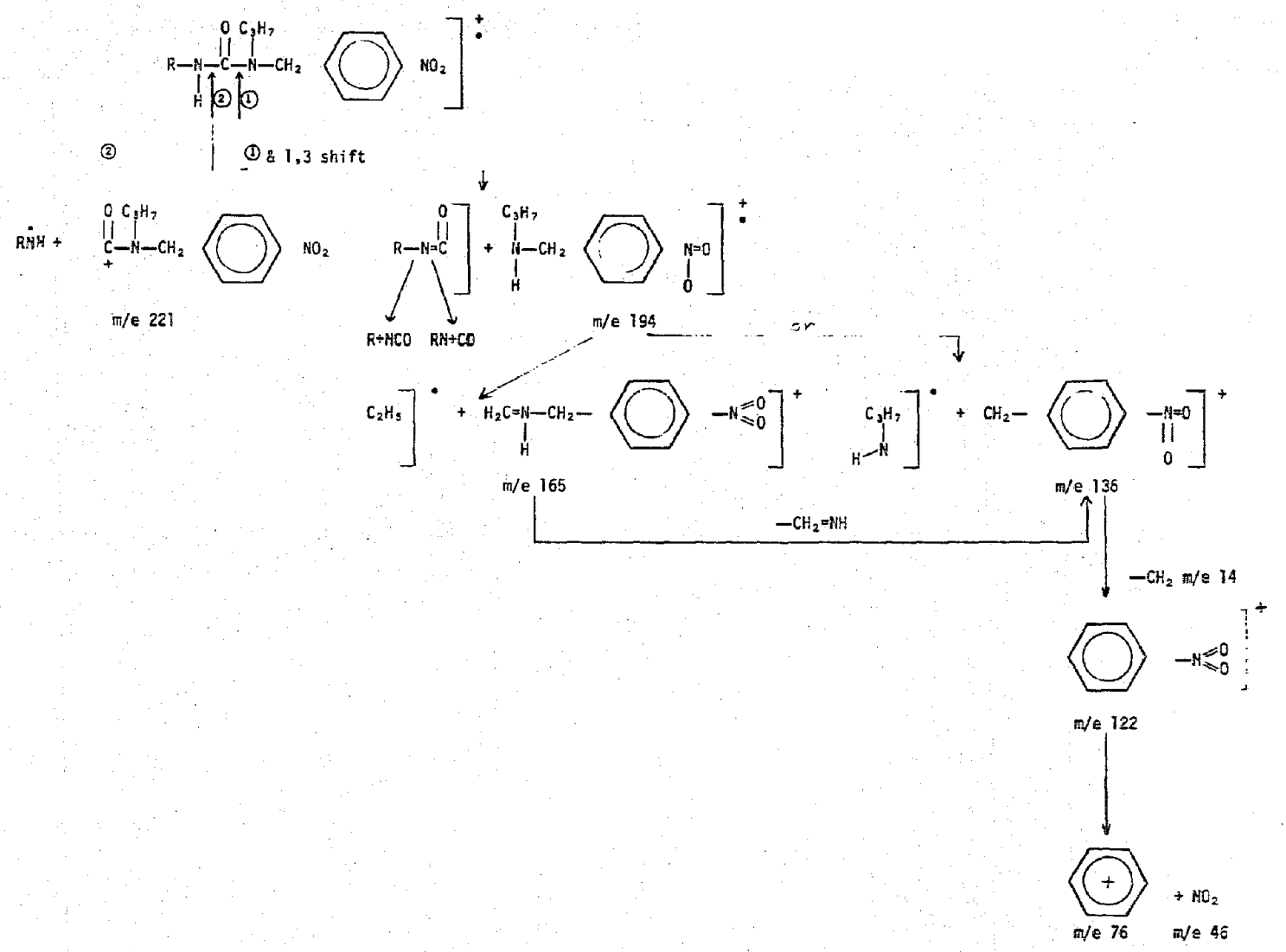
m/e	Relative Intensity	Characteristic Ions	Descriptions
528	2.5	$M^+-C_2H_2$ or M^+-CO	
423	1.5		
416	1.5		
409	1.0		
389	1.8		
362	6.0	M^+-NR	
333	1.8		
322	1.0		
250	8.0		
234	8.0		
197	8.0		
196	26.0		
195	180.0	$NR+H^+$	
194	13.0	NR	
169	2.0	$HDI+H^+$	
166	8.0		
165	20.0	$NR-C_2H_5$	

Table 8

SUGGESTED GENERAL FRAGMENTATION PATTERN OF THE UREAS



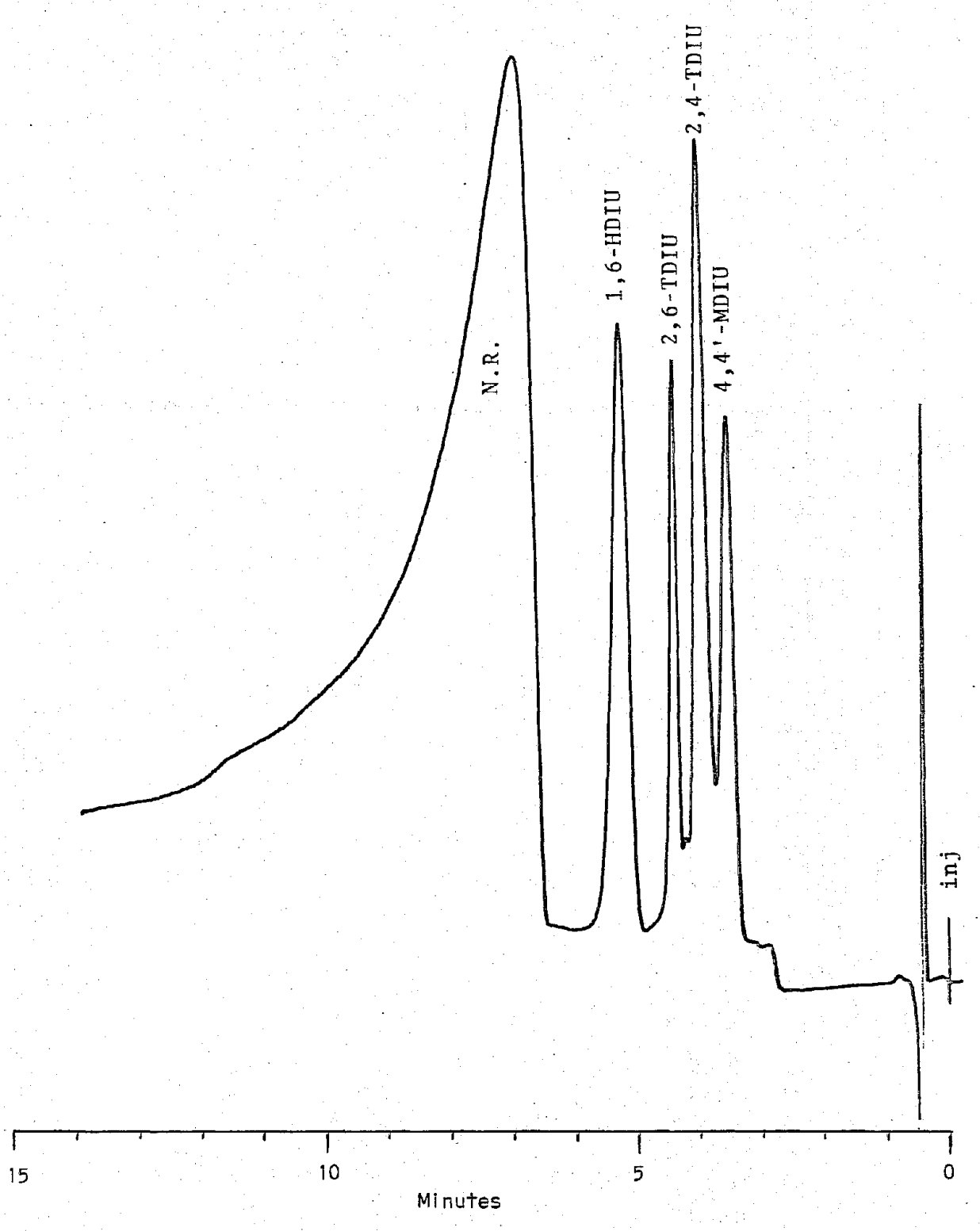


Fig. 2. Liquid chromatography of the urea test mixture on Corasil II, 37-50μ. Dry packed, 40 cm x 2.1 mm i.d., linear gradient from 5% CH_3CN/CH_2Cl_2 to 100% CH_3CN in 10 min., 2.0 ml/min., Schoeffel Spectroflow Monitor at 254 nm.

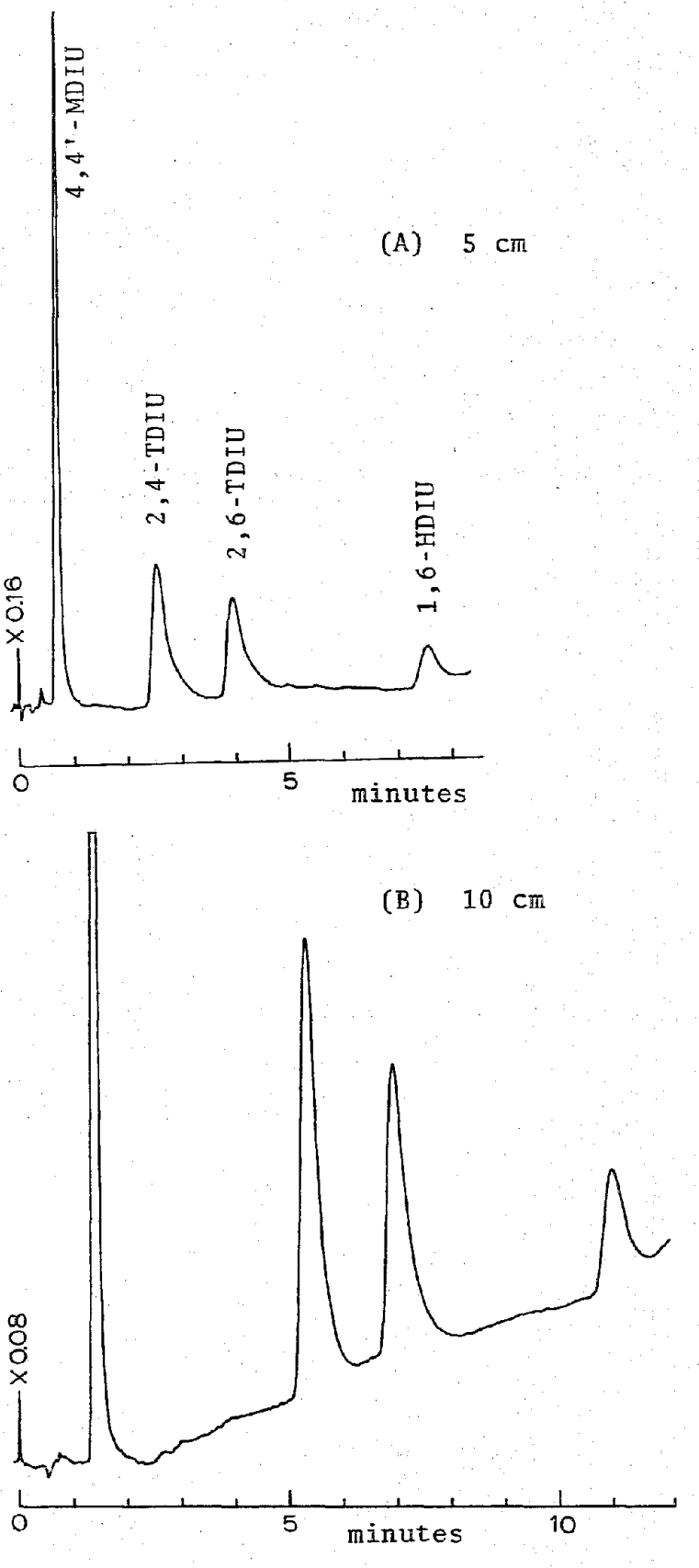


Fig. 3. Comparison of the LC separation of the urea test mixture on the 5 cm and the 10 cm Partisil 5 column. Slurry packed in 4.5 mm i.d., linear gradient from 20% CH_3CN/CH_2Cl_2 ⟶ 50% CH_3CN/CH_2Cl_2 in 10 min., 2 ml/min. Schoeffel Spectroflow Monitor at 254 nm.

that the surface of the silica gel adsorbent seemed to be "modified" by small amounts of polar solvent, for example C_2H_5OH (approx. 0.75%), to prevent tailing. Therefore, this information was used to obtain a mobile phase that will curtail tailing. This more optimized mobile phase composition was found to be 5% B/A → 100% B where B = 9.1% i-C_3H_7OH and A = CH_2Cl_2.

Chromatograms on Figure 4 show the excellent separations on the 5 cm Partisil 5 column of various urea test mixtures using a linear gradient of 5% B/A → 100% B (as defined above) completed in 10 min., and a flow rate of 2 ml/min. The complete analysis for 4,4'-MDIU, 2,4-TDIU, 2,6-TDIU, 1,6-HDIU and the nitro reagent took 14 minutes, and the elution order was as given. Using 2,4-TDIU as the solute and 50% B/A, where B = 9.1% i-C_3H_7OH/CH_2Cl_2 and A = CH_2Cl_2, as the isocratic mobile phase, this column was determined to have 5,500 theoretical plates/meter.

The performance of the pre-packed, 25 cm Partisil 10 column is shown on Figure 5. This particular chromatography was obtained by direct "nitro reagent-plus-isocyanate" reaction. It is one in the series of injections to form the calibration curves. The chromatogram shows an injected amount of 400 ng of each of the isocyanate and 23 µg of the N.R.

THE NITRO REAGENT-PLUS-ISOCYANATE REACTION TIME

The apparent reaction time of the "nitro reagent-plus-isocyanate" solutions is presented on Figure 6. It shows that the 4,4'-MDI, 2,4-TDI and 2,6-TDI react to completion with the N.R. solution within a few minutes. However, the apparent reaction time for 1,6-HDI was more than an hour. This information may prove very useful in working out the conditions for impinger collections.

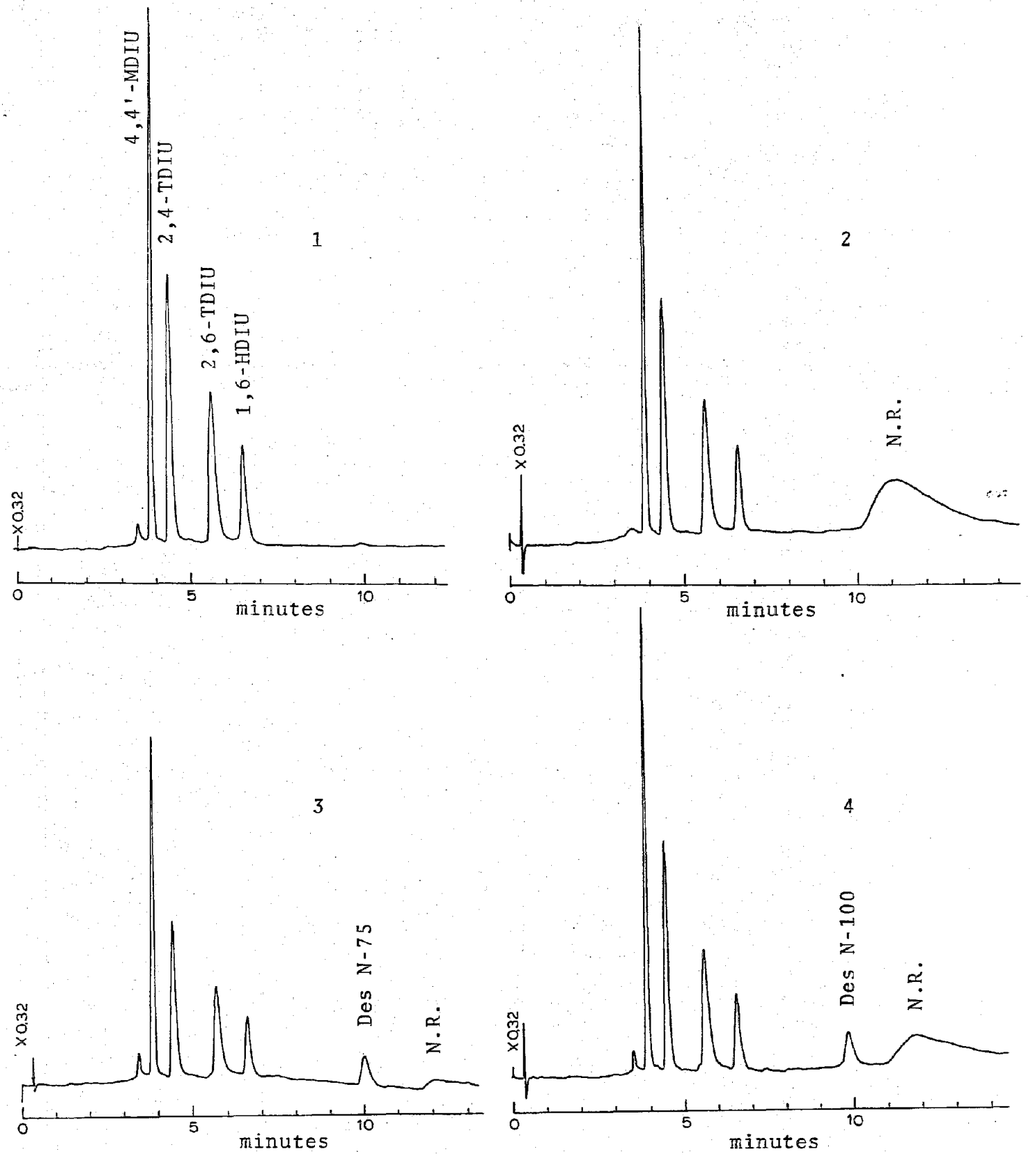


Fig. 4. Liquid chromatographic separations of various test mixtures on Partisil 5, 5 cm x 4.5 mm i.d. Linear gradient from 5% B/A ⟶ 100% B where B = 9.1% i-C_3H_7OH and A = CH_2Cl_2 in 10 min., Schoeffel Spectroflow Monitor at 254 nm, test mixtures: (1) the ureas; (2) the ureas plus 23 μg N.R.; (3) test mixture 2 plus 800 ng Des N-75; (4) test mixture 2 plus 800 ng Des N-100. Each mixture contained about 240 ng each of the ureas.

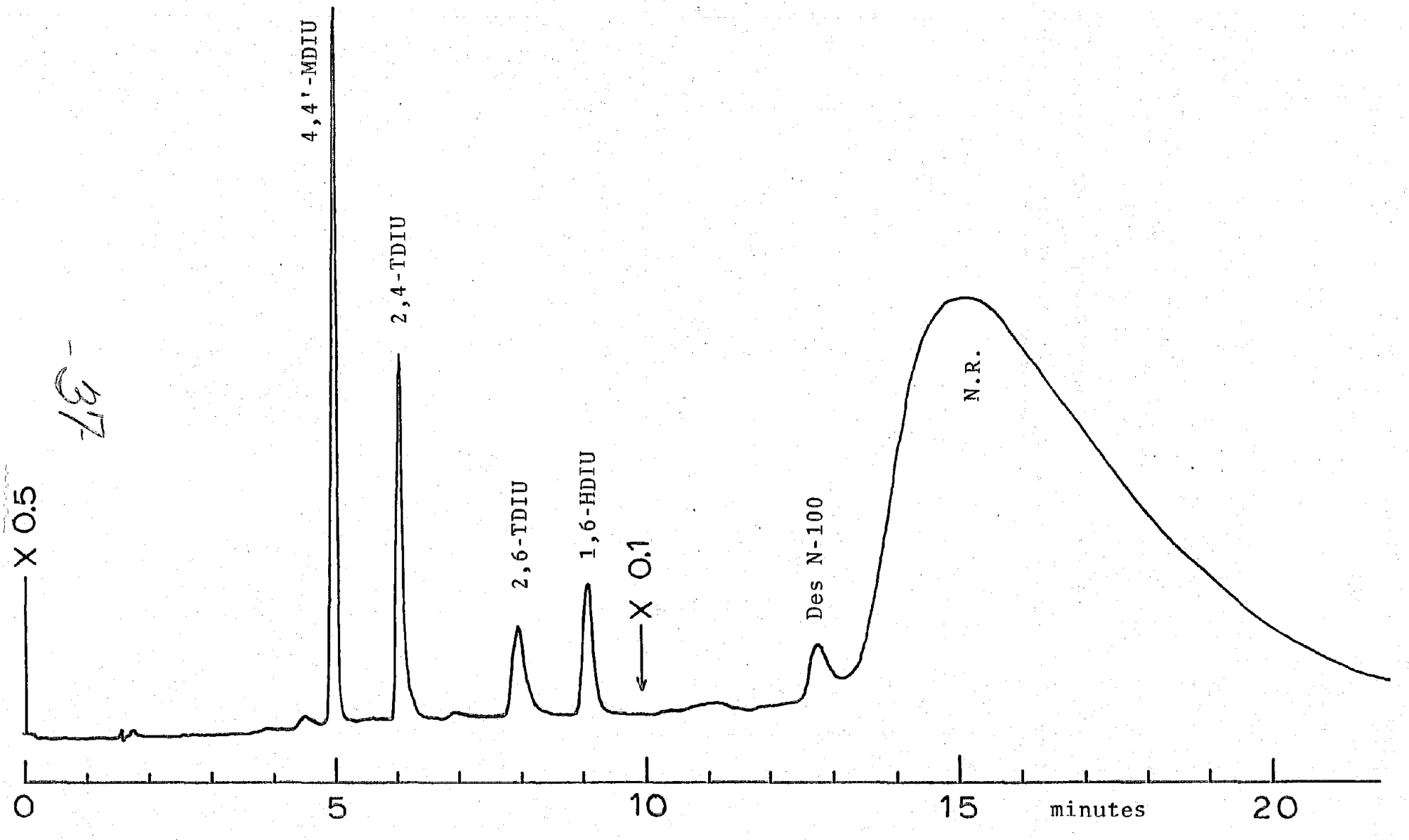


Fig. 5. Linear gradient chromatography of the nitro reagent-plus-isocyanate reaction mixture on a 25 cm x 4.5 mm i.d. pre-packed Partisil 10 column. Mobile phase from 10% B/A ⟶ 100% B, in 10 min., 2 ml/min., where B = 9.1% i-C_3H_7OH/CH_2Cl_2 and A = CH_2Cl_2, Waters Associates Model 440 absorbance detector at 254 nm.

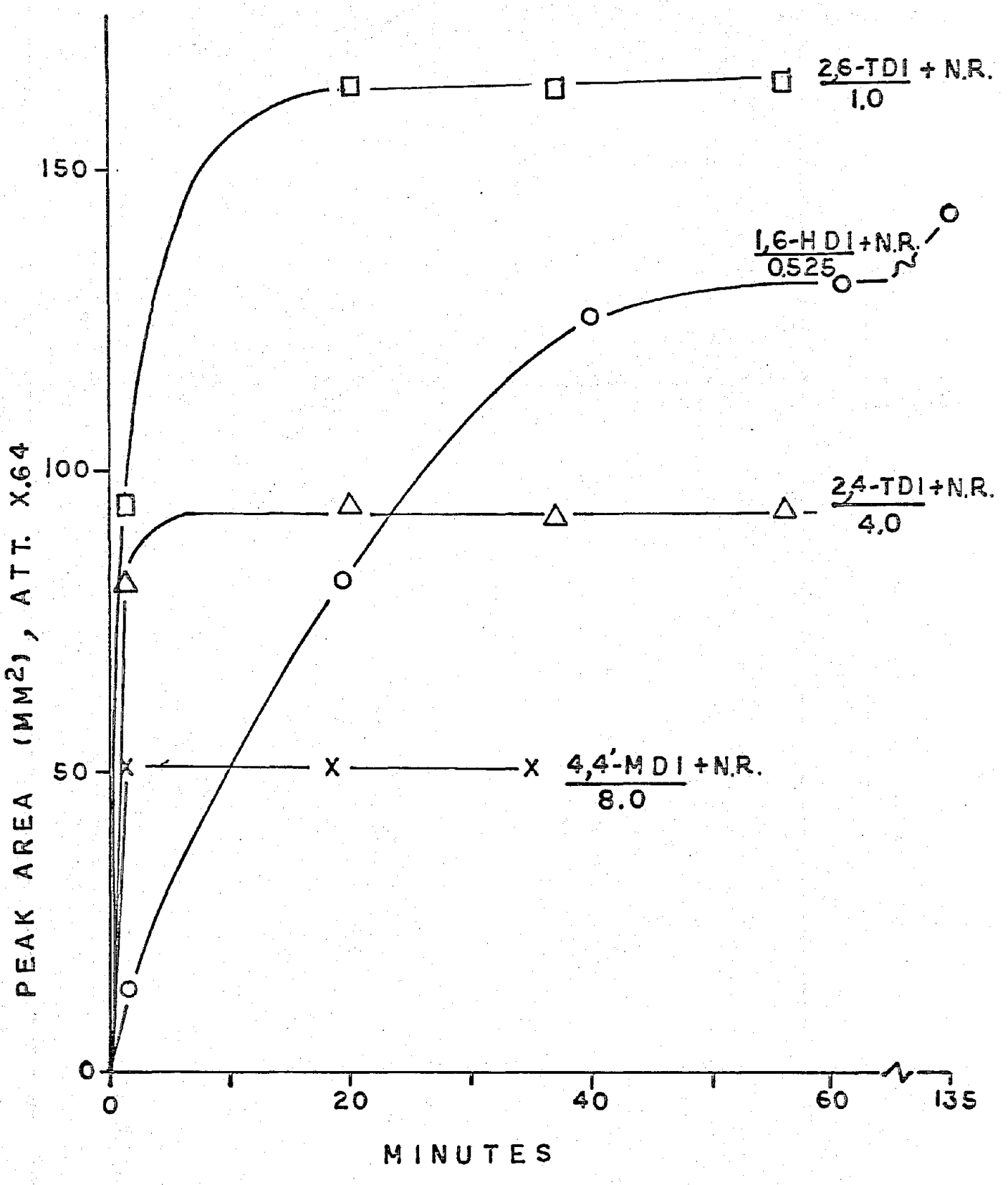


Fig. 6. The apparent reaction time of the nitro reagent-plus-isocyanate solutions. LC on Corasil II, 37-50u, 40 cm x 2.1 mm i.d., linear gradient from 5% CH_3CN/CH_2Cl_2 to 100% CH_3CN in 10 min., 2.0 ml/min., Schoeffel Spectroflow Monitor at 254 nm.

CALIBRATION CURVE, LINEAR DYNAMIC RANGE AND DETECTION LIMIT

Chromatograms on Figures 7 and 8 show a few typical injections of the urea standard mixtures on the 5 cm Partisil 5 column. The experimental minimum detectable limit (MDL) is 1.2 ng for 4,4'-MDIU, 2,4-TDIU and 2,6-TDIU and 6.2 ng for 1,6-HDIU. This instrument must be well optimized, and with the least noise, as shown on these two figures. Figure 9 depicts the calibration curves on Partisil 5.

Liquid chromatograms on Partisil 5 of the nitro reagent-plus-isocyanate reaction mixture are shown on Figures 10 and 11. The nanograms shown refer to the injected amount of each of the isocyanates except for the 2,6-TDI which is 53.8% of 2,4-TDI (i.e., composition of Modur TD). Figure 12 shows the plotted calibration curves. Linearity was established up to 1,600 ng, beyond which no further test was made. Minimum detectable limits were 2 ng except for 1,6-HDI (5 ng) and Desmodur N-100 (240 ng).

It is obvious from the results seen above that the 5 cm Partisil 5 column is capable of doing the simultaneous analysis of the isocyanates in the presence of some unreacted N.R. Both minimum detectable limit and linearity are better than par. Nonetheless, to conveniently adapt this analytical method in any laboratory, a pre-packed, commercially available column was tested. This column was 25 cm x 4.5 mm i.d. packed with Partisil 10. Figure 13 shows the chromatograms on this column at the lower nanogram range and Figure 14 is a continuation of the same at the upper range. Again, the amount shown on each of the chromatograms refers to all the isocyanates except for 2,6-TDI which is 53.8% of 2,4-TDI. The calibration curves are shown on Figure 15. Linear dynamic range was observed up to 1,200 ng, the highest amount injected. The experimental detected amounts were 2 ng for 4,4'-MDI and 2,4-TDI, 5 ng for 2,6-TDI and 1,6-HDI and 40 ng for Des. N-100.

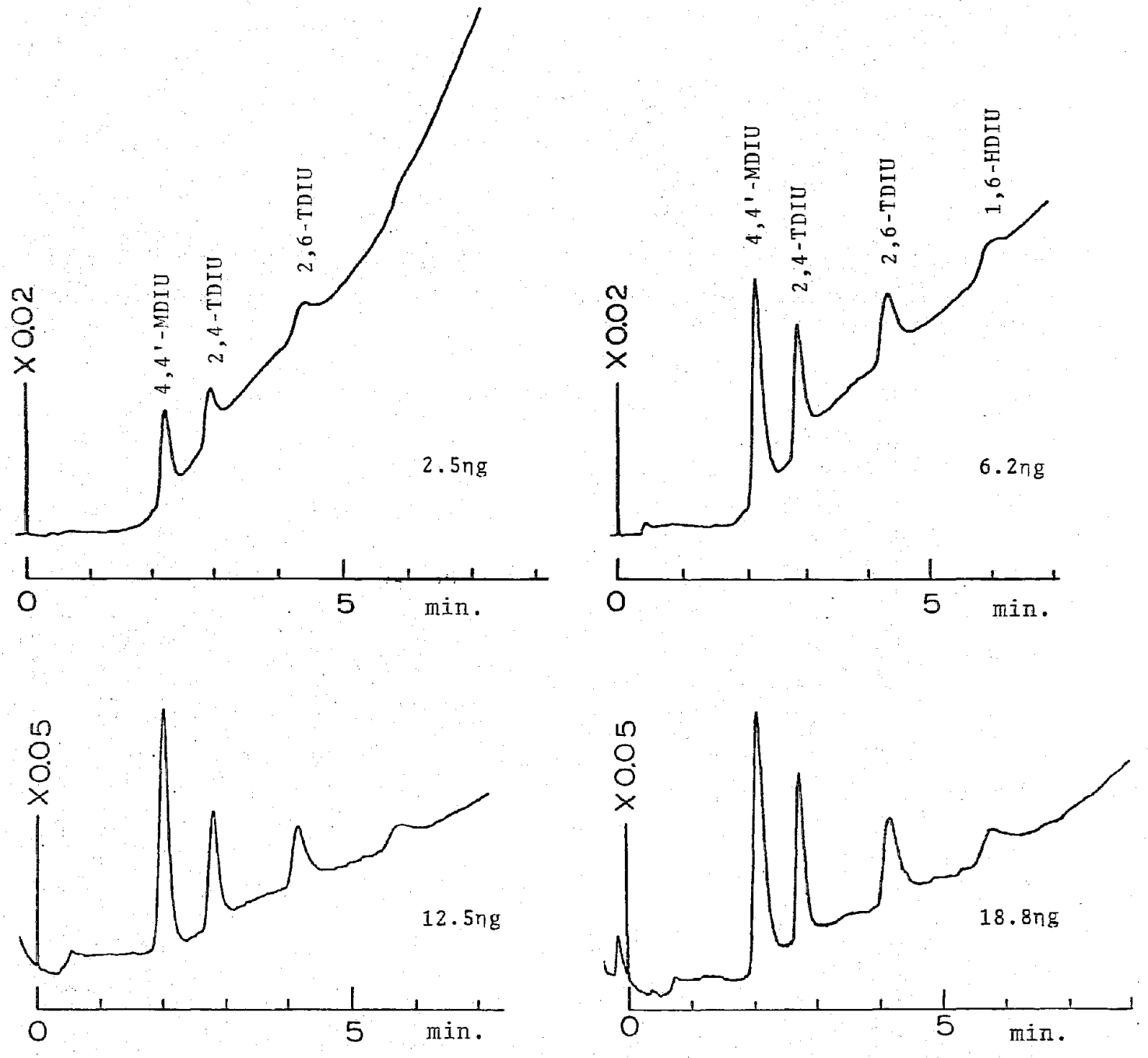


Fig. 7. Typical chromatograms of urea standard solutions at the lower nanogram range. Partisil 5, 5 cm x 4.5 mm i.d., linear gradient from 10% B/A → 100% B, in 10 min., 2 ml/min., where B = 9.1% i-C_3H_7OH and A = CH_2Cl_2, Waters Associates Model 440 absorbance detector at 254 nm. Amount injected for each compound as shown.

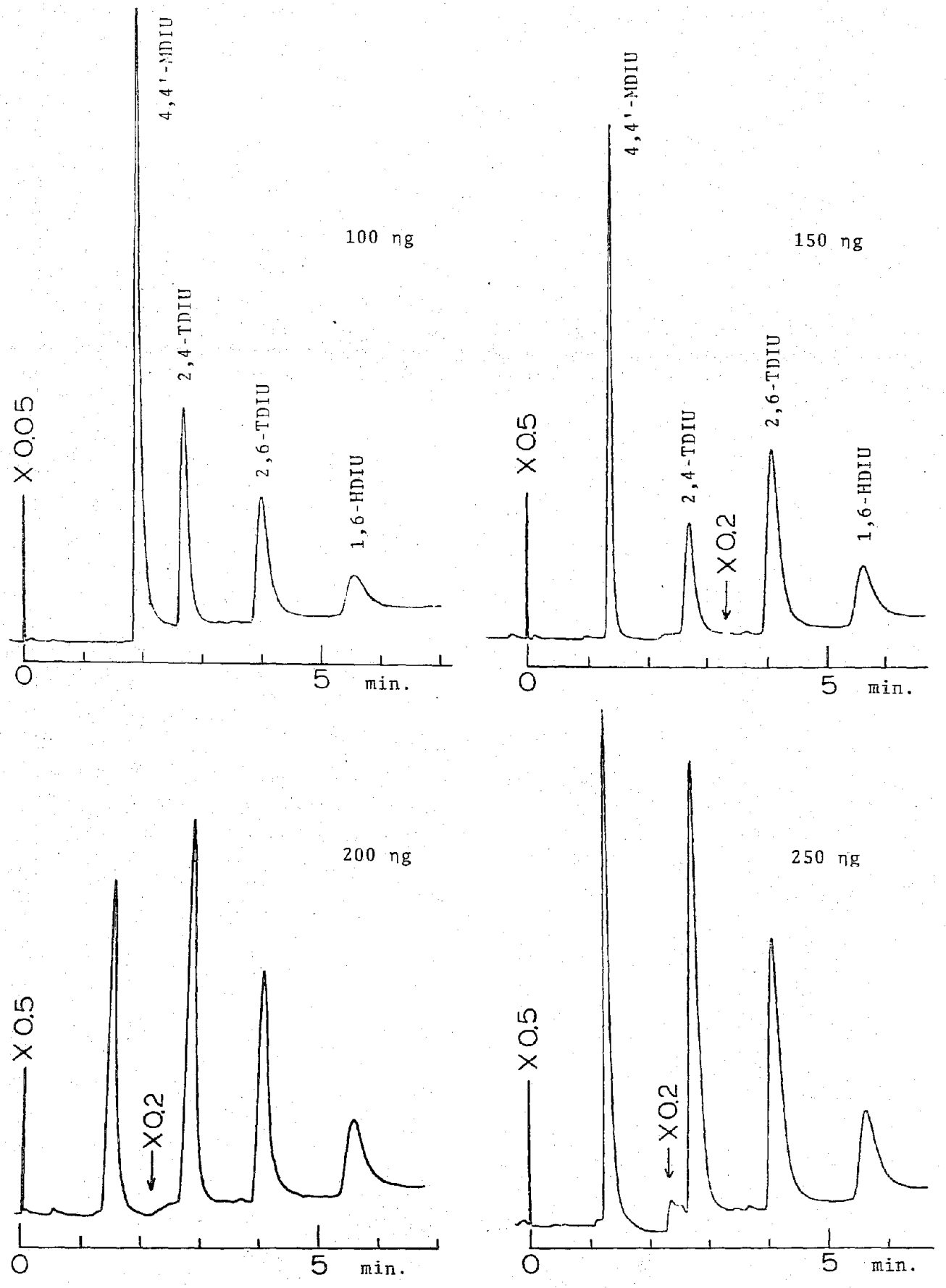


Fig. 8. Chromatograms of urea standard solutions. LC conditions same as on Fig. 7.

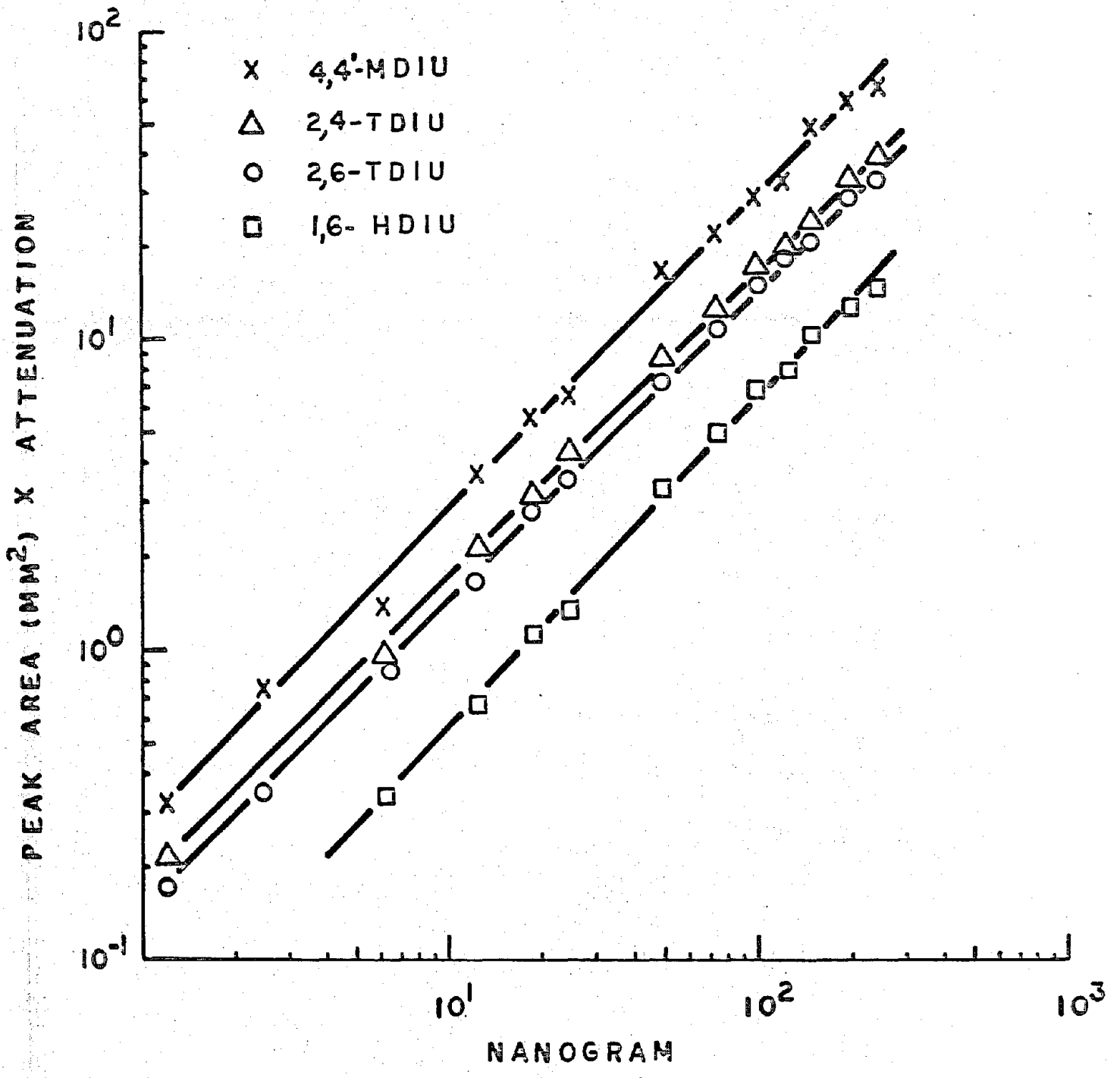


Fig. 9. Urea calibration curves on Partisil 5.

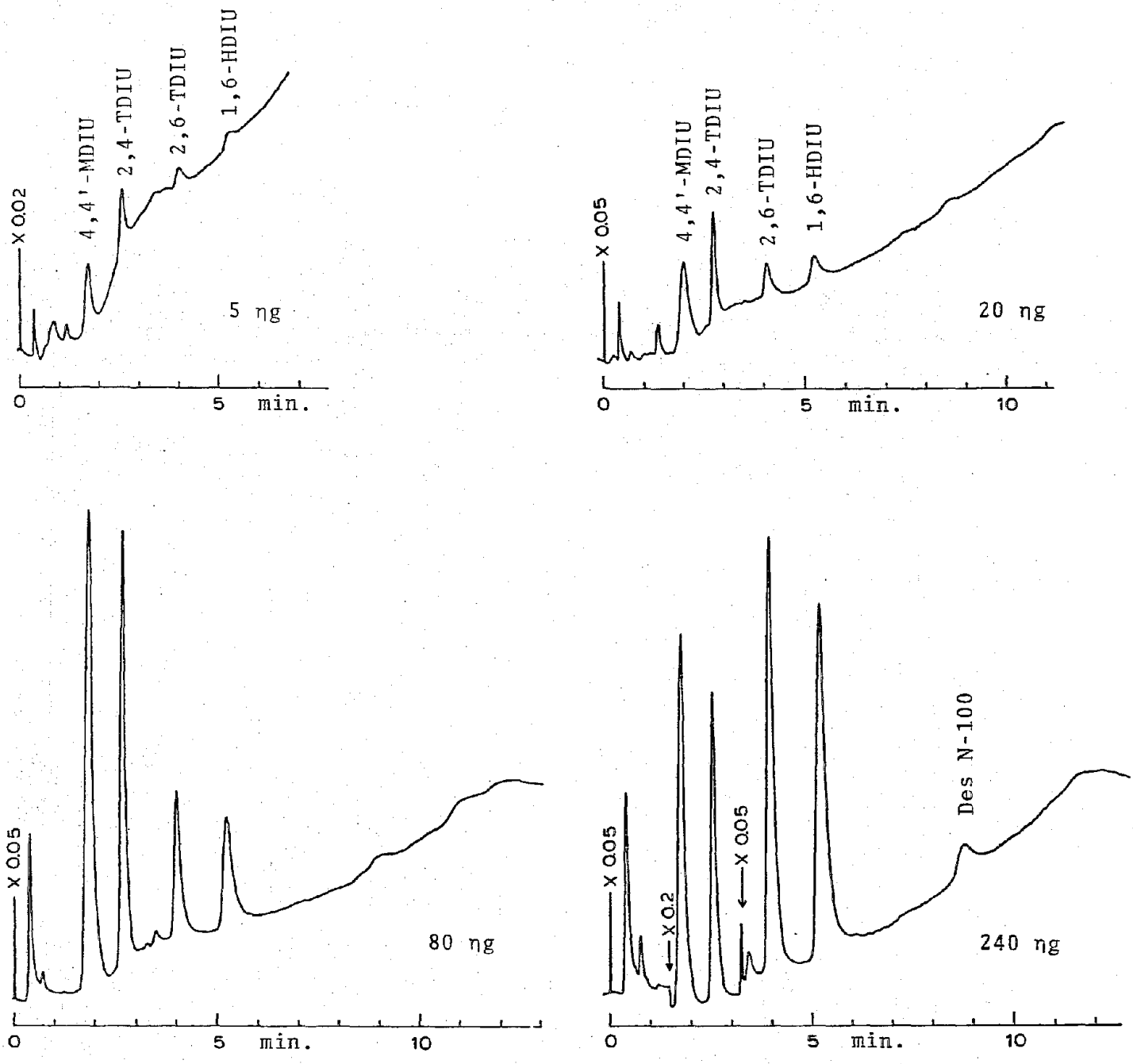


Fig. 10. Chromatograms of the nitro reagent-plus-isocyanate reaction mixtures on Partisil 5, 5 cm x 4.5 mm i.d., linear gradient from 10% B/A → 100% B in 10 min., 2 ml/min., where B = 9.1% i-C_3H_7OH and A = CH_2Cl_2, Waters Associates Model 440 absorbance detector at 254 nm. The nanograms shown refer to the injected amount of each except for 2,6-TDI which is 53.8% of 2,4-TDI.

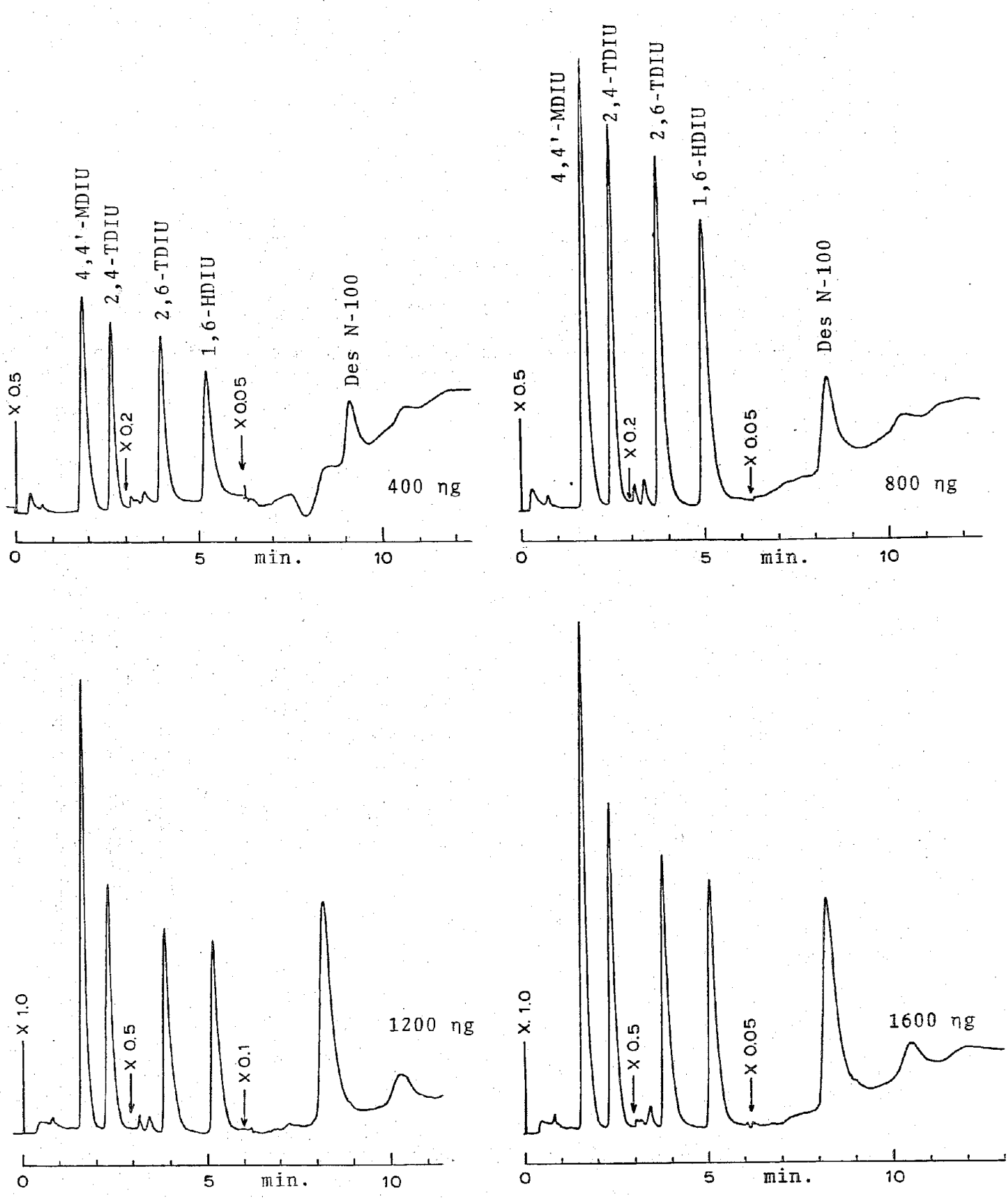


Fig. 11. Chromatograms of the nitro reagent-plus-isocyanate reaction mixture on Partisil 5. LC conditions the same as on Fig. 10.

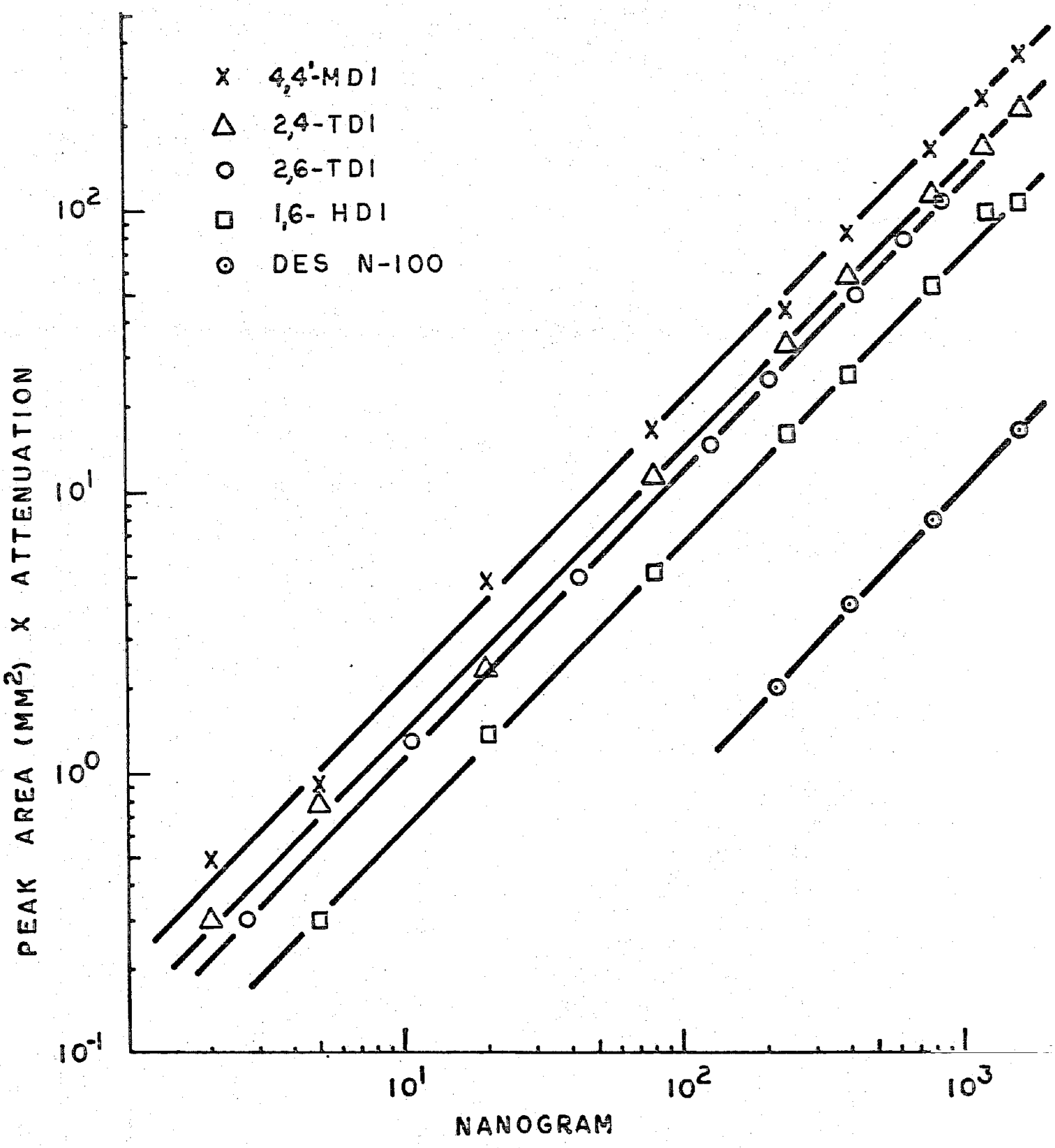


Fig. 12. Calibration curves of nitro reagent-plus-isocyanate reaction mixtures on Partisil 5.

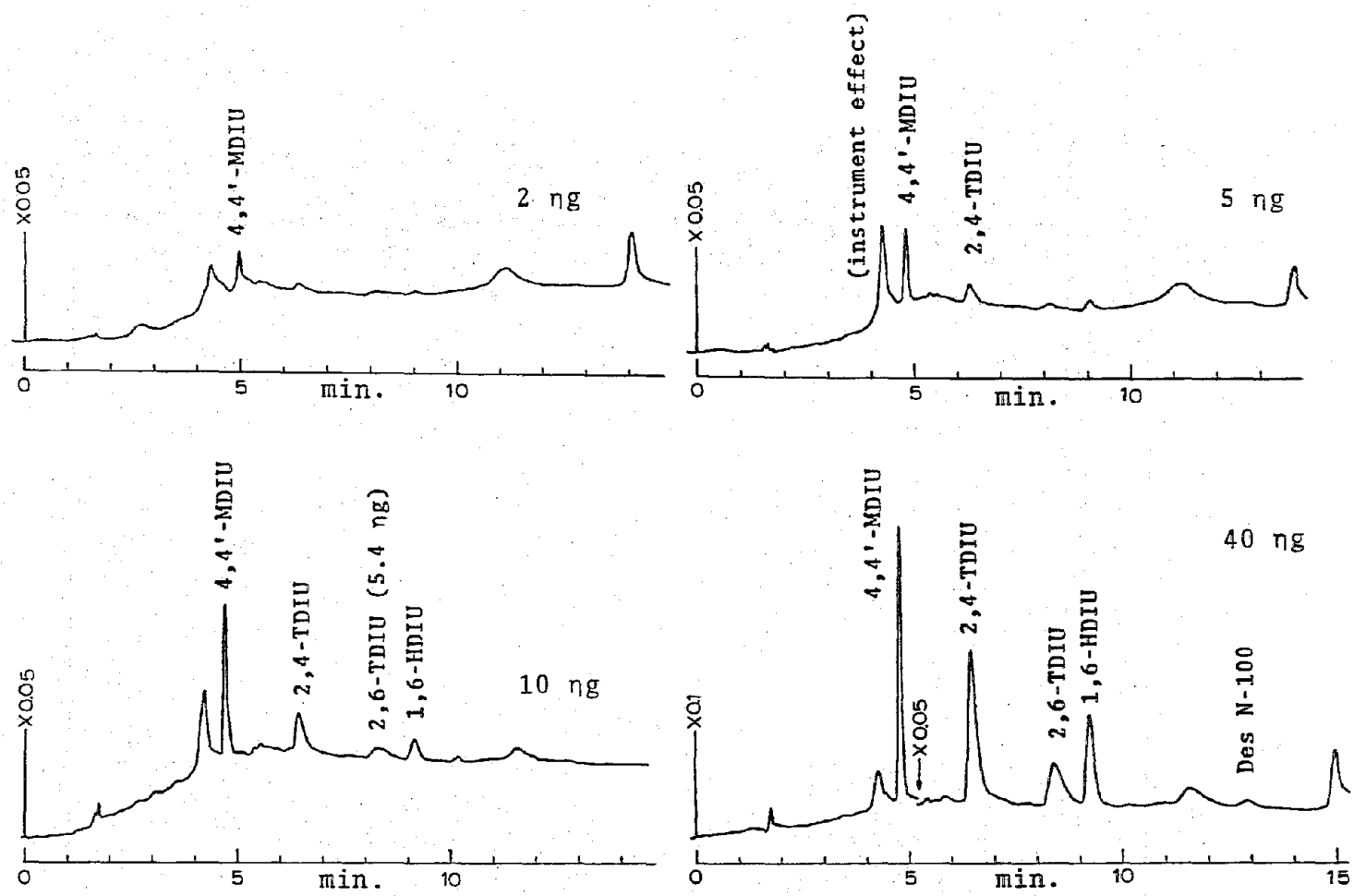


Fig. 13. Chromatograms of the nitro reagent-plus-isocyanate reaction mixtures at the low nanogram range on Partisil 10, 25 cm x 4.5 mm i.d., linear gradient from 10% B/A → 100% B in 10 min., 2 ml/min., where B = 9.1% i-C_3H_7OH and A = CH_2Cl_2, Waters Associates Model 440 absorbance detector at 254 nm. The nanograms shown refer to the injected amount of each except for the 2,6-TDI which is 53.8% of 2,4-TDI.

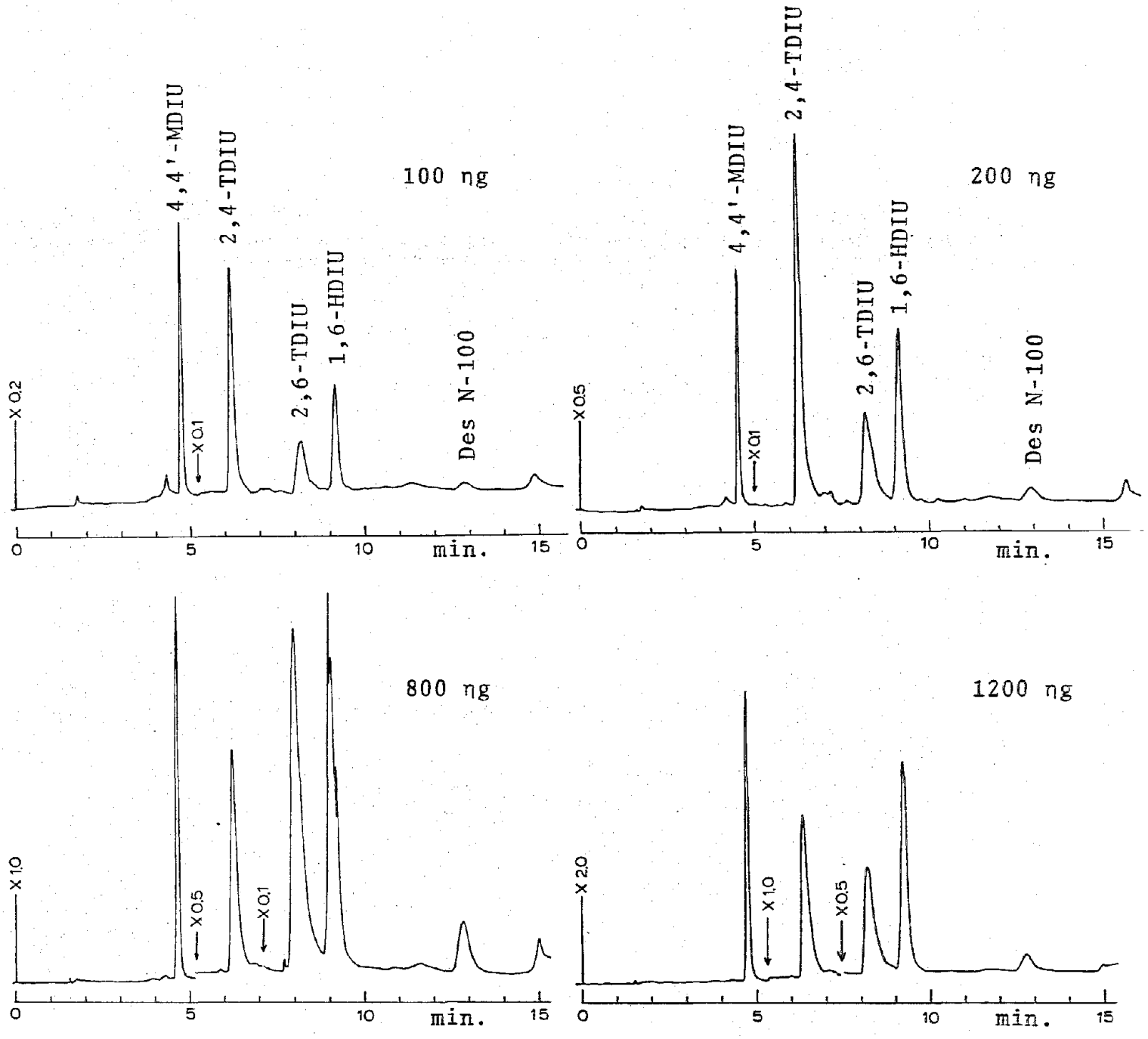


Fig. 14. Chromatograms of the nitro reagent-plus-isocyanate reaction mixtures on Partisil 10. LC conditions the same as on Fig. 13.

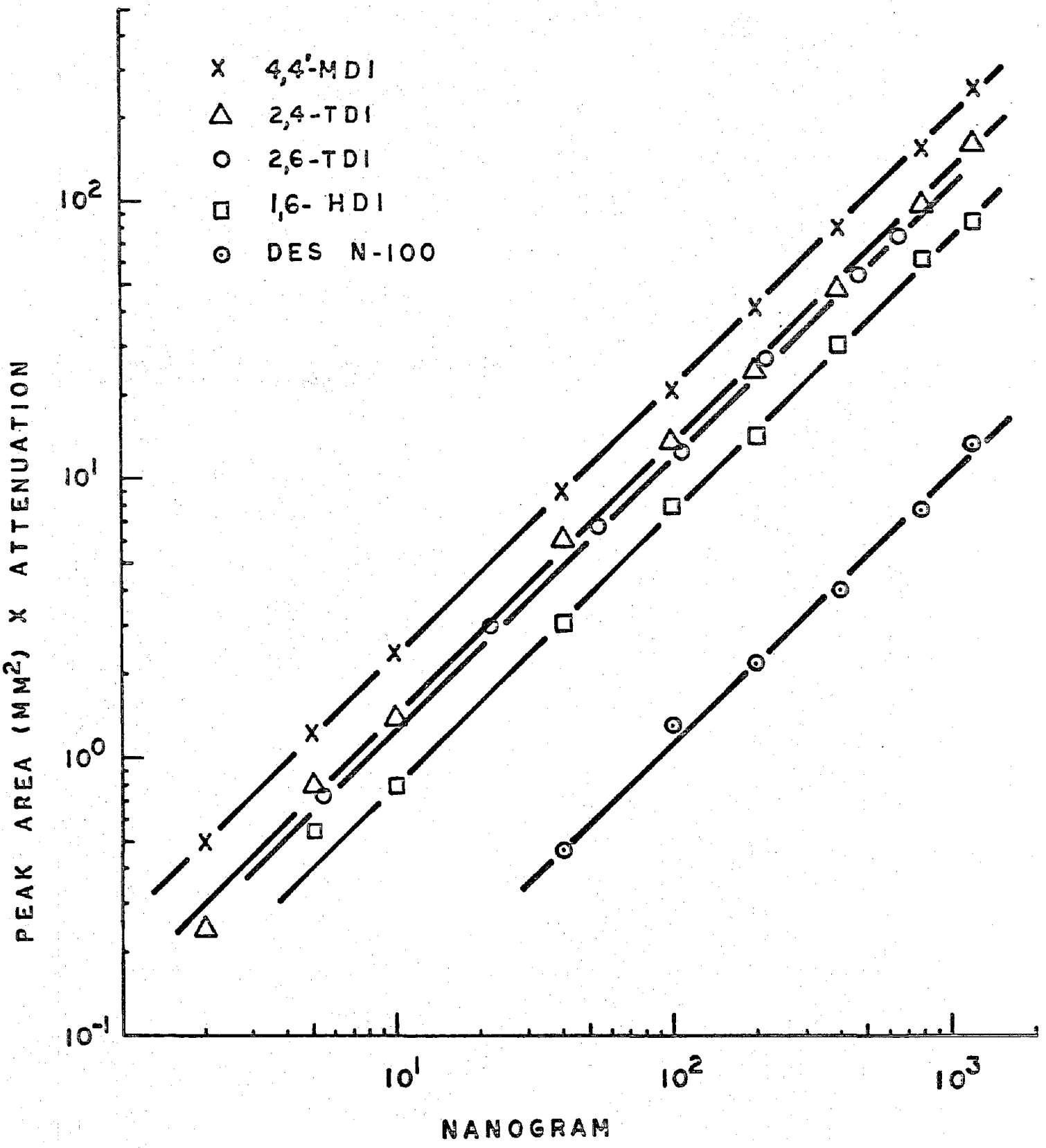


Fig. 15. Nitro reagent-plus-isocyanate calibration curves on Partisil 10.

A point of interest is to compare the uv responses of the solutes eluted from the 5 cm Partisil 5 and 25 cm Partisil 10 columns. Figure 16 reveals the superimposed responses from Figure 12 (5 cm Partisil 5) and Figure 15 (25 cm Partisil 10). Highly reversible adsorption is represented. The two columns behave very much alike, except, as expected, the retention time on the 25 cm column is longer.

THE STABILITY OF THE NITRO REAGENT-PLUS-ISOCYANATE MIXTURES

The stability of the samples (isocyanate-plus-nitro reagent) was studied for a period of 18 days. Figure 17 shows the measured peak areas plotted against time. It is apparent that 4,4'-MDI, 2,4-TDI, and 1,6-HDI are stable up to 18 days. The 2,6-TDI and Desmodur N-100 are less stable. Degradation starts after 10 days. A typical injection is shown on Figure 18. Relative standard deviations in measured peak areas for six consecutive injections in the same day were: 4,4'-MDI, 2.6%, 2,4-TDI, 3.2%, 2,6-TDI, 2.2%, 1,6-HDI, 4.0% and Desmodur N-100, 2.9%.

INTERNAL STANDARDS

An internal standard is the best reference in terms of peak areas (i.e. concentrations) and retention times. It should fulfill several requirements: The internal standard should be well resolved from peaks of interests; it should absorb in the uv region; it should not react with the analytes; if possible, it should be similar in structure to the components of interest.

The 3,5-dimethylphenol and the monourea of p-tolylisocyanate, 4(1-tolyl)-3-n-propyl-3-(4 nitrobenzyl) urea, or p-TIU, were tested. The former met part, and the latter met all of these requirements. They both elute around 1.5-2.0 minutes earlier than that of 4,4'-MDIU. The former was used

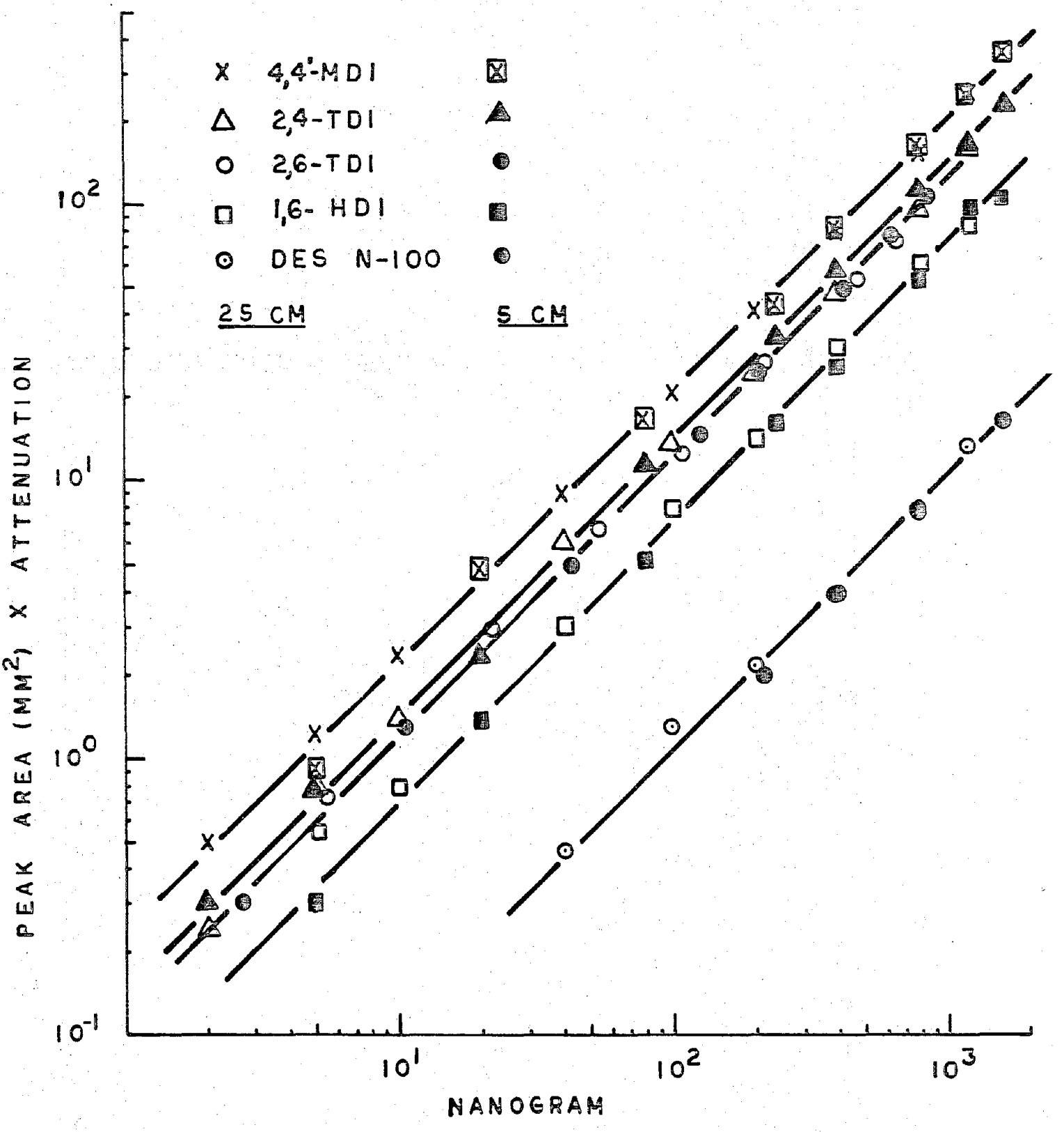


Fig. 16. Calibration curves of the different di[3-n-propyl-3-(4-nitrobenzyl)] ureas on 5 cm Partisil 5 and 25 cm Partisil 10 columns.

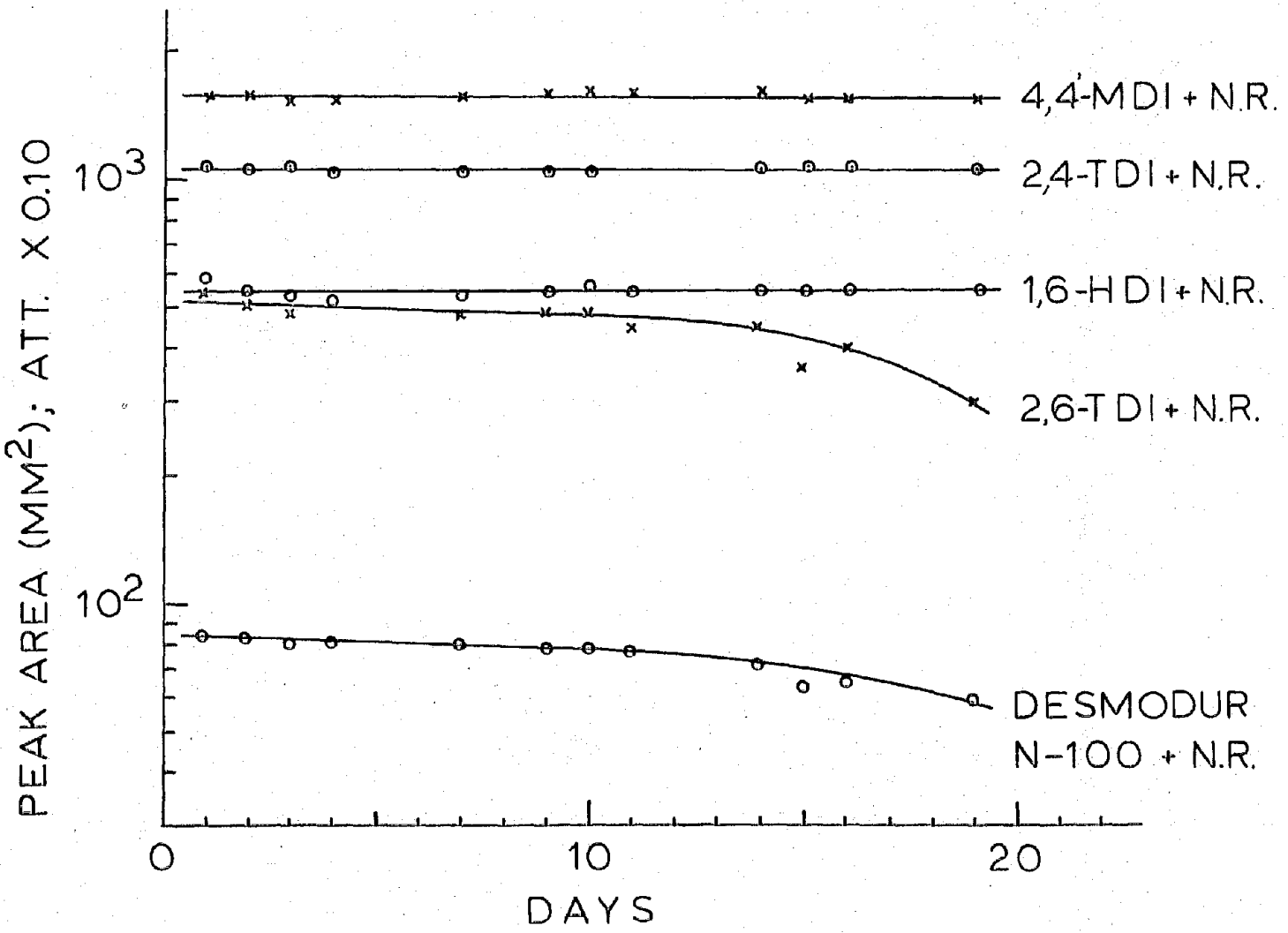


Fig. 17. The stability of the isocyanate-plus-nitro reagent solutions.

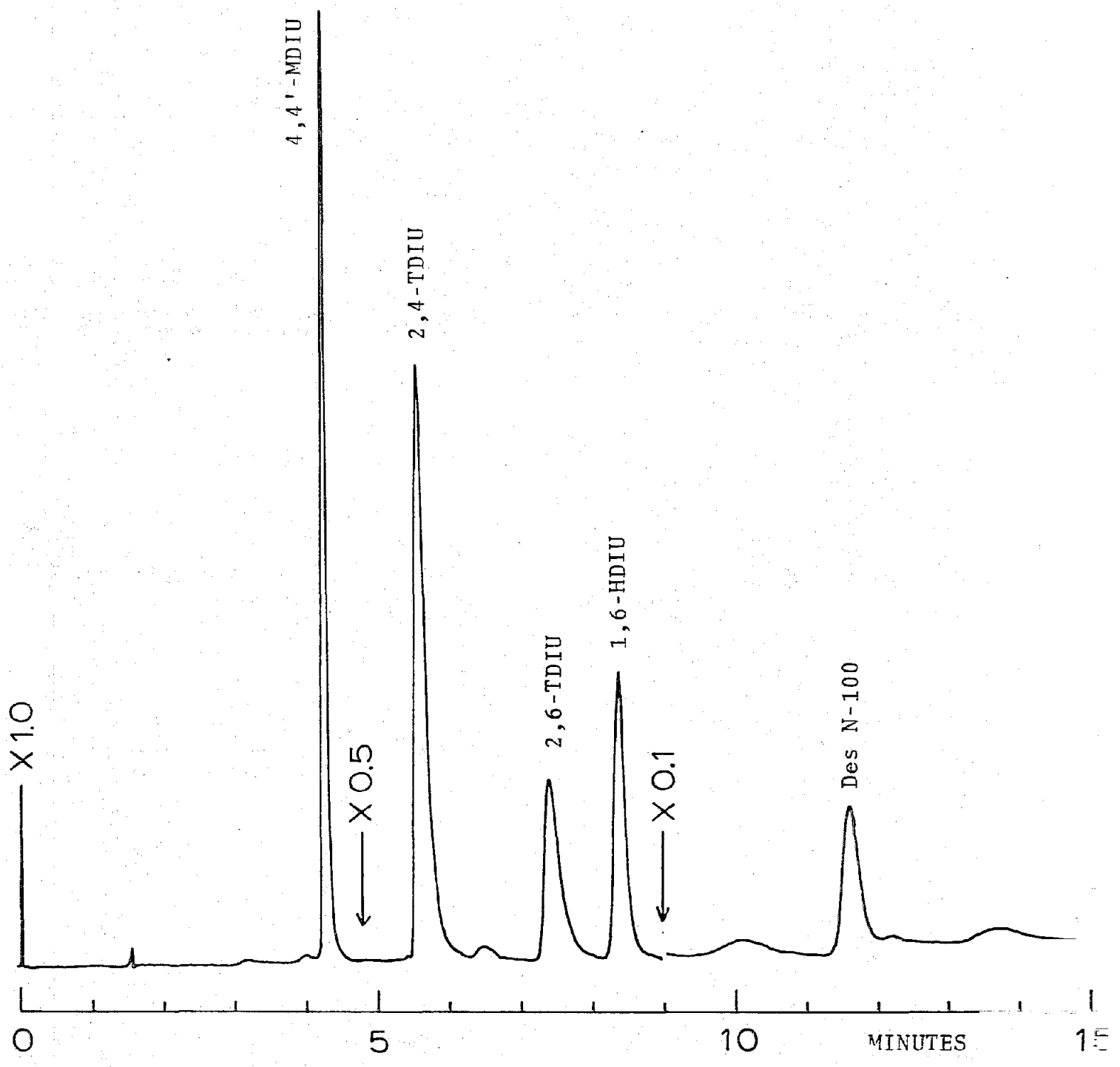


Fig. 18. A typical chromatogram of the nitro reagent-plus-isocyanate solution on a pre-packed Partisil 10 column, 25 cm x 4.5 mm i.d., 10% B/A → 100% B in 10 min., 2 ml/min., where B = 9.1% i-C_3H_7OH/CH_2Cl_2 and A = CH_2Cl_2. Waters Associates Model 440 absorbance detector at 254 nm. Each peak represents 800 ng of the isocyanates except 2,6-TDI which is 53.8% of 2,4-TDI.

for Set #2 in the reproducibility test, however, its retention time proved to be unrelated to those of the ureas at the gradient used. The latter was meant to be used for Sets #1 and #3 of the same test. However, because of the wide spread of the three sets, the problem of too much unreacted nitro reagent was encountered. Therefore, a more unique use of the p-tolylisocyanate was applied, that is, as a scrubber of the unreacted nitro reagent in the reaction mixtures. It must be emphasized here that the synthesized p-TIU can be used as internal standard if the reaction mixture has a small amount of unreacted nitro reagent; that the p-TI itself can be used to remove most of the excess N.R. Excessive amount of N.R. in the samples poses a problem. It is a serious threat to column life, chromatographic performance, and reproducibility of results.

A perfunctory test to precipitate the excess nitro reagent with HCl showed that there were other parameters to be studied in order to validate the method. For example, an attempt was made to precipitate the N.R. with 1 ml of 1.2 M HCl from a 15 ml reaction mixture from Set #1 (lowest concentrations). Chromatography showed the appearance of at least four peaks not otherwise observed. Owing to the time confinement, it was not further studied.

REPRODUCIBILITY TEST

The absolute retention times, relative retention times and peak areas for the three sets of samples are given on Tables 9-17. In Set #1, the percent relative standard deviations are 0.8-1.6% and 2.0-3.5% for absolute and relative retention times, respectively, and 11.1-16.5% for peak areas. For Set #2 these numbers are 1.4-4.1%, 2.7-8.3%, and 2.8-4.5%. Likewise, these numbers for Set #3 are 0.6-2.6%, 2.2-8.2%, and 4.6-11.3% (excluding 4,4'-MDI which showed possible chemical change as evidenced by its altered solubility in CH_2Cl_2 and the appearance of an unknown chromatographic peak from 4,4'-MDI

solution). All these numbers are well within the experimental error, although the measured peak areas have a wider range of standard deviations than is acceptable. A problem with the column started when excessive N.R. was used to accomodate the three sets of samples in the reproducibility tests. The column efficiency changed during the tests, causing peak broadening , tailing and, in some cases, peaks overlap. Subjecting the column to excessive N.R. made it impossible to regain its efficiency.

CONCLUSION

The analysis of the industrial isocyanates using high speed (HSLC) liquid chromatography is shown and the advantages of HSLC over that of the TLC counterpart are demonstrated. Minimum detectable limit of 2 ng was observed. Linearity up to 1,600 ng has been shown on the 25 cm Partisil 10 and 5 cm Partisil 5 columns. Reproducibility of repeated injections are within experimental errors. Samples are Stable for at least 10 days. Column life and efficiency can be preserved by flushing the column daily and minimizing the amount of unreacted nitro reagent injected.

Ureas that may serve as "primary standards" can be synthesized, isolated, purified and characterized.

Table 9

Set #1

Absolute Retention Times (in mm)

Sample Number	p-TI*	4,4'-MDI	2,4-TDI	2,6-TDI	1,6-HDI	Desmodur N-100
1	38.0	67.5	89.0	114.0	148.0	
2	39.0	67.0	88.0	112.0	142.0	
3	40.0	68.0	88.0	111.0	143.0	
4	39.0	69.0	87.0	112.0	146.0	
5	40.0	69.0	88.0	111.0	144.0	
6	39.0	68.0	86.5	111.0	146.0	
7	39.5	66.5	87.0	111.0	147.0	
8	39.5	67.0	86.5	112.0	146.0	
9	40.0	67.0	88.0	111.0	145.0	
10	40.0	68.0	88.5	111.0	143.0	
Average	39.4	67.7	87.6	111.6	145.0	
Standard Deviation	±0.62	±0.91	±0.81	±0.89	±1.85	
Percent Relative Standard Deviation	1.6%	1.3%	0.9%	0.8%	1.3%	

Not Detected

* The p-tolylisocyanate was used primarily to react with the excess N.R.

Table 10

Set #1

Sample Number	$t_R/t_{R\ ref}$ 4,4'-MDI	2,4-TDI	2,6-TDI	1,6-HDI
1	1.78	2.34	3.00	3.89
2	1.72	2.26	2.87	3.64
3	1.70	2.20	2.78	3.58
4	1.77	2.23	2.87	3.74
5	1.73	2.20	2.78	3.60
6	1.74	2.22	2.85	3.74
7	1.68	2.20	2.81	3.72
8	1.70	2.19	2.84	3.70
9	1.68	2.20	2.78	3.63
10	1.70	2.21	2.78	3.58
Average	1.72	2.23	2.84	3.68
Standard Deviation	±0.16	±0.04	±0.06	±0.03
Percent Relative Standard Deviation	9.3%	1.8%	2.3%	0.8%

Table 11

Set #1

Peak Areas (mm^2 at x .02 Attenuation)

Sample Number	4,4'-MDI	2,4-TDI	2,6-TDI	1,6-HDI
1	108	188	(184*)	183
2	104	184	109	243
3	89.0	125	117	158
4	111	148	97.5	158
5	110	144	132	202
6	94.5	140	103	127
7	116	148	118	179
8	(180*)	127	98.0	183
9	99.5	142	135	204
10	132	124	123	177
Average	107	147	115	181
Standard Deviation	±11.9	±21.5	±13.2	±29.9
Percent Relative Standard Deviation	11.1%	14.6%	11.5%	16.5%

* Deleted in calculating averages by Q test

Table 12

Set #2

Absolute Retention Times (in mm)

Sample Number	Internal* Standard	4,4'-MDI	2,4-TDI	2,6-TDI	1,6-HDI	Des N-100
1	44.5	66.5	88.0	107.5	123.5	171.0
2	47.5	68.5	88.0	108.0	124.0	170.0
3	44.5	68.5	91.5	113.0	130.0	178.5
4	46.5	68.5	91.0	113.0	130.0	179.5
5	45.0	67.0	88.0	108.5	125.5	175.5
6	45.0	68.5	89.5	112.0	129.0	179.0
7	46.0	69.0	91.0	113.0	131.5	178.0
8	46.5	68.0	89.5	111.0	129.0	177.5
9	47.0	69.0	91.0	113.5	130.5	178.5
10	44.0	48.5**	86.0	108.0	127.0	176.0
Average	45.8	68.1	89.7	111.0	128.1	176.4
Standard Deviation	±1.0	±2.8	±1.3	±1.96	±2.81	±3.34
Percent Relative Standard Deviation	2.18%	4.1%	1.4%	1.8%	2.2%	1.9%

* Internal standard is 3,5-dimethyl phenol.

** Deleted in calculating averages by Q test

Table 13

Set #2

	$t_R/t_{R\ ref}$				
Sample Number	4,4'-MDI	2,4-TDI	2,6-TDI	1,6-HDI	Des N-100
1	1.49	1.98	24.16	27.75	38.43
2	1.44	1.85	22.74	26.10	35.79
3	1.54	2.05	25.39	29.21	40.11
4	1.47	1.96	24.30	27.96	38.60
5	1.49	1.95	24.11	27.89	39.00
6	1.52	1.99	24.89	28.66	39.77
7	1.50	1.98	24.56	28.58	38.69
8	1.46	1.92	23.87	27.74	38.17
9	1.47	1.94	24.15	27.76	37.98
10	1.10	1.95	24.54	28.86	40.00
Average	1.45	1.98	2.43	2.81	4.15
Standard Deviation	±0.12	±0.07	±0.66	±0.87	±2.99
Percent Relative Standard Deviation	8.3%	3.6%	2.7%	3.1%	7.2%

Table 14

Set #2

Peak Areas (mm^2 at x .02 Attenuation)

Sample Number	Internal Standard	4,4'-MDI	2,4-TDI	2,6-TDI	1,6-HDI	Des N-100
1	75.0	62.9	117.3	49.9	142.0	56.6
2	71.3	57.4	116.6	47.2	132.6	64.1
3	67.8	65.1	106.4	47.6	135.2	54.5
4	69.2	(76.7)*	114.0	50.1	142.8	54.4
5	67.0	67.8	106.4	48.4	133.9	53.6
6	68.5	67.8	106.4	51.0	143.1	56.7
7	68.7	65.1	109.2	52.7	(177.0)** peaks overlapped	58.7
8	70.4	64.2	105.1	47.9	144.7	52.5
9	70.0	66.0	105.3	48.4	144.7	54.6
10	67.8	66.3	109.8	48.5	140.4	57.6
Average	69.57	65.93	109.65	49.17	139.93	56.33
Standard Deviation	±1.96	±2.97	±4.55	±1.55	±4.60	±2.11
Percent Relative Standard Deviation	2.82%	4.50%	4.15%	3.15%	3.29%	3.75%

* Deleted in calculating averages by Q test.

** Deleted because an unknown peak overlapped with 1,6-HDI.

Table 15
Set #3

Absolute Retention Times (in mm)

Sample Number	p-TI*	4,4'-MDI	2,4-TDI	2,6-TDI	1,6-HDI	Des N-100
1	39.0	67.5	79.5	104.0	135.0	190.0
2	38.0	65.0	78.0	103.0	132.5	190.0
3	37.0	66.0	78.0	102.0	134.0	190.0
4	39.5	67.5	78.5	103.5	134.0	192.0
5	39.0	66.0	78.5	105.0	135.0	191.0
6	38.5	66.0	79.5	105.0	134.5	193.0
7	39.0	66.5	79.0	104.0	134.0	191.0
8	38.0	65.0	77.0	103.0	132.0	190.0
9	38.0	66.0	78.5	104.0	133.5	190.0
10	36.0	67.0	79.0	104.0	134.5	190.0
Average	38.2	66.2	78.5	103.8	133.9	190.7
Standard Deviation	±1.01	±1.05	±0.72	±0.88	±1.02	±1.05
Percent Relative Standard Deviation	2.6%	1.6%	0.9%	0.8%	0.8%	0.6%

* The p-tolylisocyanate was used primarily to react with the excess N.R.

Table 16

Set #3

Sample Number	$t_R/t_{R\ ref}$ 4,4'-MDI	2,4-TDI	2,6-TDI	1,6-HDI	Des N-100
1	1.73	2.04	2.67	3.46	4.87
2	1.71	2.05	2.71	3.49	5.00
3	1.78	2.11	2.76	3.62	5.13
4	1.71	1.99	2.62	3.40	4.86
5	1.69	2.01	2.69	3.46	4.90
6	1.71	2.06	2.73	3.49	5.01
7	1.71	2.03	2.67	3.44	4.90
8	1.71	2.03	2.71	3.47	5.00
9	1.74	2.07	2.74	3.51	5.00
10	1.86	2.19	2.89	3.74	5.28
Average	1.74	2.06	2.72	3.51	5.00
Standard Deviation	±0.05	±0.05	±0.06	±0.09	±0.41
Percent Relative Standard Deviation	2.9%	2.7%	2.2%	2.6%	8.2%

Table 17

Set #3

Peak Areas (mm^2 at x .02 Attenuation)

Sample Number	4,4'-MDI	2,4-TDI	2,6-TDI	1,6-HDI	Des N-100
1	800	5958	2074	4760	76.5
2	554	6638	2120	5000	175*
3	920	6102	2138	4920	100
4	486	6800	2272	4880	80.5
5	622	6082	2250	4880	81
6	478	6490	1960	4760	66
7	528	6160	2160	5040	80
8	968	6624	2224	5420	95
9	644	6090	2312	5520	79.5
10	924	7920*	2210	5560	81
Average	693	6328	2172	5080	82
Standard Deviation	±183	±292	±99	±292	±9.3
Percent Relative Standard Deviation	26.4%	4.61%	4.56%	5.75%	11.3%

* Deleted in calculating averages by Q test.

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2. K. Mercali, "Microdetermination of Toluene Diisocyanates in Atmosphere," Anal. Chem., 29, 552-58 (1957).

3. K. E. Grim and A. L. Linch, "Recent Isocyanate-in-Air Analysis Studies," Am. Ind. Hyg. Assoc. J., 25, 285-90 (1964).

4. R. L. Larkin and R. E. Kupel, "Microdetermination of Toluene Diisocyanate Using Toluenediamine as the Primary Standard," Am. Ind. Hyg. Assoc. J., 30, 640-42 (1969).

5. K. L. Dunlap, R. L. Sandridge and J. Keller, "Determination of Isocyanates in Working Atmospheres by High Speed Liquid Chromatography," Anal. Chem., 48, 497-99 (1976).

APPENDIX

SPECTRUM NO. 1

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SAMPLE Nitro Reag·HCl

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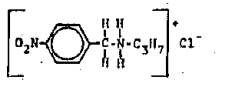


PATH mm

SOLVENT

CONCENTRATION

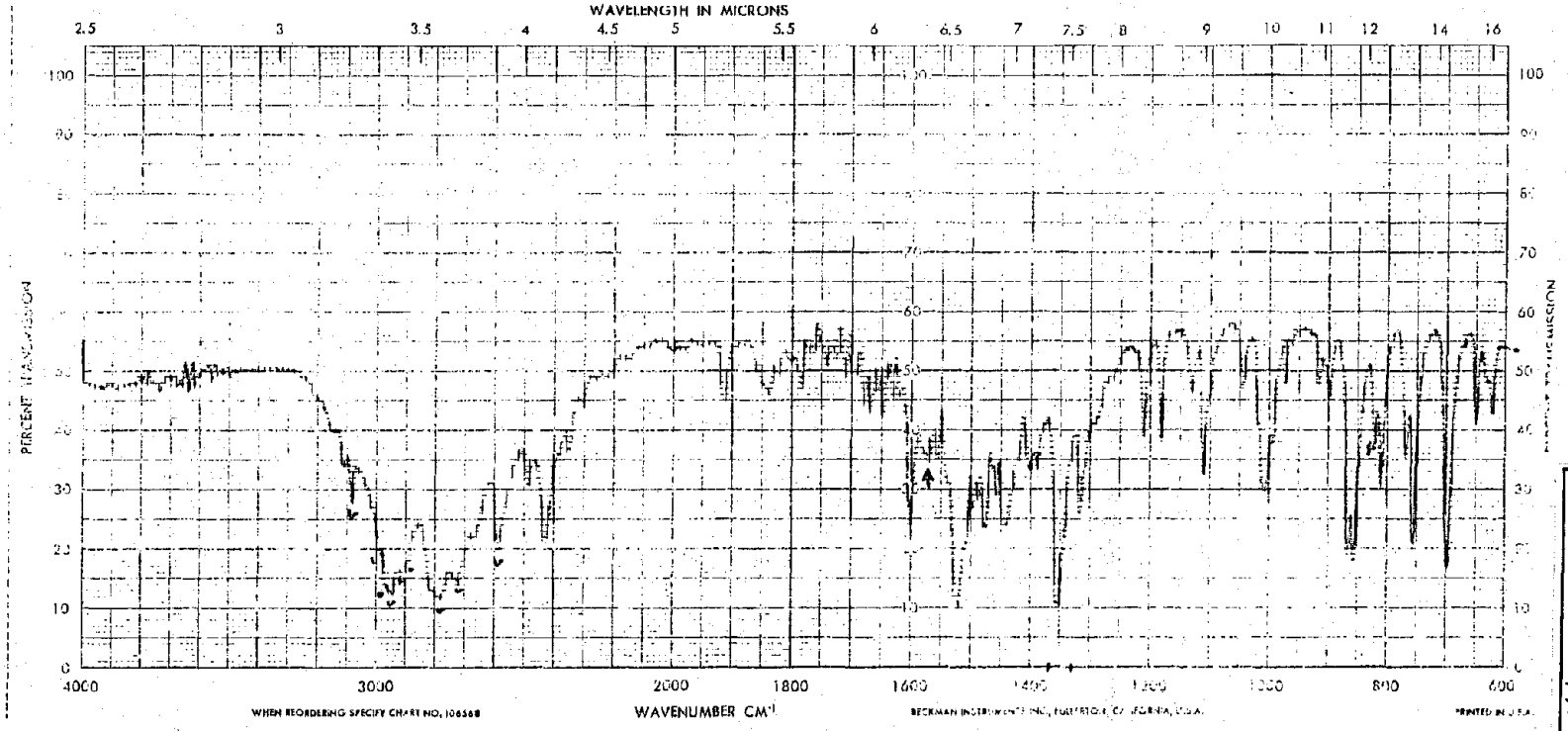
PHASE

COMMENTS KBr disc

ANALYST Ryan

Beckman®

INFRARED SPECTROPHOTOMETER



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SAMPLE Nitro Reag

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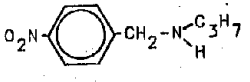

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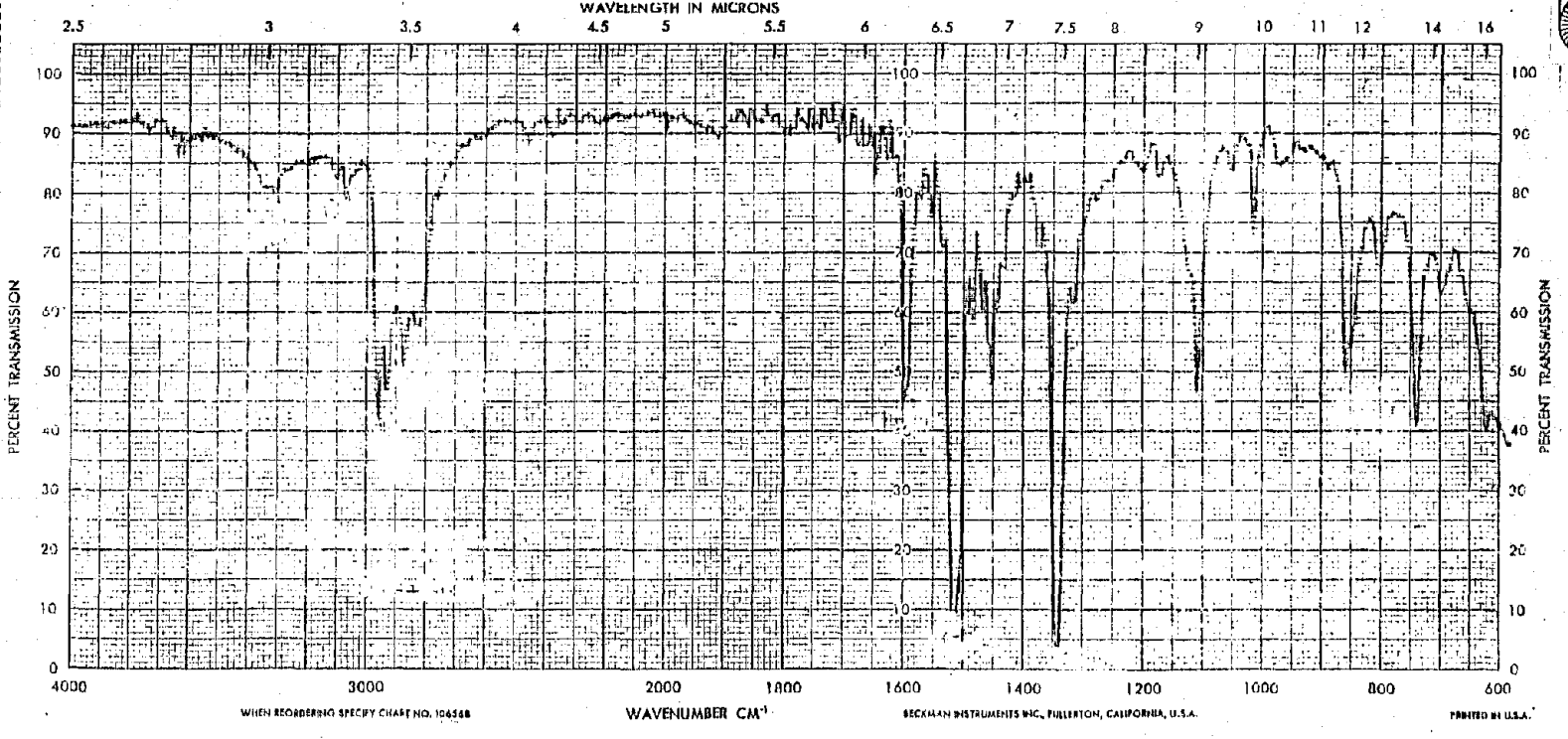

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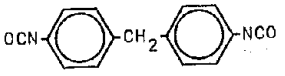
DATE 8/5/75

SAMPLE 4,4-MDI-UCC-P

SOURCE Mobay



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SOLVENT

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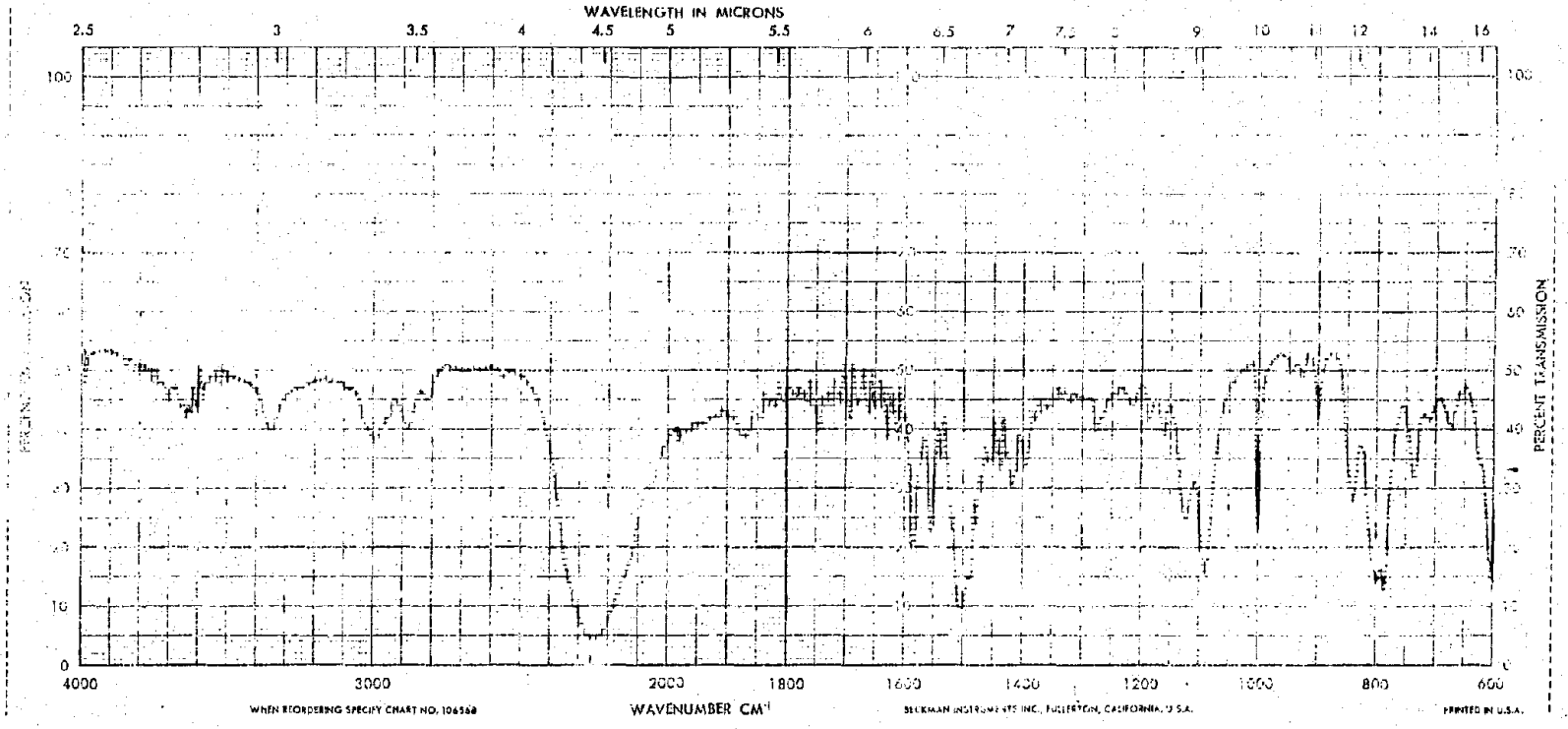

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DATE 8/5/75

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SOLVENT

CONCENTRATION

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Beckman®

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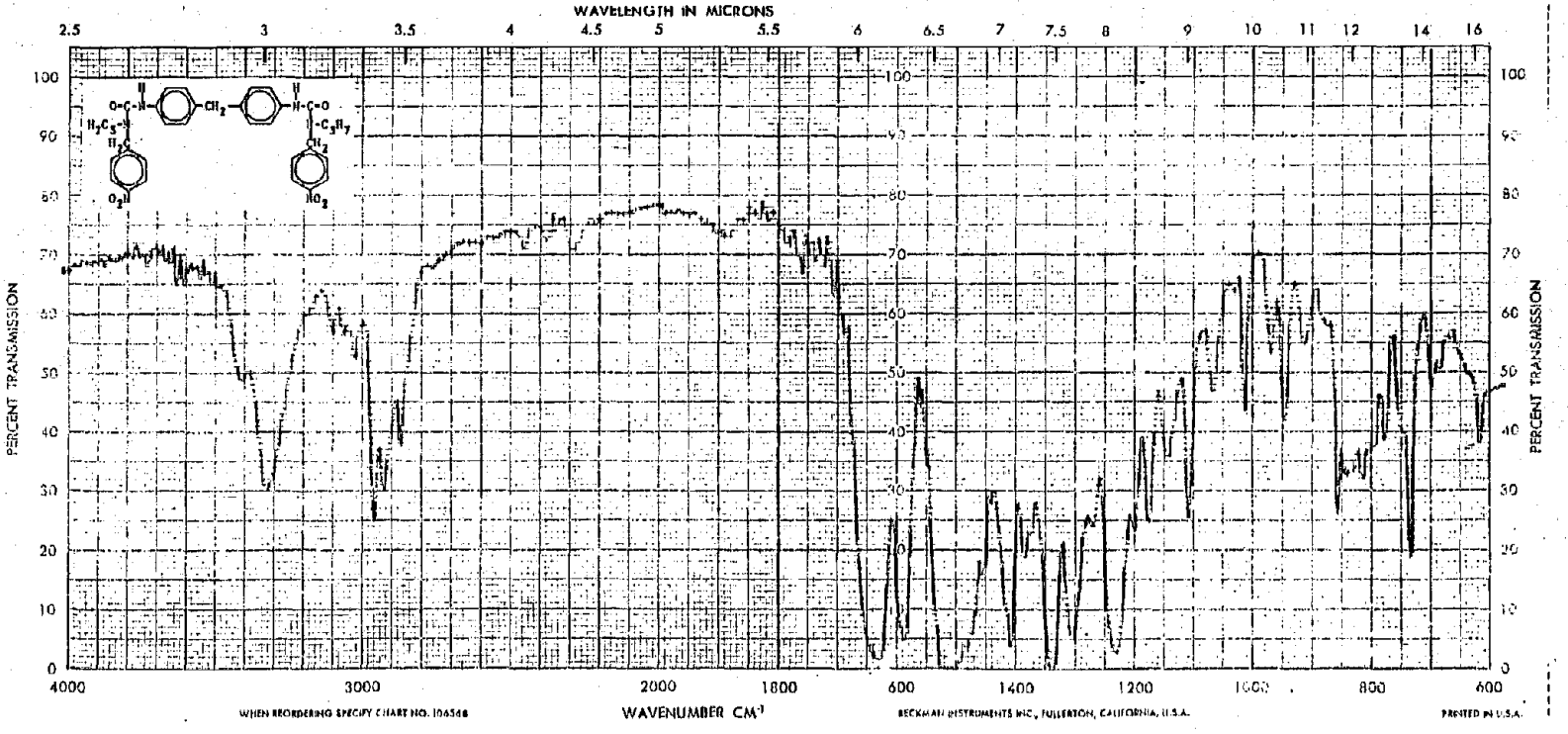

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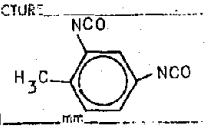
STRUCTURE 
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COMMENTS KBr plates
ANALYST Ryan

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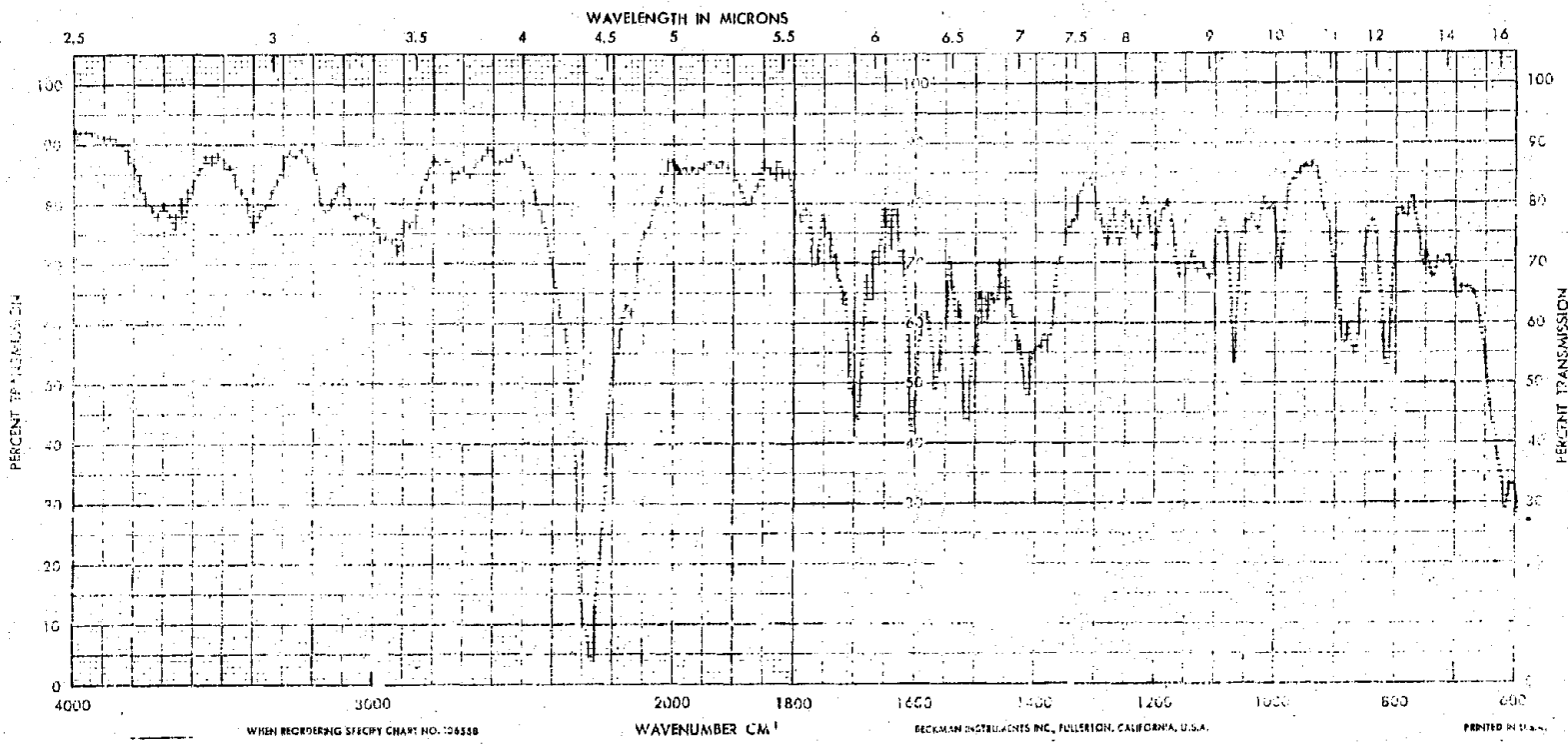

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DATE 7/17/75
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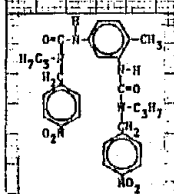
STRUCTURE 
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ANALYST Ryan

Beckman®
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SPECTRUM NO. 7

DATE 8/11/75

SAMPLE 2,6-TDIU-3c

SOURCE

STRUCTURE

PATH mm

SOLVENT

CONCENTRATION

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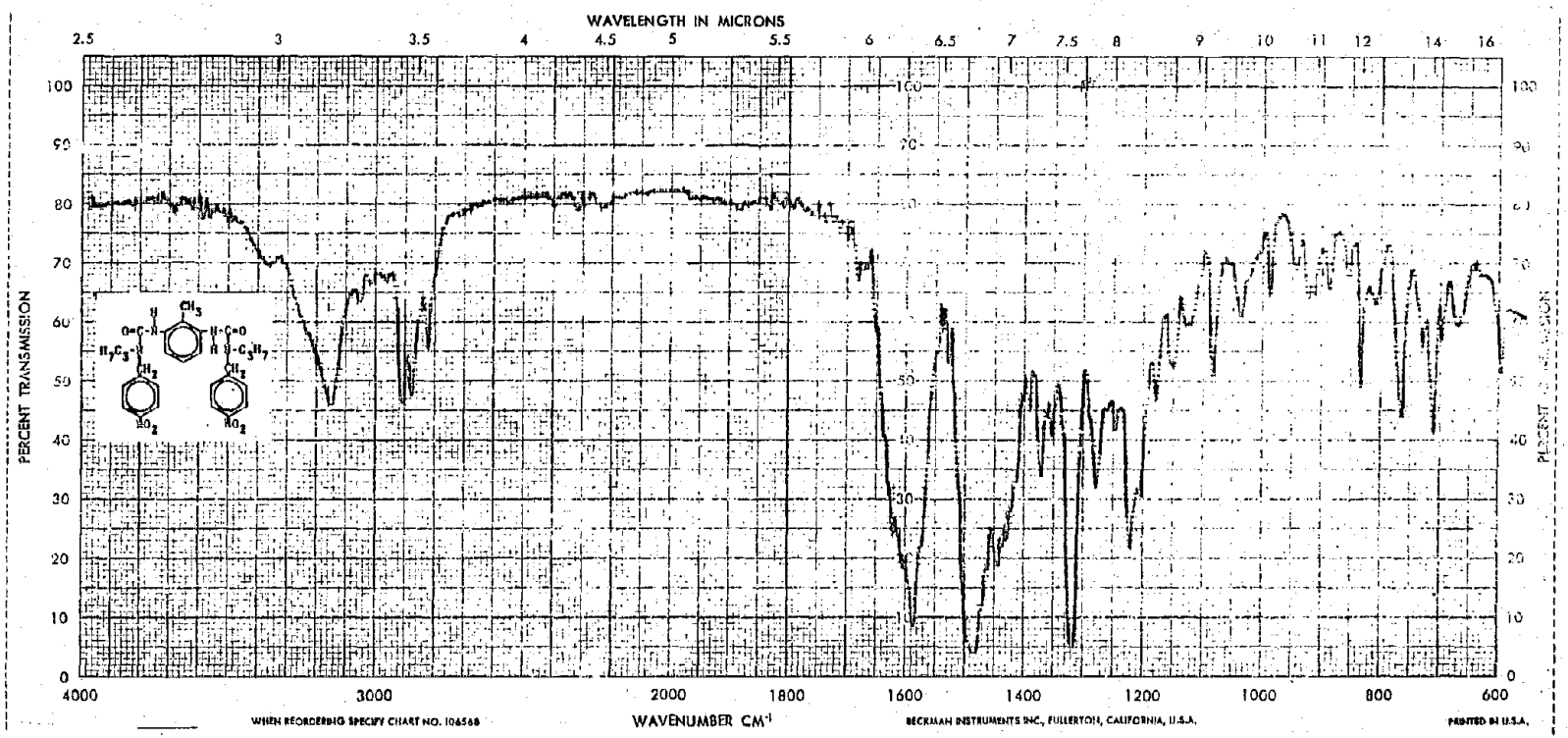
COMMENTS KBr disc

ANALYST Ko

Beckman®

INFRARED SPECTROPHOTOMETER



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STRUCTURE
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SOLVENT none
CONCENTRATION
PHASE
COMMENTS KBr plates
ANALYST Ryan

Beckman®
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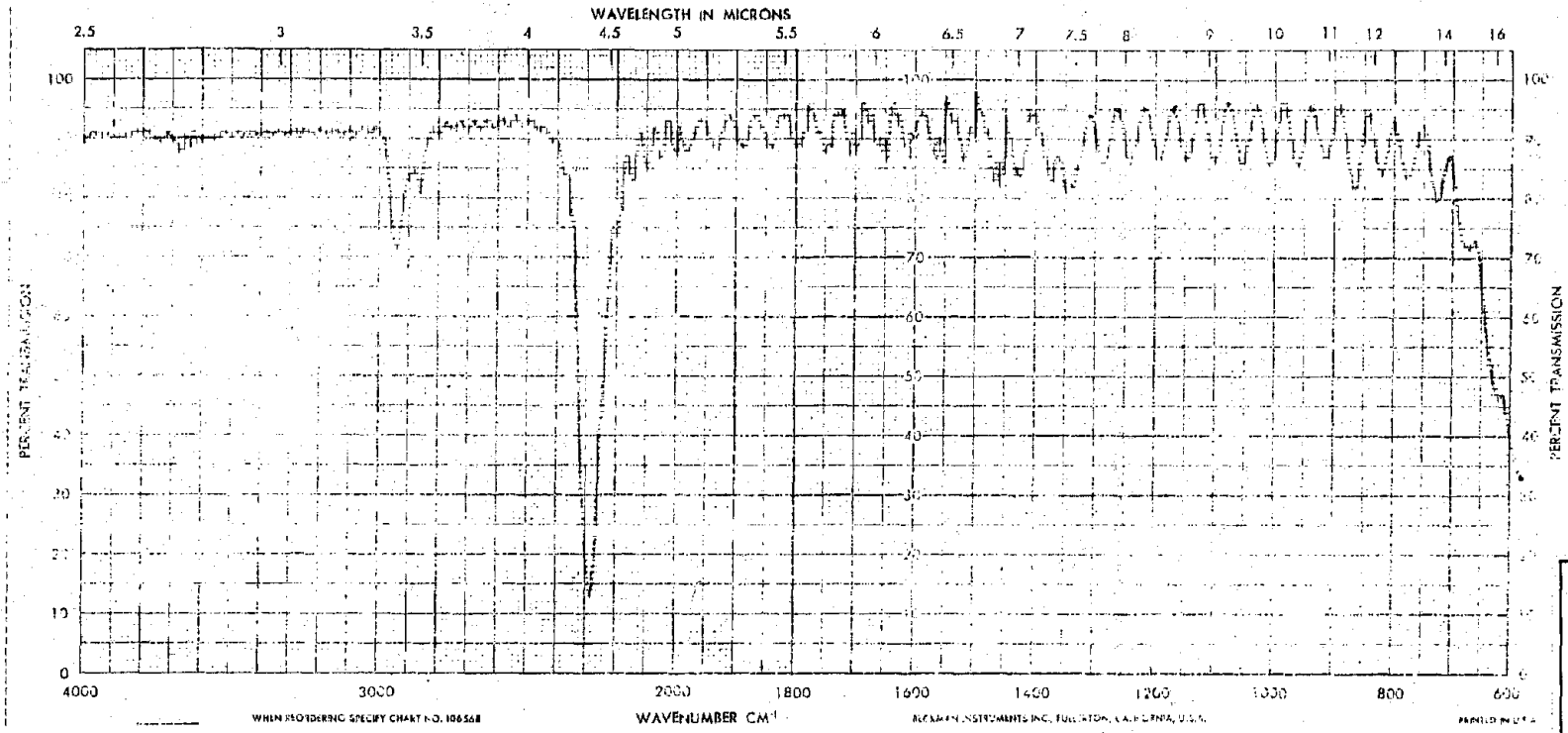

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DATE 7/17/75
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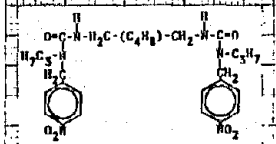


PATH mm
SOLVENT
CONCENTRATION
PHASE
COMMENTS KBr disc
ANALYST Ryan

Beckman®
INFRARED SPECTROPHOTOMETER

SPECTRUM NO. 10

DATE 3/3/76

SAMPLE p-TIU

SOURCE

STRUCTURE

PATH mm

SOLVENT

CONCENTRATION

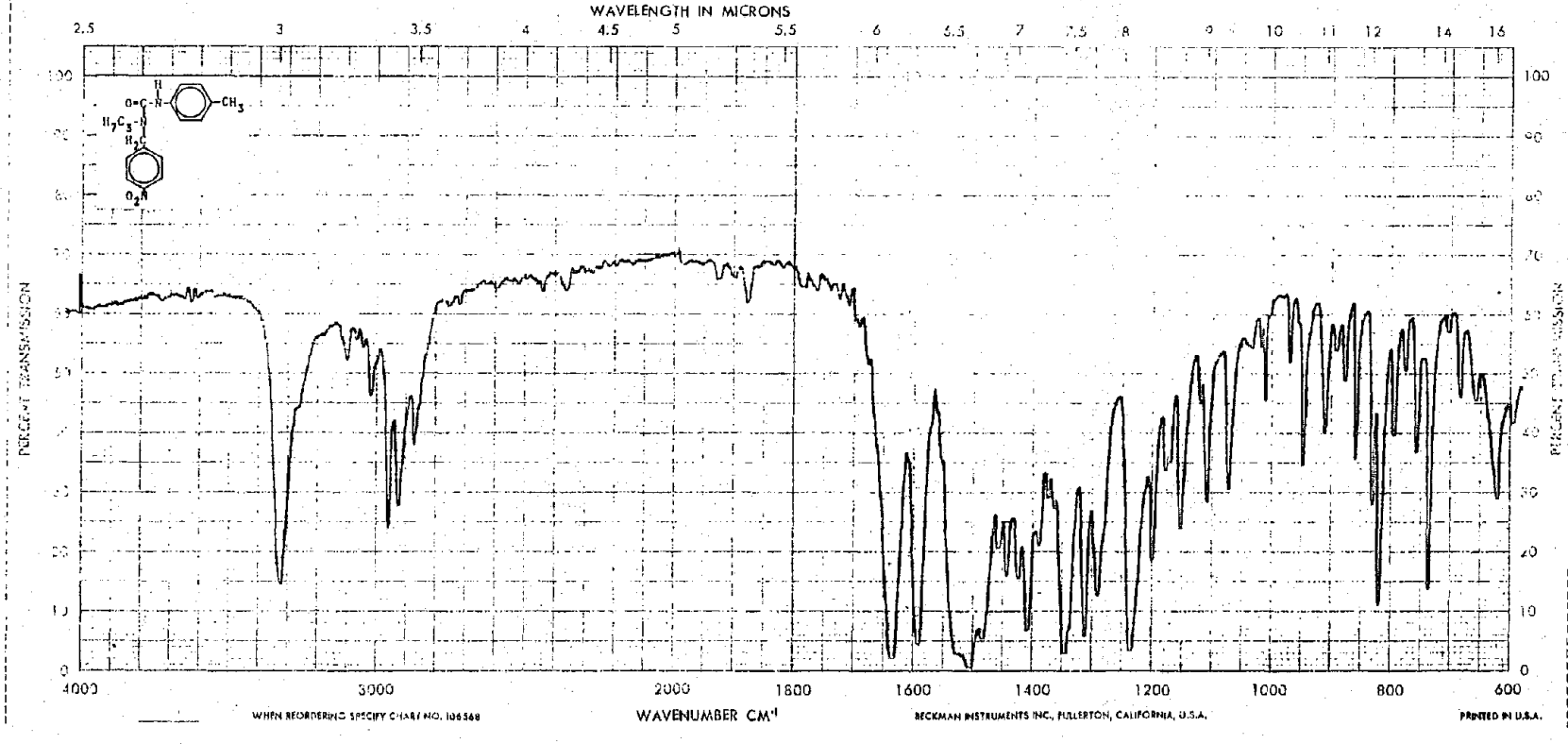
PHASE

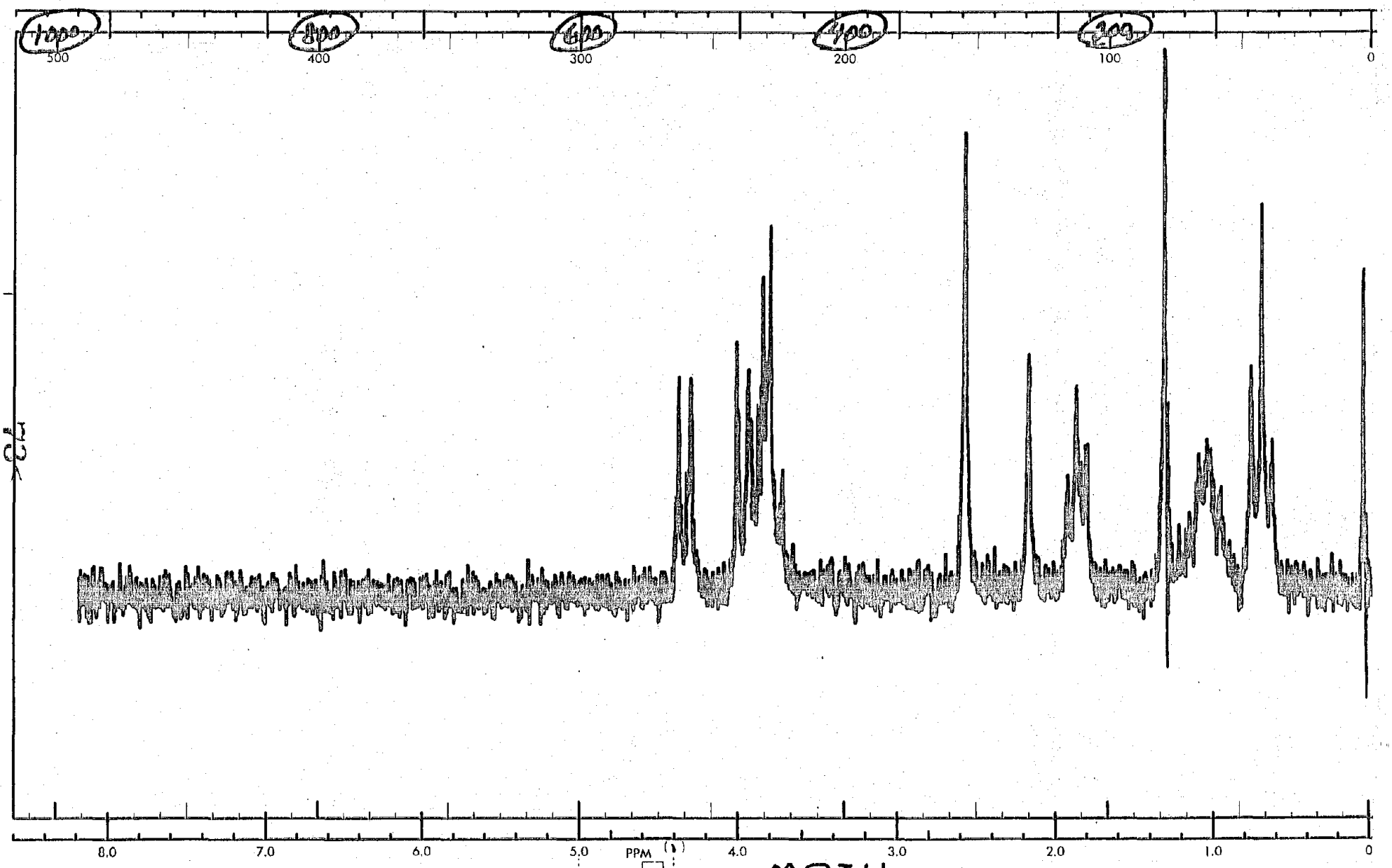
COMMENTS KBr disc

ANALYST Ko

Beckman

INFRARED SPECTROPHOTOMETER





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SPECTRUM AMPLITUDE: 8.0
INTEGRAL AMPLITUDE:
SPINNING RATE (RPS): 40

MANUAL
SWEEP TIME (SEC): 50 | 250 | (500)
SWEEP WIDTH (Hz): 25 | 50 | 100 | 250 | 500 | (1000)
FILTER: 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8
RF POWER LEVEL: .08

AUTO ☐
(250)
(500)
(2)
(.05)

SAMPLE: MDIU
SOLVENT: $CDCl_3$

REMARKS:

DATE: 3/4/76
OPERATOR: SS

60 MHz NMR SPECTRUM NO. 2143 A

GRAPHIC CONTROLS CORPORATION
BUFFALO, NEW YORK
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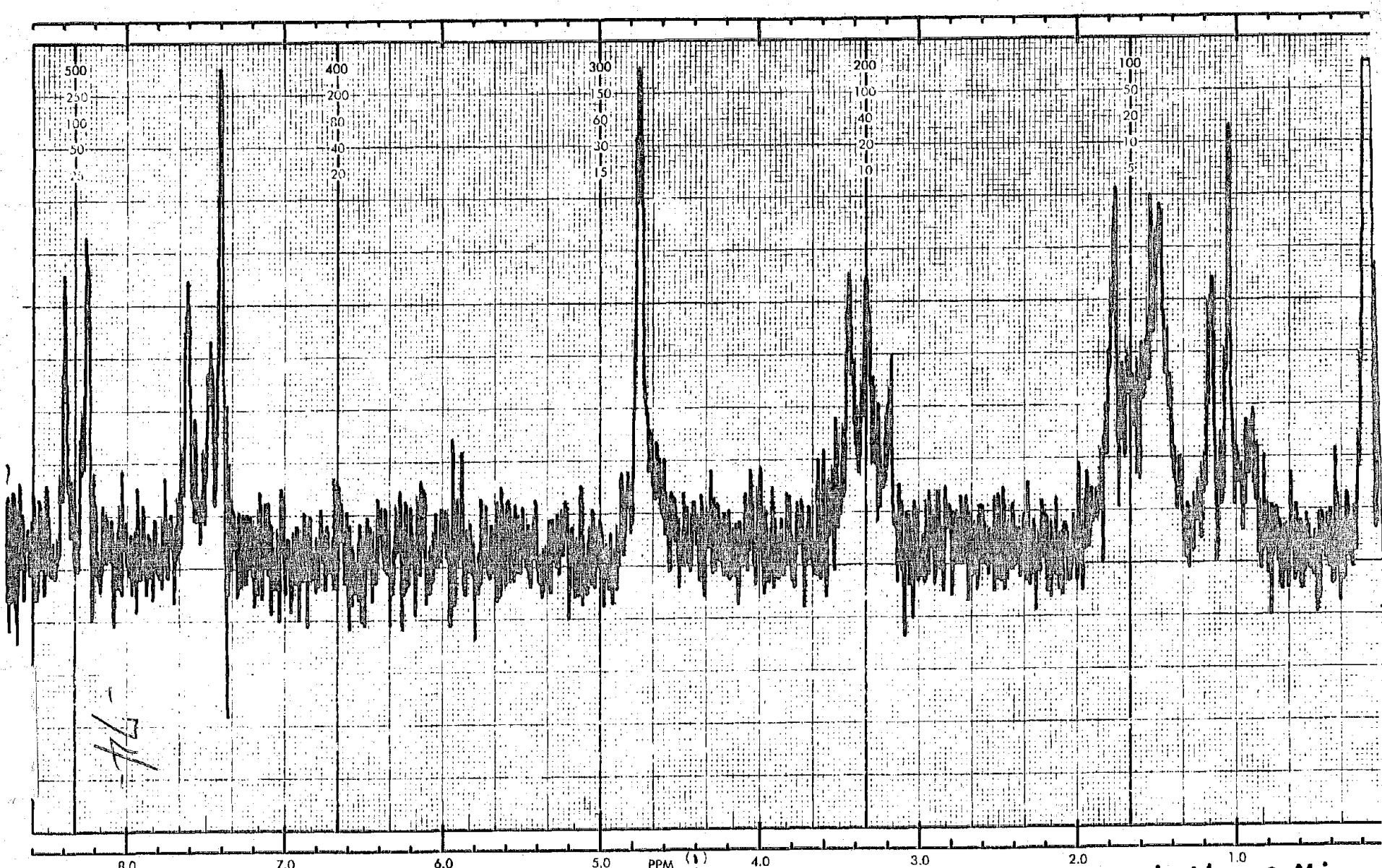
No. VN 1009 (S-60T)



SWEEP OFFSET (Hz): 0
SPECTRUM AMPLITUDE: 1.6 x100
INTEGRAL AMPLITUDE: —
SPINNING RATE (RPS): 40

MANUAL
SWEEP TIME (SEC): 50 250
SWEEP WIDTH (Hz): 25 50 100 250 500
FILTER: 1 2 3 4 5 6 7 8
RF POWER LEVEL: .5

AUTO ☐
(250)
(500)
(2)
(.05)

SAMPLE: # 4 1,6-HDIU Vogt

REMARKS: University of Missouri

SOLVENT: $CDCl_3$

DATE: 11/18/75

OPERATOR: SS

60 MHz NMR SPECTRUM NO. 1816

RECORDING CHARTS
GRAPHIC CONTROLS CORPORATION
BUFFALO, NEW YORK
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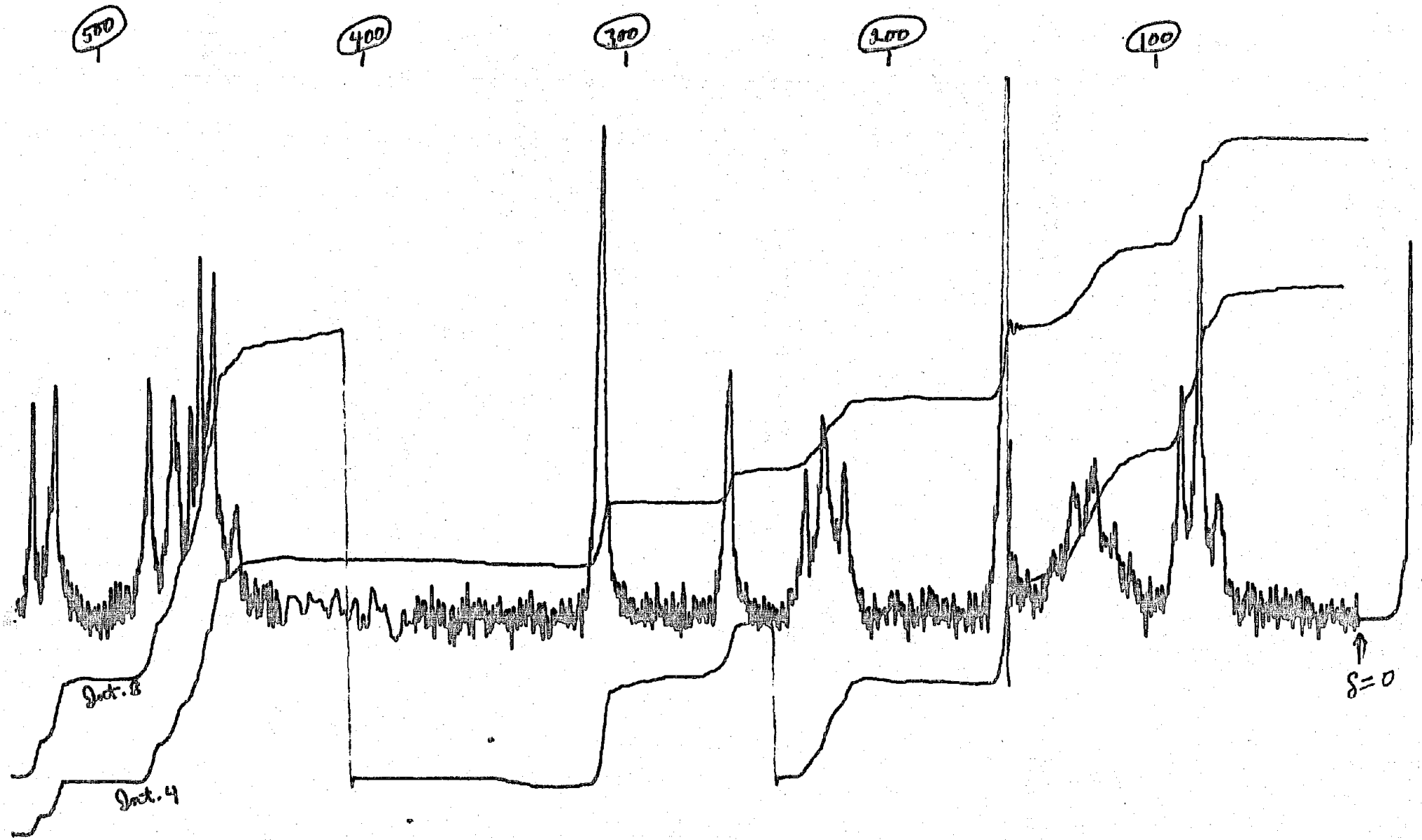
NO. VN 1009 (S-60T)



CV

0
80
40

350
500

.08

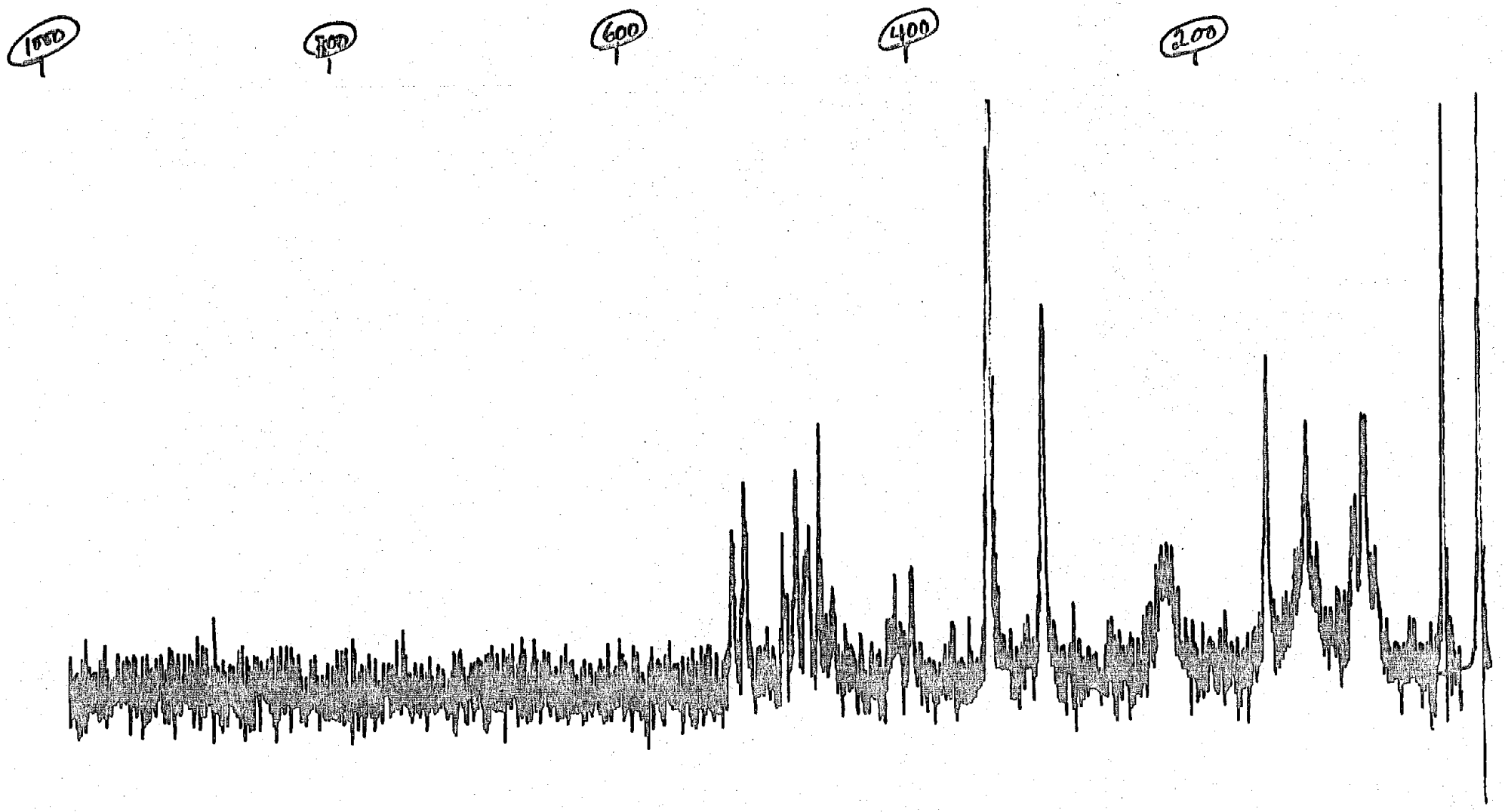
MDIU

$CDCl_3$

3/4/76

SS

2143B



76

0
125
40

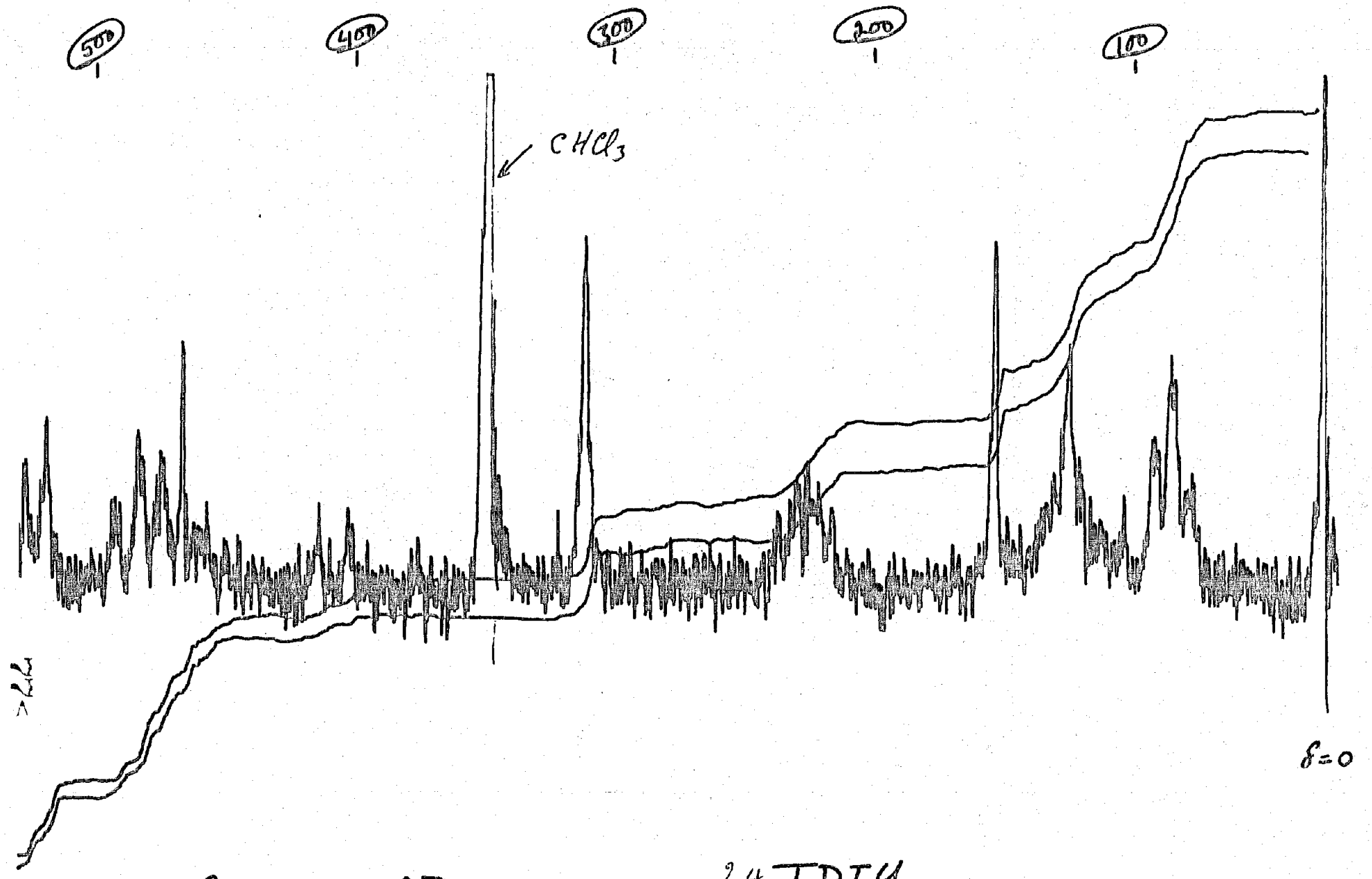
500
1000
.08
3/4/76

2,4-TDIU

$CDCl_3$

58

2144 A



2,4-TDIU

$CDCl_3$

SS

125.0

40

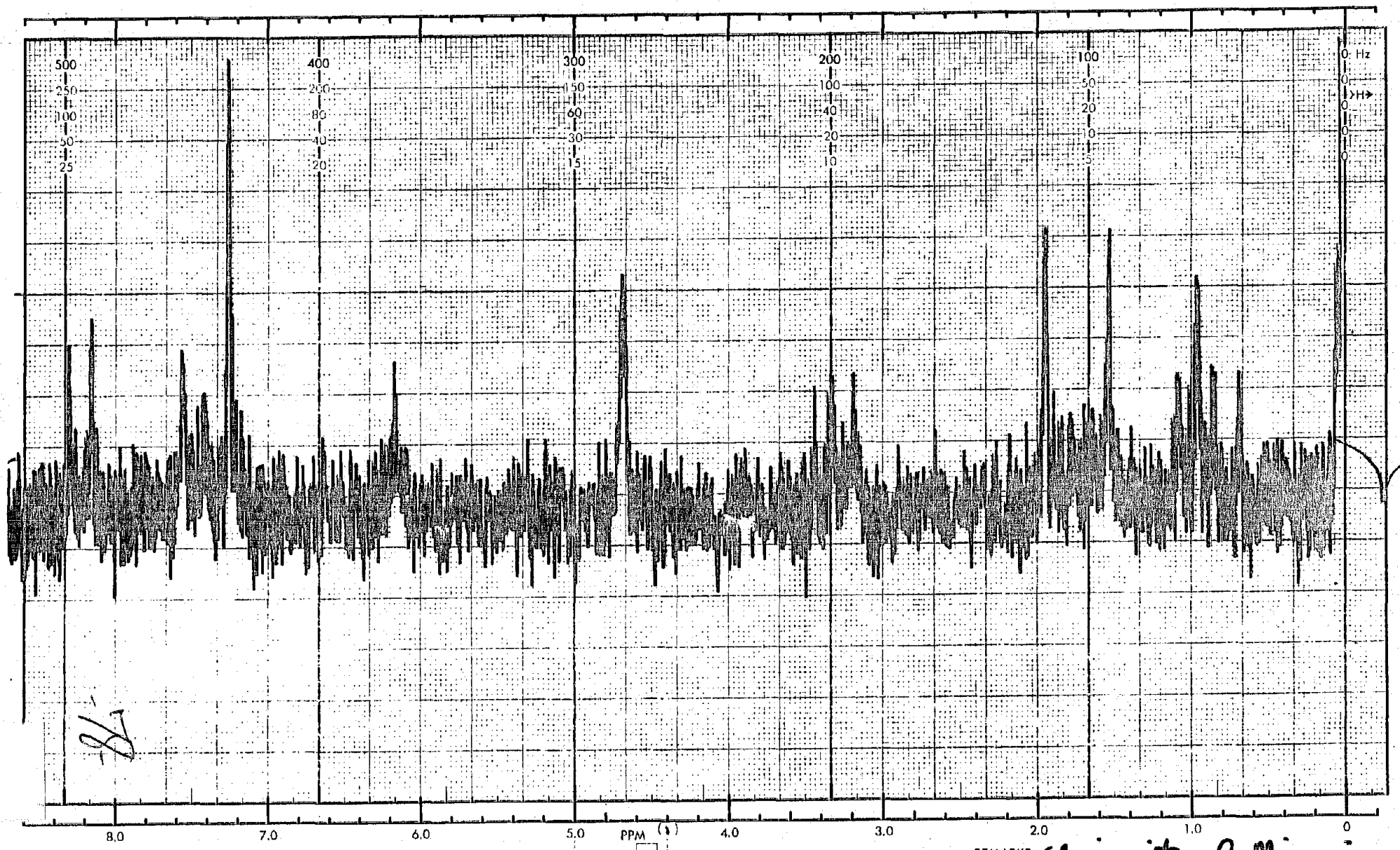
250

500

.08

3/4/76

21444 B



SWEEP OFFSET (Hz): 0
SPECTRUM AMPLITUDE: 1.6 X 100
INTEGRAL AMPLITUDE: —
SPINNING RATE (RPS): 40

MANUAL ○ AUTO ☐

SWEEP TIME (SEC): 50 250 ○ (250)
SWEEP WIDTH (Hz): 25 50 100 250 500 ○ (500)
FILTER: 1 2 ○ 4 5 6 7 8 (2)
RF POWER LEVEL: .5 (.05)

SAMPLE: #2 2,6-TDIU Vogt

SOLVENT: $CDCl_3$

REMARKS: University of Missouri

DATE: 11/18/75

OPERATOR: SS

60 MHz NMR SPECTRUM NO. 1814

RECORDING CHARTS
GRAPHIC CONTROLS CORPORATION
BUFFALO, NEW YORK
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No. VN 1009 (S-60T)

Isocyanates in Air

Measurements Research Branch
Analytical Method

Analyte: See Table I	Method No.: MR 240
Matrix: Air	Range: 0.004 mg/m³ - 0.6 mg/m³ (Analytical)
Procedure: Impingers; Formation of urea, LC	Precision:
Date Issued:	Classification: E (Proposed)
Date Revised:	

1. Principle of the Method

1.1 A known volume of air is drawn through two midget gas impingers (connected in series at 1 liter/min.) containing the nitro reagent absorber solution to collect the air sample.

1.2 The two solutions are combined and carefully rotary evaporated to dryness. The residue is then dissolved in 1.0 ml of CH_2Cl_2 containing an internal standard.

1.3 An aliquot of the solution is injected into a liquid chromatograph.

1.4 The area of the resulting peak is determined and compared with areas obtained from injection of standards.

2. Range and Sensitivity

2.1 The upper limit of the range of the method depends on the

concentration of the nitro reagent in the midget gas impingers. For a ten liter air sample, the limit of diisocyanates that can be absorbed is 1.5×10^{-6} moles/m^3 using a 15 ml of 2×10^{-4} M nitro reagent solution.

2.2 The minimum detectable limit is 2 ng, except for Desmodur N-100 which is 40 ng per injection. Maximum injection volume is 50 µl. Hence for a 10 liter sample, the useful range is 4 µg/m^3 - 600 µg/m of total diisocyanates. If a particular atmosphere is suspected of containing a large amount of contaminant, a smaller sampling volume should be taken. (Limit of detection for Desmodur N-100 is 80 µg/m^3).

3. Interferences

3.1 Any compound which reacts with nitro reagent and has the same retention time as the analyte is an interference. Retention time alone cannot be considered as proof of chemical identity.

3.2 When the possibility of interference exists, chromatographic conditions have to be changed (modes of gradient, concentration of mobile phases, packings, etc.) to circumvent the problem.

4. Precision and Accuracy

Precision and accuracy for the total analytical and sampling method have not been determined. However, the analytical method has been shown to have relative standard deviations within experimental error for peak areas and retention times, 2.8-16.5% and 0.6-4.1%, respectively, depending on the concentration of the analytes.

5. Advantages and Disadvantages of the Method

5.1 The sampling device is portable. Collection of samples is specific. Interferences are minimal. Simultaneous

analysis of 5 substances can be carried out routinely.

5.2 The nitro reagent has to be fairly freshly prepared. It is recommended not to store over 3 weeks and should keep the solution in darkness.

5.3 The ureas formed in solution are generally stable up to 7 days. Degradation or polymerization may occur after this period.

5.4 Excess nitro reagent should be removed before injecting into the LC to maintain longer column life and precision.

6. Apparatus

6.1 An approved and calibrated personnel sampling pump whose flow rate can be determined within ±5% at the recommended flow rate.

6.2 Two midget gas impingers connected in series and each containing 15 ml of 2×14^{-4} M nitro reagent solution.

6.3 A liquid chromatograph capable of gradient elution and equipped with a uv detector, 254 nm.

6.4 A commercially available 25 cm Partisil 10, 4.5 mm, i.d., stainless steel column.

6.5 A recorder or computing integrator for measuring peak areas.

6.6 Two milliliter sample containers with teflon lined caps.

6.7 Microliter syringes: 10-microliter, 50-microliter, and other convenient sizes.

6.8 Pipets: 1.0 ml type graduated in 0.01 ml increments, 15.0 ml type and other convenient sizes for making standard solutions.

6.9 Volumetric flasks: convenient sizes for making standard solutions.

7. Reagents

7.1 Isopropanol, certified grade or reagent grade

7.2 Methylene chloride, pesticide grade (Certified ACS)

7.3 Toluene, certified grade

7.4 2,4-Toluene Diisocyanate, 98% pure

7.5 2,6-Toluene Diisocyanate (not available in pure form commercially; found as mixture with 2,4-TDI).

7.6 4,4'-Methylenebis (phenyl isocyanate), <98% pure

7.7 1,6-Hexane Diisocyanate, 99% pure

7.8 p-Tolylisocyanate as excess nitro reagent scrubber

7.9 Desmodur N-100

7.10 Hydrochloride of N-4 nitrobenzyl-N-n-propylamine

The Preparation of Hydrochloride of N-4-nitrobenzyl-N-n-propylamine: Fifty g (0.29 moles) of 4-nitrobenzyl chloride (99% pure, Aldrich Chemical Co., Inc., Milwaukee, Wis. 53233) is dissolved in 240 ml of benzene. The solution is brought to boiling under reflux conditions. Then 36 g (0.61 moles) of n-propylamine (98% pure, Aldrich Chemical Co., Inc.) is added dropwise to the refluxing solution over a 15 minute period. It is refluxed for five hours. The solvent is stripped off in a rotary evaporator (Büchi Rotavapor-R, distributed by Fisher Scientific Co., Fairlawn, N.J. 07410) at 50°C. The residue is dissolved in 80 ml of double distilled water, and 30 ml of a 45% NaOH solution is slowly added. Then 100 ml of benzene is added and the mixture is stirred for five minutes. The benzene layer is separated. The benzene and the excess n-propylamine are stripped off in a rotary evaporator. The product (N-4-nitrobenzyl-N-n-propylamine) is dissolved in 50 ml of acetone and 34 g of concentrated HCl is added to form its salt. The mixture is evaporated to dryness at 50°C in a

rotary evaporator. The salt is washed with a 1:1 mixture of acetone:benzene followed by suction filtration. The washing step is repeated three times. The solid salt (about 25 g) is dried in a vacuum oven at 50°C. mp 230-232°C, ir (KBr) 1340, 1520cm^{-1} (C-NO_2). In addition to the 2 ir bands of the salt, the free amine (see below) shows a band at 3320cm^{-1} (N—H). From here on the N-4-nitrobenzyl-N-n-propylamine is referred to as "nitro reagent" or "N.R."

Preparation of Nitro Reagent Solution: A typical procedure for the routine preparation of the N.R. solution is as follows:

About 120 mg (5.2×10^{-4} moles) of the hydrochloride of nitro reagent is dissolved in 25 ml of distilled water. Thirteen ml of 1 N NaOH is added to precipitate the free amine. The free amine is extracted with 50 ml of toluene. The toluene layer is dried over anhydrous $CaSO_4$ (Drierite, W.A. Hammond Drierite Co., Xenia, Ohio) and the resulting solution is diluted to 250 ml to prepare the 2×10^{-3} M solution. The nitro reagent solution is stored in the refrigerator. The solution is not used after five days of storage.

8. Procedure

8.1 Cleaning of Equipment

All glassware used should be detergent washed and thoroughly washed with tap water and distilled water.

8.2 Collection and Shipping of Samples

The sample solution should be transferred to a 20 ml glass tube with a teflon cap. Use one ml of toluene to wash each impinger. Repeat twice. Combined with the sample solution. Keep cap tight and wrap with paper tapes. Ship out to place of analysis immediately.

8.3 Analysis of Samples

8.3.1 Preparation of Samples:

The sample is transferred to a round bottom flask. The sample tube is washed twice with 1 ml toluene and combined with the sample. The round bottom flask is attached to a rotary evaporator and the sample is evaporated to dryness at 50 degrees C. It is then dried over dry N_2 for two minutes. Redissolved into 1.0 ml of CH_2Cl_2 containing an internal standard. An aliquot is submitted to LC analysis.

8.3.2 LC Conditions:

The chromatographic conditions are:

1. Flow rate, 2.0 ml/min.
2. Gradient elution, 10% B/A to 100% B in 10 min.
   B = 9.1% isopropanol/CH_2Cl_2
   A = 100% CH_2Cl_2
3. Detector, uv at 254 nm
4. Room temperature
5. Recorder chart speed 0.5"/min.
6. Injection port with a loop

8.3.3 Injection:

The syringe must be cleaned thoroughly between injections. Dry. The syringer is then ready to take up sample for injection. A known amount of sample is injected into LC. Size of injections may vary from 1 µl up to 50 µl.

8.3.4 Measurement of peak area:

The peak area is measured by peak height x peak width at 1/2 height or by an electronic integrator such as a computing integrator. Preliminary results are read from a standard curve prepared as discussed below.

9. Calibration and Standards

A series of standards, varying in concentration over the range of interest are prepared. Calibration curves

are established prior to sample analysis each day.

When an internal standard is used, the analyte concentration is plotted against the area ratio of the analyte to that of the internal standard.

Typical Preparation of Stock Standard Solutions

The following weights of the isocyanates are dissolved in 4.0 ml portions of CH_2Cl_2: 2.12 mg MDI; 29.60 mg of TDI (i.e., 19.30 mg of 2,4-TDI and 10.30 mg of 2,6-TDI), 21.14 mg of HDI and 22.78 mg of Desmodur N-100.

Then 755 l of MDI, 83.1 l of TDI, 75.5 l of HDI, and 70.1 l of Desmodur N-100 are mixed and 1.017 ml CH_2Cl_2 is added to make a total volume of 2.00 ml (200 ng/ l of each). Then 1.0 ml nitro reagent (2.06 mg/ml or $8.9 \times 10^{-}$ M in hexane) is added to 1.0 ml of the isocyanate mixture. The total NCO/N.R. mole ratio in this solution is 1:1. The reaction mixture is stored overnight. Dilutions are made from this solution. The solvent is evaporated in a rotary evaporator and the residue redissolved in 1 ml CH_2Cl_2. These solutions are used to establish the calibration curves, linear dynamic range and minimum detectable amount in the 25 cm Partisil 10 column.

10. Calculations

10.1 Read the weight corresponding to each peak area from the standard calibration curve.

10.2 The concentration of the analyte in the air sampled can be expressed in µg per cu m.

$$\mu g/m^3 = \frac{\text{Amount of analyte } (\mu g) \times 1000\ \mu l \times 1000\ (\text{liter}/m^3)}{\text{Air volume sampled (liter)} \times \text{volume of injection } (\mu l)}$$

10.3 Another method of expressing concentration is ppb (corrected to standard conditions of 25 degrees C and 760 mm Hg).

$$\text{ppb} = \frac{\mu g}{m^3} \left(\frac{24.45}{MW}\right) \left(\frac{760}{P}\right) \left(\frac{(T + 273)}{298}\right)$$

where: P = pressure (mm Hg) of air sampled
T = temperature (°C) of air sampled
24.45 = molar volume (liter/mole)
MW = molecular weight
760 = standard pressure (mm Hg)
298 = standard temperature (°K)

11. References

11.1 Final Report, NIOSH Contract 210-750052, April, 1976.

11.2 J. Keller, K. L. Dunlap and R. L. Sandridge, Anal. Chem., 46, (1974) 1845-6.

Table I

Analyte	Lower Detection Limit
2,4-Toluene diisocyanate (2,4-TDI)	2 ng
2,6-Toluene diisocyanate (2,6-TDI)	2 ng
4,4'-Methylenebis(phenylisocyanate) (MDI)	2 ng
1,6-Hexane diisocyanate (HDI)	5 ng
Desmodur N-100	40 ng