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DEVELOPMENT OF METHODS FOR THE DETECTION OF TRACE AMOUNTS OF SELECTED CARCINOGENIC AND MUTAGENIC AMINES IN WATER

by

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ABSTRACT

A great deal of concern exists over the presence of potentially carcinogenic and/or mutagenic substances in drinking water supplies. The general analytical schemes currently applied to water are less suited than specific methods for the detection of certain classes of organic contaminants such as aromatic amines because of their reactivity. We have concentrated our efforts at developing analytical schemes by which we are able to reliably detect, separate, and quantitate trace levels of a number of aromatic and heterocyclic amines. Both liquid and gas chromatographic methods have been developed. The relative strengths and limitations of the methods are discussed. Field evaluations of the final methods were carried out and reported.

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KEYWORDS: aromatic amines/ carcinogens/ estuaries/ gas chromatography/ liquid chromatography/ mutagens/ water pollution/ water quality

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INTRODUCTION

Aromatic amines have widespread use in both industry and agriculture and may occur as contaminants in groundwater and surface water as well as in biological tissues. These compounds exert physiological effects including toxicity, mutagenicity, and carcinogenicity. Several aromatic amines are listed by the Occupational Safety and Health Administration as carcinogens (*Fed. Reg.* 1973; *Fed. Reg.* 1974). Although aromatic amines present a potential environmental problem, relatively little data assessing the presence of the compounds in the environment have been reported. Most of the available literature deals with the separation and detection of physiological amines.

The concern for the presence of amines in natural waters seems to be worldwide. The presence of aromatic amines has been confirmed in fish taken from the Buffalo, Delaware, and other rivers, in the vicinity of textile and dyestuff manufacturers. Diachenko (1979) detected in fish the presence of 1-naphthylamine, N-ethyl-N-phenylbenzylamine, and N-ethyl-N-(*m*-tolyl)-benzylamine by using gas chromatography, after digesting the amines with NaOH, partitioning with benzene, and cleaning up with gel permeation chromatography.

A study conducted in the USSR (Vlasova 1974) has reported the screening of some lakes for amines and other organic compounds. Many such compounds have been found to exhibit toxic, mutagenic, and carcinogenic effects in experimental animals. Weisburger et al. (1978) tested 21 environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity in male rats and male and female mice. They found that *o*-toluidine, toluenediamine, and trimethylanilines led to tumors in all three of the animal models. Other amines studied had varying degrees of activity in one animal model or the other. Most recently, aromatic amines have been identified by gas chromatography/mass spectrometry (GC/MS) in dye-manufacturing waste leachates. The amines were linked with fish tumors (Nelson and Hites 1980).

Aromatic amines have been detected or determined by using various analytical techniques: titration with $NaNO_2$ (Zelenina and Krasnov 1974), microdiffusion by bromocresol purple reaction (Semenov et al. 1974),

and measurement of the melting points of their reaction products with N-chloroacetanilide (Legradi 1975). Legradi (1975) identified the products by nuclear magnetic resonance spectroscopy. More recently gas chromatography and liquid chromatography have been applied to the analysis of aromatic amines. A review and discussion of analytical techniques for the detection of aromatic amines has been presented by Yllo (1977).

DEVELOPMENT OF LIQUID CHROMATOGRAPHIC METHODS

REVIEW OF LITERATURE

Many of the high-performance liquid chromatographic (HPLC) separations of aromatic amines which have been reported were accomplished using silica gel modified with metal ions. Chow and Grushka (1977, 1978) obtained better separations of several sets of aromatic amine isomers using copper(II)-alkylamine-bonded silica gel, copper(II)-alkyldithiocarbamate-bonded silica gel, and copper(II)-alkyldiketone-bonded silica gel than using silica gel. Increased resolution of the three chloroaniline isomers was achieved by Vogt et al. (1977) using silica gel hydrothermally treated with $CdCl_2$ as compared with silica gel. Similar results were also reported for 1-naphthylamine and 2-naphthylamine (Vogt et al. 1977). Kunzru and Frei (1974) report baseline separation of the three chloroaniline isomers and near-baseline separation of the three toluidine isomers using cadmium-impregnated silica gel which had been pretreated with NaOH. Silver has also been used to modify silica gel to enhance separations of aromatic amines. By hydrothermally treating silica gel with $AgNO_3$, Vogt et al. (1978) have achieved near-baseline resolution of the three isomers of chloroaniline. Aigner et al. (1976) and Vivilecchia et al. (1972) have reported separations of aza-heterocyclics using silver-modified silica gel. Silica gel modified with metal ions has also been used in the separations of various unsaturated acetates, aldehydes, and hydrocarbons (Heath et al. 1975; Mikeš et al. 1973).

Various other methods of separating aromatic amines have been reported. Englehardt et al. (1974) separated N,N-diethylaniline, N,N-dimethylaniline, and N-methylaniline using silica gel coated with H_2O (0.6g H_2O/g silica gel). Gel permeation chromatography has been used by Protivová and Pospíšil (1974) to separate amine antioxidants and antiozonants, including

aniline, 2-naphthylamine, benzidine, and *o*-tolidine. Reverse-phase separations using mixtures of CH_3CN and 0.15M phosphate buffer at pH 2.1 were reported by Lores et al. (1978). Aniline, 2-amino-5-chlorophenol, *p*-chloroaniline, *p*-bromoaniline, *m*-chloroaniline, *o*-chloroaniline, and 3,4-dichloroaniline were separated using an 8-min gradient from 80% 0.15M phosphate buffer/20% CH_3CN to 40% 0.15M phosphate buffer/60% CH_3CN. A variety of HPLC conditions with capacity factors is given for approximately 50 aromatic amines by Young and McNair (1976).

The use of three common methods of detection—ultraviolet absorbance, fluorescence, and electrochemical potential—with aromatic amines has been reported. Clark et al. (1977) and Clark and Wells (1978) have reported enhancement of the UV absorbance of amines by forming the corresponding 4-methoxybenzamides or 4-nitrobenzamides. Seiler (1977) has written a review of the chromatography of biogenic amines which includes a good section on the use of fluorescent derivatives of amines. Included in this review are the following agents used to form fluorescent derivatives of amines: 4-chloro-7-nitrobenzo-[c]-1,2,5-ozydiazole, sulphonyl chlorides, isothiocyanates, fluorescamine, pyridoxal, pyridoxal-5-phosphate, ω-formyl-*o*-hydroxyacetophenone, and benzo-γ-pyrene. Samejima (1974) and Samejima et al. (1976) report the synthesis and separation of fluorescamine derivatives of diamines and polyamines. *o*-Phthalaldehyde was found to form more fluorescent derivatives of primary amines than either ninhydrin or fluorescamine by Benson and Hare (1975). The most sensitive method of detection of aromatic amines is electrochemical detection. Using electrochemical detection, subnanogram and picomole quantities of aromatic amines have been detected (Lores et al. 1978; Mefford et al. 1977). Using solvent extraction of aqueous solutions, detection limits can be lowered. Riggin and Howard (1979) have compared the detection limits of benzidine, 3,3'-dichlorobenzidine, and 1,2-diphenylhydrazine using direct injection, solvent extraction, and resin adsorption. Less than 1 μg/l could be detected by direct injection. These limits were improved to 50 ng/l by solvent extraction and 100 ng/l by resin adsorption.

EXPERIMENTAL MATERIALS

Equipment

1. Dual Waters 6000A Solvent Delivery System with Model 660 Gradient Programmer
2. ISCO Model UA-5 Fixed Wavelength Ultraviolet Detector
3. Schoeffel Model 970 Variable Wavelength Fluorescence Detector
4. Waters Model 440 Fixed Wavelength Ultraviolet Detector
5. ISCO Model 1440 Isocratic Liquid Chromatograph

Reagents and Supplies

1. Amine standards were prepared from reagent grade (99+% pure) materials obtained from Aldrich Chemical Company.
2. All solvents used were of distilled-in-glass quality (Burdick and Jackson Laboratories, Inc., Muskegon, MI). All other chemicals were Baker analyzed reagent grade.
3. The following chromatographic columns were used in these studies:
   a. Waters μBondapak C_{18}
   b. Waters μBondapak CN
   c. Waters μBondapak Phenyl
   d. Waters Corasil I impregnated with cadmium
   e. Waters Poragel PS
   f. Dupont ODS
   g. Waters Porasil A
4. Amberlite XAD-4 and XAD-8 resins were purchased from Rohm and Haas.

EXPERIMENTAL PROCEDURES

High-performance liquid chromatographic (HPLC) analyses were run on an ISCO model 1440 liquid chromatograph and a Waters Associates gradient elution pumping system including two model 6000A chromatography pumps and a model 660 solvent programmer. The amines were detected using both a fixed wavelength ISCO model UA-5 ultraviolet-visible detector and a Schoeffel 970 variable wavelength fluorescence detector. Separations were attempted using each of the following columns: Poragel PS (Waters Associates), a vinyl pyridine, styrene, vinyl benzene terpolymeric adsorption material; ODS (Dupont), a reverse-phase partition material; Corasil I (Waters Associates), a pellicular silica adsorption material; Corasil I impregnated with cadmium(II); Porasil A (Waters Associates), a porous silica adsorption material, impregnated with cadmium; μBondapak C_{18} (Waters Associates), a reverse-phase partition material;

and μBondapak CN (Waters Associates), a reverse-phase cyano-derivatized adsorption/partition material.

Normal-Phase Separations (Adsorption)

Standard amines were dissolved singly and in mixtures in methanol and diluted to known concentrations in the ppm (μg/ml) range. Twenty-microliter aliquots of these solutions were injected at a time onto the columns which were being evaluated and eluted with the appropriate solvent system. The areas under the peaks observed were measured by an electronic integrator or by planimetry. Peak height measurements were also used in some cases. Retention indices (k') and linear response curves were prepared. Flow rates of 0.5 to 2.0 ml per minute were used.

Reverse-Phase Separation (Absorption)

The same series of standard amines and mixtures of amines were used to evaluate the utility of four reverse-phase columns. Twenty-microliter samples of each solution were injected onto the columns and eluted using solvent gradients of 0-100% methanol in water or 0-100% acetonitrile in water. Flow rates of 0.5 to 2.0 ml per minute were used. Retention indices (k') values and linear response curves were prepared as above.

SAMPLE EXTRACTION/CONCENTRATION STUDIES

One-liter samples of pure water or natural water were spiked with standard amines at the 1-100 ppb level. Samples were prepared in triplicate and treated in one of two ways.

One set of samples was adjusted to pH 12 with 50% NaOH and extracted with methylene chloride. Three extractions were performed using a total of 200 ml (100 + 50 + 50) of solvent. The combined extracts were dried over anhydrous sodium sulfate and evaporated in a Kuderna-Danish evaporator to a final known volume of one milliliter.

A second set of samples was concentrated using XAD-4 or XAD-8 resin. The liter of water, adjusted to pH 10, passed through a 20g bed of pre-extracted XAD-4 resin at a rate of 3-5 ml per minute. The resin was precleaned by soaking in 5% ammonium carbonate and sequential reflux extraction with distilled water, acetone, methanol, methylene chloride, methanol, and water.

The bed was washed with 50 ml of deionized water to remove excess base, and then the resin was extracted with 100 ml 10% methanol in methylene chloride. The solvent was dried over anhydrous sodium sulfate and evaporated in a Kuderna-Danish evaporator to a known volume of one milliliter.

FIELD EVALUATION OF METHODS

A series of fourteen field samples was collected in fresh and estuarine regions, concentrated using the XAD-4 resin, and analyzed using reverse-phase gradient separation on a μBondapak C_{18} column. Quantitation was achieved using ultraviolet absorbance at 254 nm and peak area integration with an internal standard (diphenyl amine). Two of the samples were analyzed in duplicate, and two were analyzed with a known spike of 10 ppb of several amines. The concentrations of identified amines as well as recoveries of the spiked compounds were calculated.

RESULTS

Evaluation of Normal-Phase Column Separations

The cadmium-impregnated Corasil I column was prepared using a modification of the procedure reported by Kunzru and Frei (1974). Corasil I was baked overnight at ∿120°C. After cooling in a vacuum desiccator, the Corasil I was stirred with a 15% solution of NaOH in CH_3OH for one hour. This suspension was vacuum filtered and washed three times with CH_3OH. The Corasil I was then then stirred with a 50% solution of CdI_2 in CH_3OH for 90 minutes. This slurry was vacuum filtered and dried at ∿120°C. The cadmium-impregnated Corasil I was packed in a stainless steel column 57.6 cm in length by 2.5 mm ID using the "tap-fill" method (Snyder and Kirkland 1976, p. 189). Prior to packing, the empty column was cleaned by the method described by Karger et al. (1970).

The cadmium content of the Corasil I was analyzed using a method similar to that described by Kunzru and Frei (1974). A known amount (∿0.2 g) of the cadmium-impregnated Corasil I was stirred with 10 ml of ∿5N HNO_3 for two hours. The suspension was centrifuged, and one ml of the supernatant was diluted to 50 ml with H_2O. The resulting solution, analyzed with atomic absorption spectrophotometry, contained 0.7 mg/l cadmium. This indicates that the cadmium-impregnated Corasil I is 0.2% cadmium. This value is much lower than that reported by Kunzru and Frei (1974). An attempt was made to increase the

cadmium concentration by treating the Corasil I with 50% CdI_2 in CH_3OH for 150 minutes instead of 90 minutes. This procedure failed to increase the cadmium concentration. Treatment with a saturated solution of CdI_2 in CH_3OH also failed. This failure can be explained by the structural differences of the silica packing materials used. Corasil I is a thin layer of silica gel fused to a glass bead, whereas LiChrosorb SI 60, used by Kunzru and Frei (1974), is a completely porous silica gel packing material. LiChrosorb SI 60, being porous, has a greater capacity for impregnation by cadmium than Corasil I.

Separation of amine mixtures on the cadmium-impregnated Corasil I column was initially attempted using 2% CH_3OH in hexane as the solvent system, as reported by Kunzru and Frei (1974). With this solvent system, there was no resolution of N-methylaniline, aniline, and 2,4,6-trichloroaniline. This is explained by the lower cadmium content of the cadmium-impregnated Corasil I column as compared to the cadmium-impregnated LiChrosorb SI 60 column used by Kunzru and Frei. Using the cadmium-impregnated Corasil I column, the best separations were achieved using mixtures of ethyl acetate and hexane. With a 100-to-1 ratio of hexane to ethyl acetate, near-baseline separation of 1-naphthylamine, 2,4-dichloroaniline, and N-methylaniline was achieved (Fig. 1). However, using the same solvent, a mixture of N-methylaniline, 2,4-dichloroaniline, 1-naphthylamine, *p*-chloroaniline, and 2-naphthylamine could not be separated. Complete resolution of the above five amines was also not achieved using a 400-to-1 ratio of hexane to ethyl acetate.

On the assumption that the failure of this column to resolve the mixture is due to the structure as described above, a new column was prepared using Porasil A, a completely porous silica adsorption material. This column was prepared using the method described above with the exception that a 25%-CdI_2 solution was substituted for the 50%-CdI_2 solution. Separation of N-methylaniline, 2,4-dichloroaniline, 1-naphthylamine, 2-naphthylamine, and *p*-chloroaniline was attempted. Using hexane, the amines were not separated. Using either ethyl acetate or a one-to-one ratio of ethyl acetate to hexane, N-methylaniline, 2,4-dichloroaniline, and 1-naphthylamine were incompletely separated, and *p*-chloroaniline and 2-naphthylamine were excessively retained.

The Poragel PS column gave the best separations using mixtures of CH_3OH and H_2O. With a CH_3OH/H_2O (10/1) mixture, baseline separation of

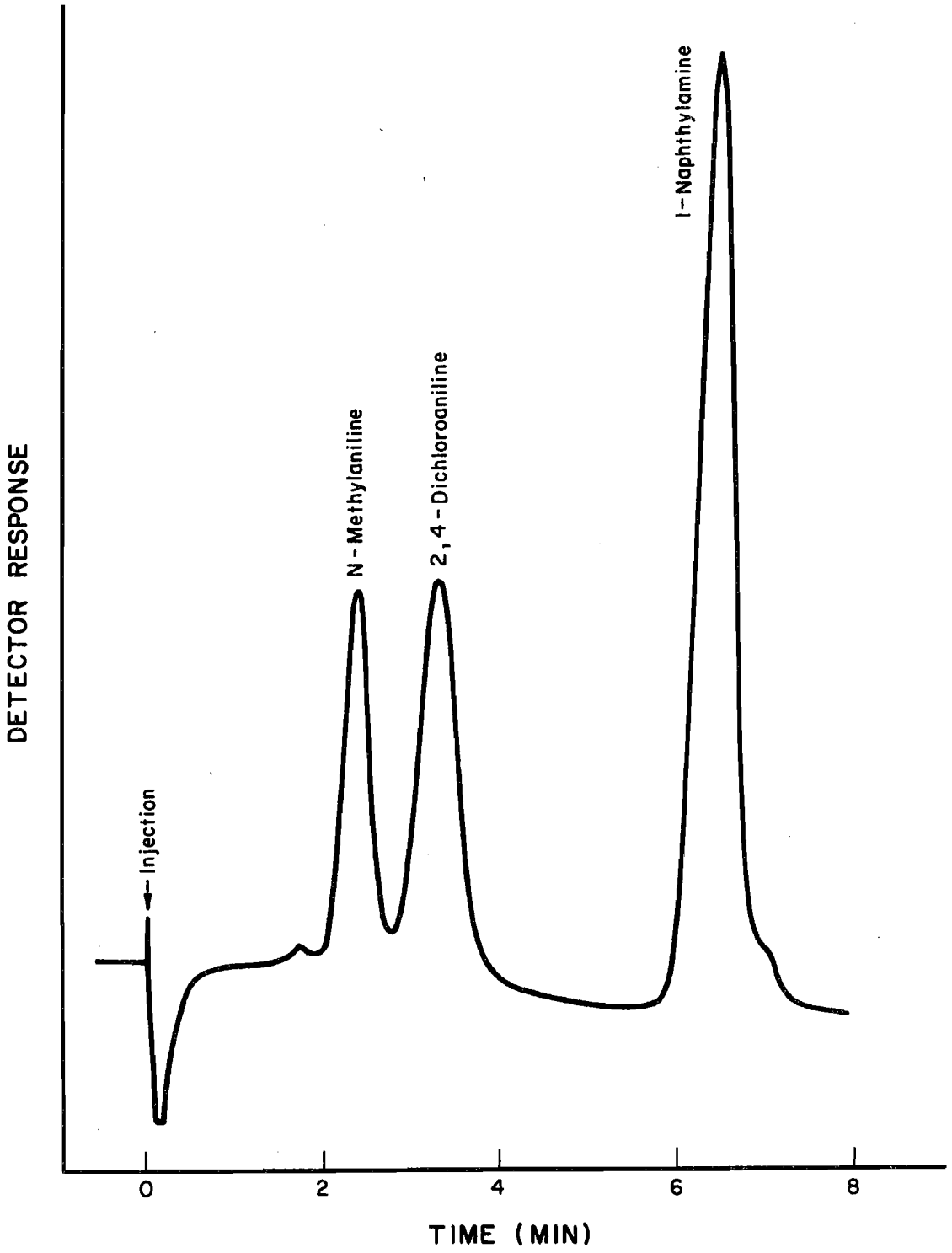

Figure 1. Separation of 1-naphthylamine, 2,4-dichloroaniline, and N-methylaniline.

Column: Corasil I/Cd. Solvent: ethyl acetate/hexane (1/100). Flow rate: 1 ml/min. UV absorbance range: 0.05 AUFS (absorbance units full scale).

aniline and N-methylaniline was accomplished, but 2,4,6-trichloroaniline was excessively retained. When the ratio of CH_3OH to H_2O was changed to 20 to 1, the peaks from aniline and N-methylaniline became merged, but 2,4,6-trichloroaniline was no longer excessively retained. These results suggested the use of a gradient system to resolve the amines; however, the peaks were too broad to be resolved by this method.

Evaluation of Reverse-Phase Columns

The Dupont ODS column gave the best separations using CH_3OH and H_2O mixtures. All of the isocratic systems attempted failed to resolve a mixture of N,N-dimethylaniline, 1-naphthylamine, and 2,4-dichloroaniline. The isocratic systems used were CH_3CN, CH_3OH, a 1-to-1 ratio of CH_3CN to H_2O, and mixtures of CH_3OH and H_2O in the following ratios: 10 to 1, 1 to 1, and 1 to 3. However, near-baseline separation of 1-naphthylamine, 2,4-dichloroaniline, and N-methylaniline was achieved using a 10-min convex gradient from 100% H_2O to 85% H_2O/15% CH_3OH.

The best overall separations were obtained using the μBondapak C_{18} column. These separations were achieved using mixtures of CH_3OH and H_2O as well as mixtures of CH_3OH and an ∿0.08M aqueous solution of triethylammonium phosphate. The ∿0.08M triethylammonium phosphate was prepared by diluting 11.3 ml of concentrated phosphoric acid to 2 liters with H_2O and increasing the pH to ∿7 with triethylamine. Using a 10-min concave gradient from 50% H_2O/50% CH_3OH to 40% H_2O/60% CH_3OH, baseline separation of Bladex, atrazine, and Simazine was achieved (Fig. 2 and Table 1). Baseline separation of *p*-chloroaniline, 2,4-dichloroaniline, and 2,4,6-trichloroaniline was achieved using 75% CH_3OH/25% 0.08M triethylammonium phosphate as the solvent (Fig. 3). Using a 20-min concave gradient from 40% CH_3OH/60% 0.08M triethylammonium phosphate to 70% CH_3OH/30% 0.08M triethylammonium phosphate, benzidine, *o*-tolidine, N-methylaniline, *p*-chloroaniline, 1-naphthylamine, 2-naphthylamine, *o*-toluidine, N,N-dimethylaniline, 2,4-dichloroaniline, and 2,4,6-trichloroaniline were partially resolved (Fig. 4 and Table 2). Baseline separation of benzidine, *o*-tolidine, N-methylaniline, N,N-dimethylaniline, 2,4-dichloroaniline, and 2,4,6-trichloroaniline was achieved. N-methylaniline and *p*-chloroaniline were partially merged, and 1-naphthylamine, 2-naphthylamine, and *o*-toluidine were co-eluted.

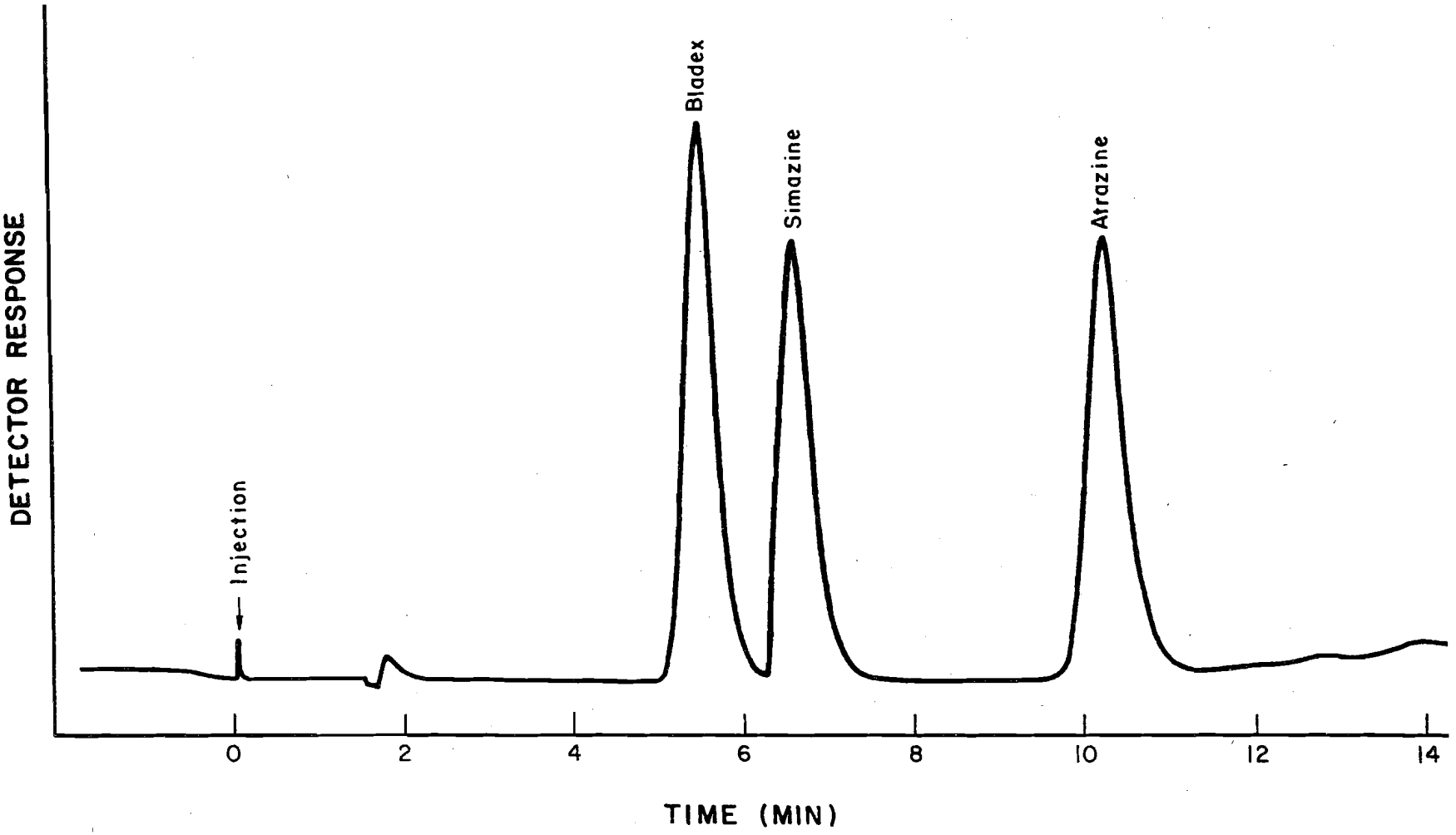

Figure 2. Separation of atrazine, Simazine, and Bladex.

Column: μBondapak C_{18}. Solvent: 10-min concave gradient from 50% H_2O/50% CH_3OH to 40% H_2O/60% CH_3OH. Flow rate: 2.0 ml/min. UV absorbance range: 0.5 AUFS (absorbance units full scale).

TABLE 1

Retention Indices of Bladex, Atrazine, and Simazine on a μBondapak C_{18} Column*

	k'
Bladex	2.52
Simazine	3.32
Atrazine	5.72

*Using a 10-minute concave gradient from 50% H_2O/50% CH_3OH to 40% H_2O/60% CH_3OH

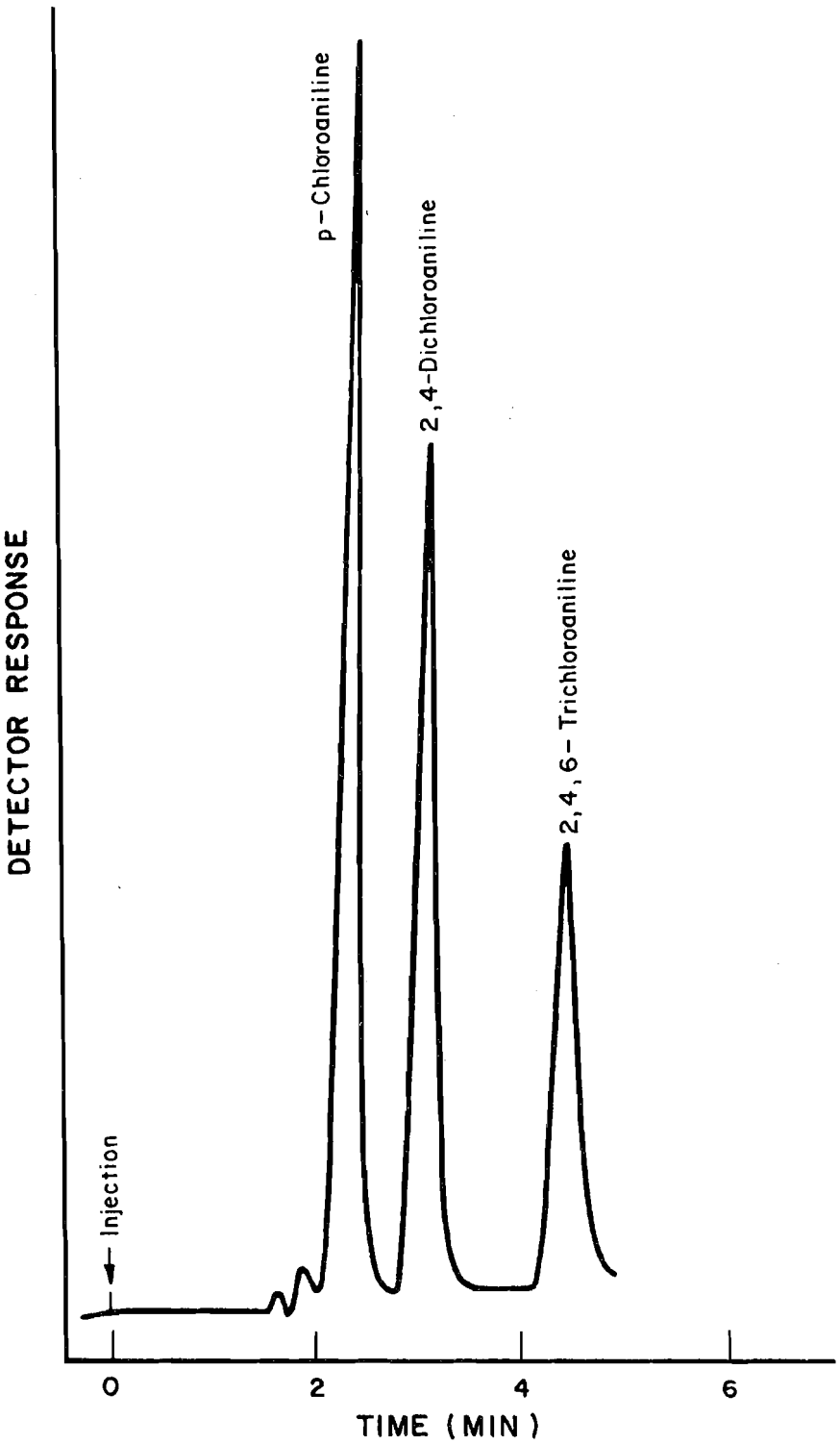

Figure 3. Separation of *p*-chloroaniline, 2,4-dichloroaniline, and 2,4,6-trichloroaniline.

Column: μBondapak C_{18}. Solvent: 75% CH_3OH/25% 0.08M triethylammonium phosphate. Flow rate: 2.0 ml/min. UV absorbance range: 0.5 AUFS (absorbance units full scale).

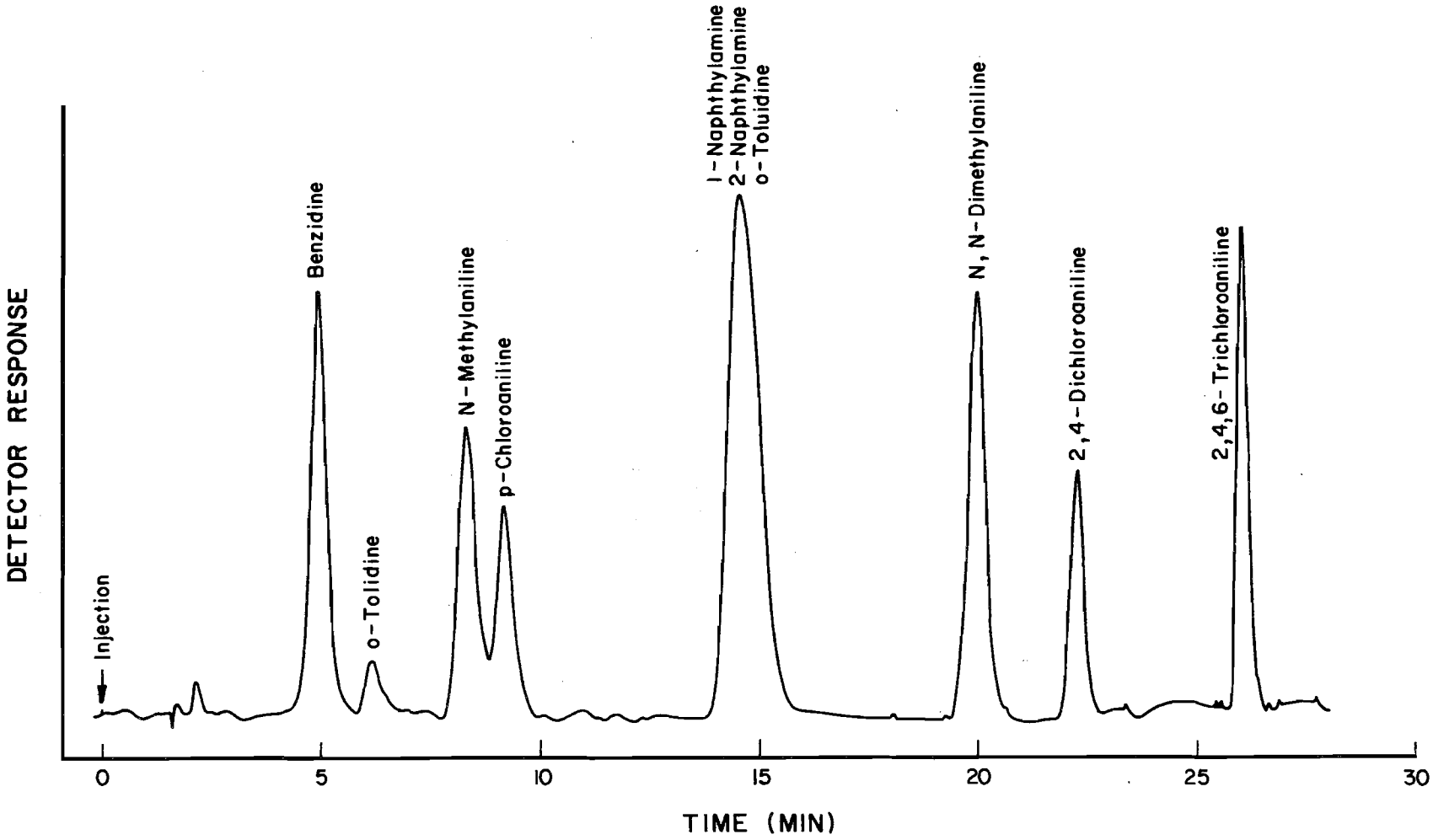

Figure 4. Separation of selected amines.

Column: μBondapak C_{18}. Solvent: 20-min concave gradient from 40% CH_3OH/60% 0.08M triethylammonium phosphate to 70% CH_3OH/30% 0.08M triethylammonium phosphate. Flow rate: 2.0 ml/min. UV absorbance range: 0.2 AUFS (absorbance units full scale).

TABLE 2

Retention Indices of Selected Amines on a μBondapak C_{18} Column*

Amine	k'
Benzidine	2.30
o-Tolidine	3.00
N-Methylaniline	4.40
p-Chloroaniline	5.03
o-Toluidine	7.66
1-Naphthylamine	8.15
2-Naphthylamine	8.64
N,N-Dimethylaniline	12.6
2,4-Dichloroaniline	13.8
2,4,6-Trichloroaniline	16.1

*Using a 20-minute concave gradient from 40% CH_3OH/60% 0.08M triethylammonium phosphate to 70% CH_3OH/30% 0.08M triethylammonium phosphate

Resolution of benzidine, *o*-toluidine, *o*-tolidine, 1-naphthylamine, 2-naphthylamine, *p*-chloroaniline, 2,4-dichloroaniline, 2,4,6-trichloroaniline N-methylaniline, and N,N-dimethylaniline was less complete using either the μBondapak CN or μBondapak Phenyl column than it was using the μBondapak C_{18} column. The best separations using these columns were achieved using either a 20-min gradient from 100% H_2O to 50% H_2O/50% CH_3OH or a 20-min concave gradient from 100% 0.08M triethylammonium phosphate to 70% 0.08M triethylammonium phosphate/30% CH_3OH. Separations using this column were not reproducible.

Linearity of Ultraviolet and Fluorescent Response of Selected Amines

Standard serial dilutions of the amines used in this study were injected singly and in mixture to determine if linear responses could be obtained using either ultraviolet absorbance at 254 nm or intrinsic fluorescence. In all cases, the ultraviolet absorbance versus concentration plots obtained for the amines were linear over at least a two-order-of-magnitude range in concentration. Figure 5 shows the linear response obtained for two amines, atrazine and Linuron. Detection limits for several amines are reported in Table 3. The detection limit was defined in this case to be the mass of material required to produce a signal twice that of the noise level at 0.02 absorbance units full scale (AUFS). All of these detection limits correspond to approximate concentrations in the analyte solution in the range of 0.1 to 0.2 ppm. Depending upon the volume extracted and the concentration factor employed, these detection limits corrrespond to concentrations at the 0.1 ppb level in natural waters.

A similar study was performed to determine the detection limits of several amines using intrinsic fluorescence. Linear response curves were obtained for all compounds tested over at least a two-order-of-magnitude range in concentration. The detection limit was again defined as the amount of a compound which gave a signal twice that of the noise level. The fluorescence was measured with an excitation wavelength of 254 nm. These detection limit studies were done using the μBondapak C_{18} column and CH_3OH as the solvent. The results of this study are presented in Table 4. The large differences between the detection limits of the s-triazines (atrazine, Bladex, and Simazine) and those of the other aromatic amines tested are probably due to the difference in the molecular structure of the aromatic rings.

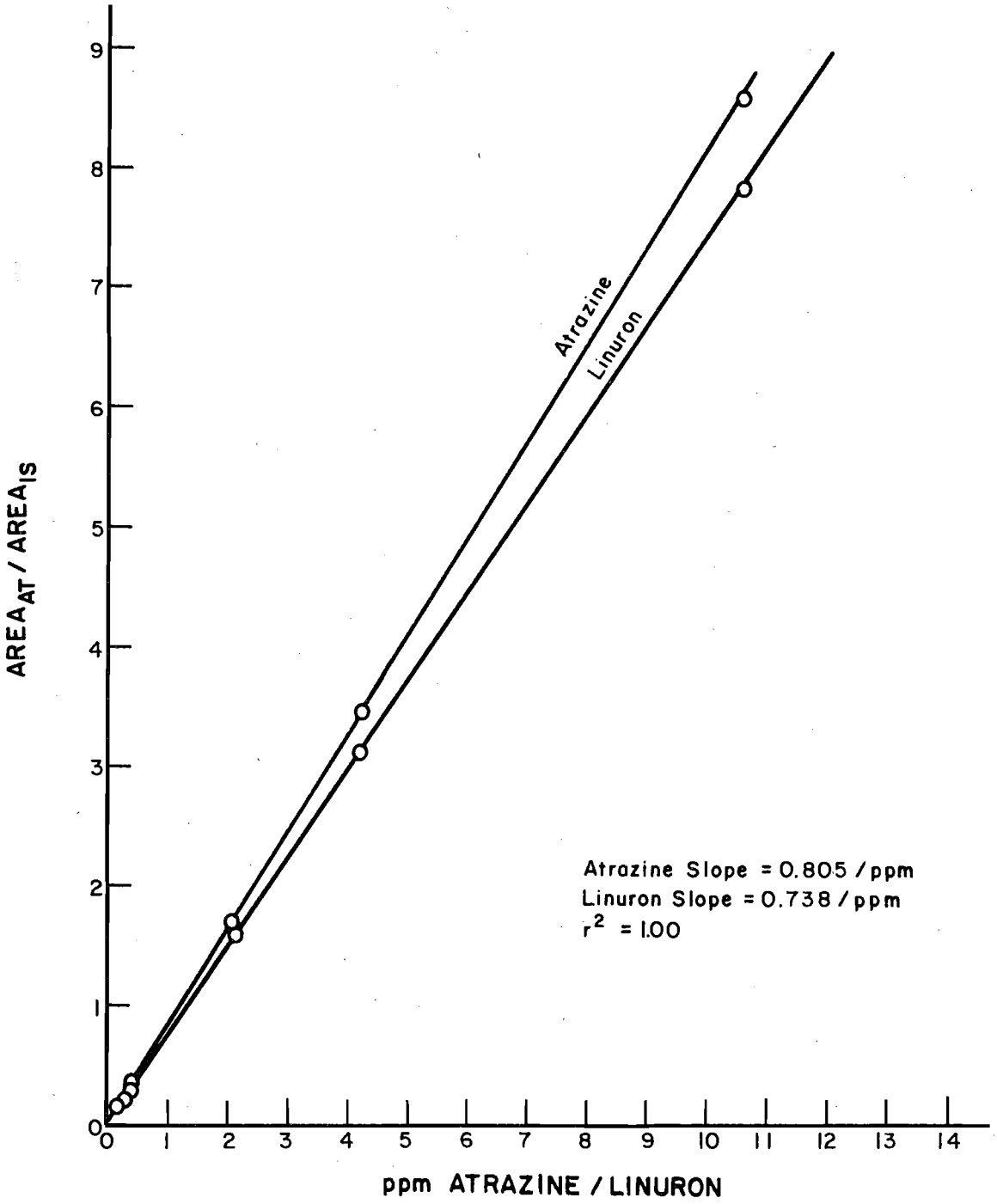

Figure 5. Linear response of ultraviolet absorbance detector to atrazine and Linuron.

$AREA_{AT}$: area of compound response.

$AREA_{IS}$: area of internal standard response.

TABLE 3

Detection Limits of Several Aromatic and Heterocyclic Amines Using Ultraviolet Absorbance (254 nm)

Amine	Detection Limit (pg)
1-Naphthylamine	1100
Benzidine	1270
o-Toluidine	1500
o-Tolidine	1300
Atrazine	2000
Simazine	2100
Bladex	1000

TABLE 4

Detection Limits of Several Aromatic and Heterocyclic Amines Using Fluorescence

Amine	Detection Limit (pg)
2-Naphthylamine	30
Benzidine	9.5
o-Toluidine	1,890
o-Tolidine	45
Atrazine	374,000
Simazine	192,000
Bladex	270,000

Benzidine, 2-naphthylamine, *o*-toluidine, and *o*-tolidine contain true carbocyclic aromatic rings, while atrazine, Bladex, and Simazine contain nitrogenous heterocyclic pseudoaromatic rings. The detection limits for the aromatic amines correspond to analyte concentration in the range of 1 to 100 ppb. Depending upon the volumes extracted and the concentration factor employed, the detection limits correspond to concentrations at the 1 to 100 part-per-trillion level in natural waters.

It is clear that, for compounds which exhibit good intrinsic fluorescence, ultra trace analysis may be accomplished. However, with both fluorescence and ultraviolet techniques, interferences from co-eluting substances are possible. This is especially true when large concentration factors are employed. A good understanding of the matrix present in any natural water sample is essential if accurate quantitation is to be achieved. Mass spectral confirmation should probably be applied as a routine measure to back up the quantitative data.

Sample Extraction/Concentration Studies

A series of amines were extracted and concentrated using methylene chloride as described in the section on sample extraction and concentration on page 5. The amines were spiked at levels of 1.0 ppb, 10 ppb and 100 ppb into laboratory water and analyzed by liquid chromatography using the ultraviolet absorbance method. The recoveries observed at the three concentration levels are reported in Table 5. It appears that all of the amines tested can be reliably and quantitatively recovered by solvent extraction. However, caution must be taken that samples are not overheated during the concentration step.

Studies were performed to determine the utility of XAD-4 and XAD-8 polymeric adsorption resins in the collection and concentration of aromatic amines. There was a two-fold purpose for this study: first, to test the retention capacity of the columns, and second, to test the recovery of the amines after adsorption to the column. First, 100 ml of a 993 ng/l aqueous solution of *p*-chloroaniline was passed through each of three columns filled with XAD-8 resin. The effluent from each column was analyzed by HPLC using the μBondapak CN column and 10% CH_3OH/90% H_2O as the solvent. No *p*-chloroaniline was detected by fluorescence in a 20-μl injection of

TABLE 5

Recoveries of Selected Amines by Solvent Extraction

Compound	1.0 ppb spike	10 ppb spike	100 ppb spike
Atrazine	97%	98%	99%
Simazine	97%	97%	98%
Benzidine	99%	95%	97%
o-Tolidine	99%	94%	97%
1-Naphthylamine	97%	95%	98%
Linuron	99%	99%	100%

this effluent, indicating that the *p*-chloroaniline was fully retained. Then, each column was rinsed with 100 ml of CH_3OH. The first 10 ml that were eluted contained no detectable *p*-chloroaniline and were discarded. The remainder of the effluent was diluted to 100 ml with CH_3OH and analyzed by HPLC using the above conditions. The average recovery was 100.4% with a standard deviation of 0.64%.

Additional work was also performed to determine if the XAD-4 resin could be used to reliably concentrate selected amines at environmental levels. The amines were spiked into laboratory water at levels of 1.0 ppb, 10 ppb, and 100 ppb, concentrated on the XAD-4 resin, recovered, and analyzed by liquid chromatography using the ultraviolet absorbance method. The recoveries observed at the three concentration levels are reported in Table 6. The recoveries of the amines at all levels tested were well within the acceptable range (90-100%).

Field Evaluation of Methods

The fourteen field samples were collected at a number of sites on the Chesapeake Bay, the Patuxent River, and the Susquehanna River at two times during the last year (October 10-12, 1979 and April 24 and 26, 1980). Figure 6 shows the locations where the samples were collected. The samples were filtered through 0.45 μm millipore filter pads at the time of collection, concentrated in XAD-4 resin, and concentrated and analyzed using reverse-phase chromatography and ultraviolet absorbance detection. The concentrations of the amines found in the fourteen samples are reported in Table 7.

Low levels of several amines associated with agriculture were detected at the sub-ppb level. No residues of dye-industry-associated amines were observed. These results are consistent with the facts that no dye manufacturing is taking place in this geographical region and that agriculture (including corn, soybean, and tobacco farming) is widespread on lands surrounding the Chesapeake and its tributaries.

Replicate analyses of the CHB-2 samples indicate that the method used can yield reliable and reproducible data at the ppb level. Table 8 shows the recoveries of several amines spiked into PAX-1 water at a level of 10 ppb after the water had been filtered. All of the recoveries were observed to be in the acceptable range (93-100%).

TABLE 6

Recoveries of Selected Amines on XAD-4 Resin

Compound	1.0 ppb	10 ppb	100 ppb
Atrazine	96%	98%	98%
Simazine	97%	99%	98%
Benzidine	95%	97%	97%
o-Tolidine	95%	98%	98%
1-Naphthylamine	96%	96%	97%
Linuron	98%	99%	99%

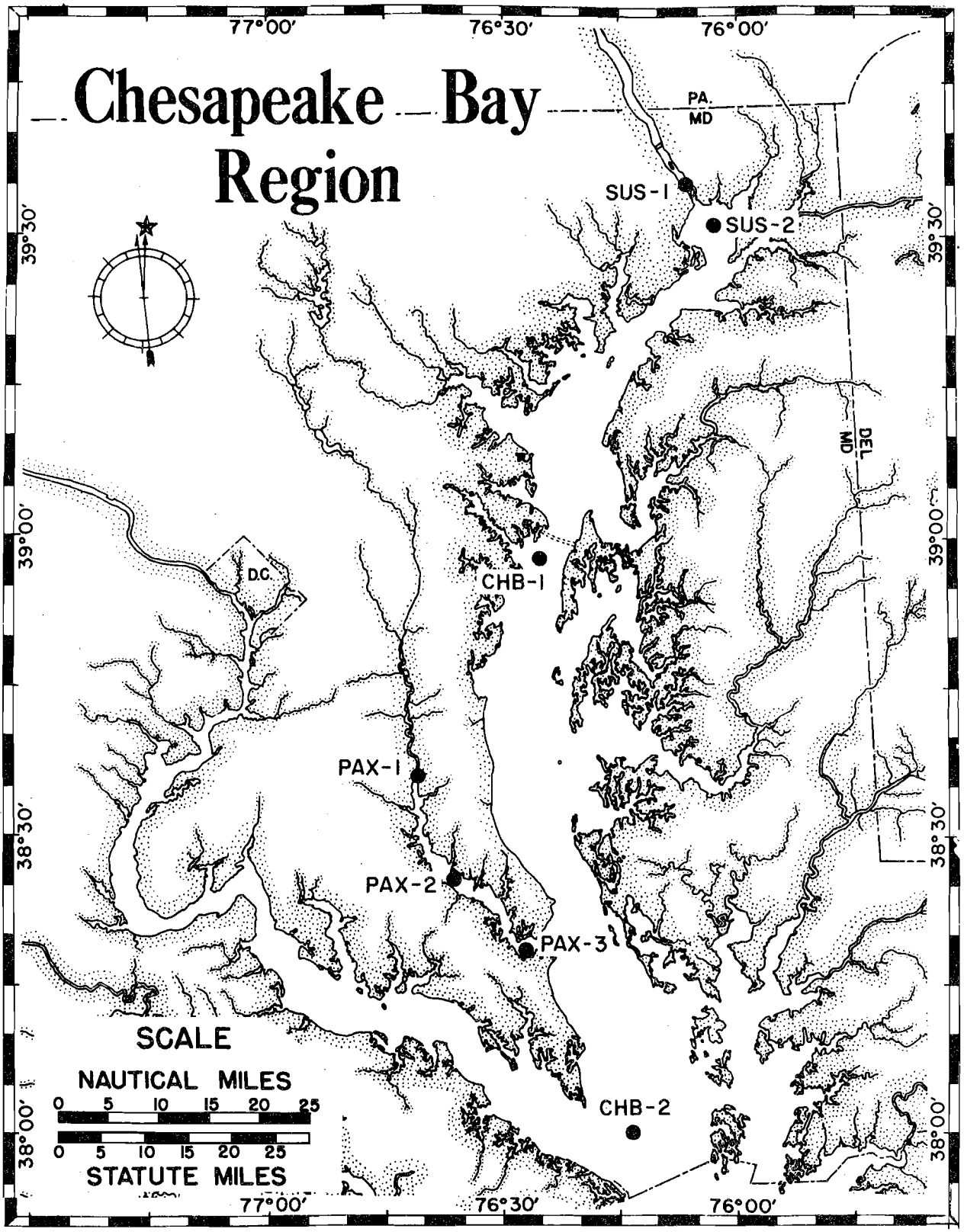

Figure 6. Sampling sites on the Chesapeake Bay and its tributaries.

TABLE 7

Concentrations (ppb) of Amines Detected in Fourteen Field Samples*

October 1979

	Atrazine	Simazine	Linuron	Benzidine	Tolidine	1-Naphthylamine
PAX-1	0.09	0.07	<0.05	N.D.	N.D.	N.D.
PAX-2	0.08	0.05	<0.05	N.D.	N.D.	N.D.
PAX-3	0.05	0.05	<0.05	N.D.	N.D.	N.D.
SUS-1	0.09	0.05	<0.05	N.D.	N.D.	N.D.
SUS-2	0.06	0.04	<0.05	N.D.	N.D.	N.D.
CHB-1	<0.05	<0.03	<0.05	N.D.	N.D.	N.D.
CHB-2a	<0.05	<0.03	<0.05	N.D.	N.D.	N.D.
CHB-2b	<0.05	<0.03	<0.05	N.D.	N.D.	N.D.

April 1980

	Atrazine	Simazine	Linuron	Benzidine	Tolidine	1-Naphthylamine
PAX-1	0.81	0.60	0.17	N.D.	N.D.	N.D.
PAX-2	0.77	0.67	0.13	N.D.	N.D.	N.D.
PAX-3	1.03	0.61	0.11	N.D.	N.D.	N.D.
SUS-1	0.33	0.21	0.13	N.D.	N.D.	N.D.
SUS-2	0.21	0.13	0.07	N.D.	N.D.	N.D.
CHB-1	0.07	0.05	0.06	N.D.	N.D.	N.D.
CHB-2a	0.07	0.05	<0.05	N.D.	N.D.	N.D.
CHB-2b	0.07	0.04	<0.05	N.D.	N.D.	N.D.

*Detection limit for benzidine, *o*-tolidine, and 1-naphthylamine is 0.05 ppb; for atrazine, also 0.05 ppb; and for Simazine, 0.03 ppb. Values preceded by (<) indicate that a trace was detected but that it was below the reliable detection limit. N.D. indicates that no peak was observed at that specific retention time.

TABLE 8

Recoveries of Several Amines from Estuarine Water

PAX-1 (October 1979)

Compound	Matrix	Spike (ppb)	Concentration (ppb)	Recovery
Atrazine	0.81	10.0	10.47	96.9%
Simazine	0.60	10.0	10.51	99.2%
Benzidine	--	10.0	9.38	93.8%
1-Naphthylamine	--	10.0	9.49	94.9%
Linuron	0.17	10.0	9.97	98.0%

PAX-1 (April 1980)

Compound	Matrix	Spike (ppb)	Concentration (ppb)	Recovery
Atrazine	0.09	10.0	9.89	98.0%
Simazine	0.07	10.0	9.73	96.6%
Benzidine	--	10.0	9.63	96.3%
1-Naphthylamine	--	10.0	9.40	94.0%
Linuron	<0.05	10.0	9.87	98.7%

DEVELOPMENT OF GAS CHROMATOGRAPHIC METHODS

REVIEW OF LITERATURE

Due to its ease of operation and versatility, gas chromatography is widely used for the detection, identification, determination, and quantitation of amines. Various conditions, column packings, and detection systems have been employed by different workers. Flame ionization detection was used by Markacheva and Kogan (1976) and Golovnya et al. (1978); electron capture detection by Bowen (1976), Pashkevich et al. (1977), and Nony and Bowman (1978); thermal conductivity detection by Pasechnik and Rogovik (1973); and nitrogen specific detection by Bowen (1976) and Diachenko (1979).

The following phases have been employed to separate aromatic amines by gas chromatography: Apiezon M (Andersons et al. 1973a and 1973b); Apiezon L (Golovnya et al. 1978); Tenax GC (Daemen et al. 1975; Bowen 1976); Triton X-305 (Golovnya et al. 1978); Silicon OV 101 (Grimmer et al. 1978); polyethylene glycol 1000 (Golovnya et al. 1978); polyethylene glycol 2000 (Andersons et al. 1973a and 1973b); Chromaton N containing 20% polyphenylether (Markacheva and Kogan 1976); 20% Reoplex-400 (Czerwiec and Markowski 1975); 25% polymethylphenylsiloxane-4 (Pasechnik and Rogovik 1973); and 5% SE 60/ Chromaton N (Pashkevich et al. 1977). Aromatic amines have also been determined by several other workers using various conditions (Smikun et al. 1974; Farroha and Emeishi 1975; Nony and Bowman 1978; and Tsuda et al. 1977).

Trace analyses of carcinogenic amines and their analogs have been carried out by Nony and Bowman (1978) in wastewater and human urine, by Diachenko (1979) in fish, by Haefelfinger (1975) in blood and plasma, and by Neurath et al. (1977) in the human environment and foods in general.

Extraction and concentration studies for amines were performed for monitoring the safe disposal of wastewater (Nony and Bowman 1978). Salient elements of the procedure described by the above workers are: extraction of phenolic and neutral residues from the acidified sample, liquid-liquid partitioning cleanup and separation of neutral from phenolic residues at pH 14 and 10.2, acid hydrolysis of the neutral component, subsequent alkalinization of the sample and extraction of the basic residues of the

free amines, conversion of all residues to the corresponding pentafluoropropionyl (PFP) derivatives, and quantification by electron capture gas chromatography. The authors were able to detect the residues in wastewater and urine at 0.1 and 1.0 ppb levels, respectively.

EXPERIMENTAL MATERIALS

Equipment

1. Hewlett-Packard 5700A gas chromatograph equipped with a flame ionization detector and a Hewlett-Packard 3385A automation system for data processing
2. Hewlett-Packard 5840A gas chromatograph equipped with a nitrogen-phosphorus specific detector and a GC terminal for data processing
3. Kuderna-Danish concentrator (Kontes Glass Company, Vineland, NJ)
4. Rotary Evaporator (Flash-Evaporator, Buchler Instruments, Fort Lee, NJ)

Reagents and Supplies

1. Carcinogenic amine standards were acquired as 20 mg/ml solutions in methanol, from Supelco, Inc., Supelco Park, Bellefonte, PA.
2. The following column packings were purchased from Supelco, Inc:
   a. 10% Apiezon L + 2% KOH on 80/100 Chromasorb W AW
   b. 3% OV 17 on 80/100 Supelcoport
   c. Tenax GC, 60/80 mesh
   d. 1.5% SP-2250/1.95% SP-2401 on 100/120 Supelcoport
   e. 10% SP-2340 on 100/120 Supelcoport
   f. 3% SP-2100 DB on 100/120 Supelcoport
   g. 3% SP-2250 DB on 100/120 Supelcoport
3. All the solvents used were of glass-distilled quality (Burdick-Jackson Laboratories, Inc., Muskegon, MI), and reagent grade chemicals were used, if needed.
4. Glass columns and gas chromatographic supplies were purchased from Hewlett-Packard, 5201 Tollview Drive, Rolling Meadows, IL.

EXPERIMENTAL PROCEDURES

The detection of carcinogenic amines was investigated with flame ionization, electron capture, and nitrogen specific detection gas chromatography. Selected amines were studied to determine their response to each type of detection at several dilutions of the standards.

Flame Ionization Detection

Standard amines dissolved in methanol were diluted to known concentrations (nanograms/μl) of each. 1- to 5-μl injections representing 10-500 nanograms of sample were introduced into the gas chromatograph. The area units reproduced by the integrator were processed to calculate the response factors and linearity of detection for various amines.

Electron Capture Detection

The selected carcinogenic amines were studied to determine their response to electron capture detection at picogram amounts. The amines were derivatized by adding 0.5 ml benzene and 0.1 ml 0.05M trimethylamine in benzene to the samples in a 5-ml centrifuge tube, followed by the addition of 10 μl of heptafluorobutyric anhydride. The reaction mixture was heated in capped tubes for 15 minutes at 50°C, then cooled; excess anhydride was hydrolyzed by adding 1.0 ml water. Heptafluorobutyric acid was removed by addition of one ml of 5% aqueous ammonia solution to the sample, followed by vigorous shaking for 5 minutes and then by centrifugation. 1- to 2-μl injections were made from the benzene phase.

Nitrogen Specific Detection

The standard amines were evaluated to determine their response at low concentrations. The amines dissolved in methanol were diluted to contain from one to several nanograms of each standard amine per μl, and 1 to 5 μl of each amine were analyzed by nitrogen specific detection. A 6-foot-long x 2-mm-ID glass column packed with 3% OV 17 on 80/100 Supelcoport was employed for the analyses. Helium was used as carrier gas, flow-controlled at 20.0 ± 0.1 ml per minute. Flow rates for hydrogen and air were 30 ml per minute and 50 ml per minute respectively. The voltage for the detector was adjusted before every set of injections to give a recorder pen deflection of 100 mm at an attenuation of 8. The area units printed out by the integrator were processed to calculate the responses per nanogram amount and to establish the linearity of detection for various amines.

SAMPLE EXTRACTION/CONCENTRATION STUDIES

A liter of distilled water was spiked with standard amines at two concentration levels for flame ionization detection. Level A contained 500 ppb each of aniline, *o*-toluidine, 2-naphthylamine, benzidine, *o*-tolidine, 2,7-dimethylquinoline, and 1.0 ppm of atrazine. Level B contained 100 ppb each of the above standard amines except atrazine, which was spiked at 200 ppb. Samples were prepared in triplicate and the pH adjusted to 11.5. The samples were extracted with chloroform. Three extractions were performed using a total of 200 ml (100 + 50 + 50) chloroform. To eliminate traces of moisture, the combined chloroform extracts were dried by passing through a column of sodium sulfate. The extracts were concentrated to approximately one ml in a Kuderna-Danish concentrator and evaporated to dryness with a stream of nitrogen. Each sample was redissolved in one ml of methanol and analyzed on a flame ionization gas chromatograph.

To evaluate the efficiency of the extraction procedure at lower concentrations, several standard amines were spiked into one liter of distilled water at 50 ppb concentration levels. The samples were extracted as described above using 200 ml of chloroform and/or methylene chloride. The solvents were evaporated from the extracts with a flash evaporator. The recovered standard amines were dissolved in one ml of methanol and analyzed with the nitrogen specific gas chromatograph.

FIELD EVALUATION OF METHODS

A sample of water acquired from the Fox River was extracted with chloroform, as previously described, to screen for the possible presence of small amounts of aromatic amines. The extract from one liter of water was redissolved into one ml of methanol; 1-5 µl were subsequently injected into a gas chromatograph equipped with a nitrogen specific detector.

Another liter of water from the Fox River was spiked with several standard amines at 50 ppb concentration levels, extracted, and concentrated as described above. 1- to 5-µl injections were made into a gas chromatograph with the nitrogen specific detector. Percent recoveries were calculated from the printouts of area responses for recovered amines compared to area responses for standard amines. The recoveries of amines from distilled water and Fox River water, both of which had been spiked at 50 ppb concentration levels, were compared.

RESULTS

Evaluation of Stationary Phases

The effects of various stationary phases of column packings on the elution patterns of selected carcinogenic amines were determined by flame ionization detection. Using isothermal conditions, the elution and separation of all the amines under study were found to be impossible. Various programmed temperature modes were then investigated for each packing and mixture of standard amines.

Figure 7 shows elution of aniline, *o*-toluidine, lepidine, and 2-naphthylamine from a 10% Apiezon L + 2% KOH column at the programmed temperature of 100°C to 200°C. Under these conditions, other amines present in the mixture were retained by the packing. The use of temperatures higher than 200°C would have deteriorated the stationary phase on the packing. A column packed with 3% SP-2340 was then used to separate a standard mixture (Fig. 8). The figure shows that, by temperature programming the column from 125°C to 270°C, excellent separations were achieved for most of the amines. But at the maximum temperature benzidine and *o*-tolidine took a much longer time to elute, and the separation between these two amines was not complete.

Evaluation of 3% SP-2250 DB, a base-treated packing, was performed by eluting the standard amines from the column at a programmed temperature of 90°C to 275°C at a rate of 8°C per minute. The representation (Fig. 9) shows good separations, but column bleed was excessive above a column temperature of 250°C; however, it took a much longer time to elute *o*-tolidine from the column at a temperature of 250°C than at 275°. The baseline drift was excessive at lower attenuations which were necessary to detect small amounts of standard amines. Another packing commonly used for pesticide analysis, 1.5% SP-2250 + 1.95% SP-2401 on 100/200 Supelcoport, exhibited similar separations, but peak tailing and baseline drift were excessive.

Finally, the nonpolar column packings containing OV 17 and Tenax GC were investigated in an effort to find the most suitable packing to resolve a mixture of amines with considerably different volatilization characteristics. Most of the amines under study were resolved on Tenax GC (Fig. 10) and 3% OV 17 (Fig. 11); 3% OV 17 also resolved the hydrocarbons. Both of these packings

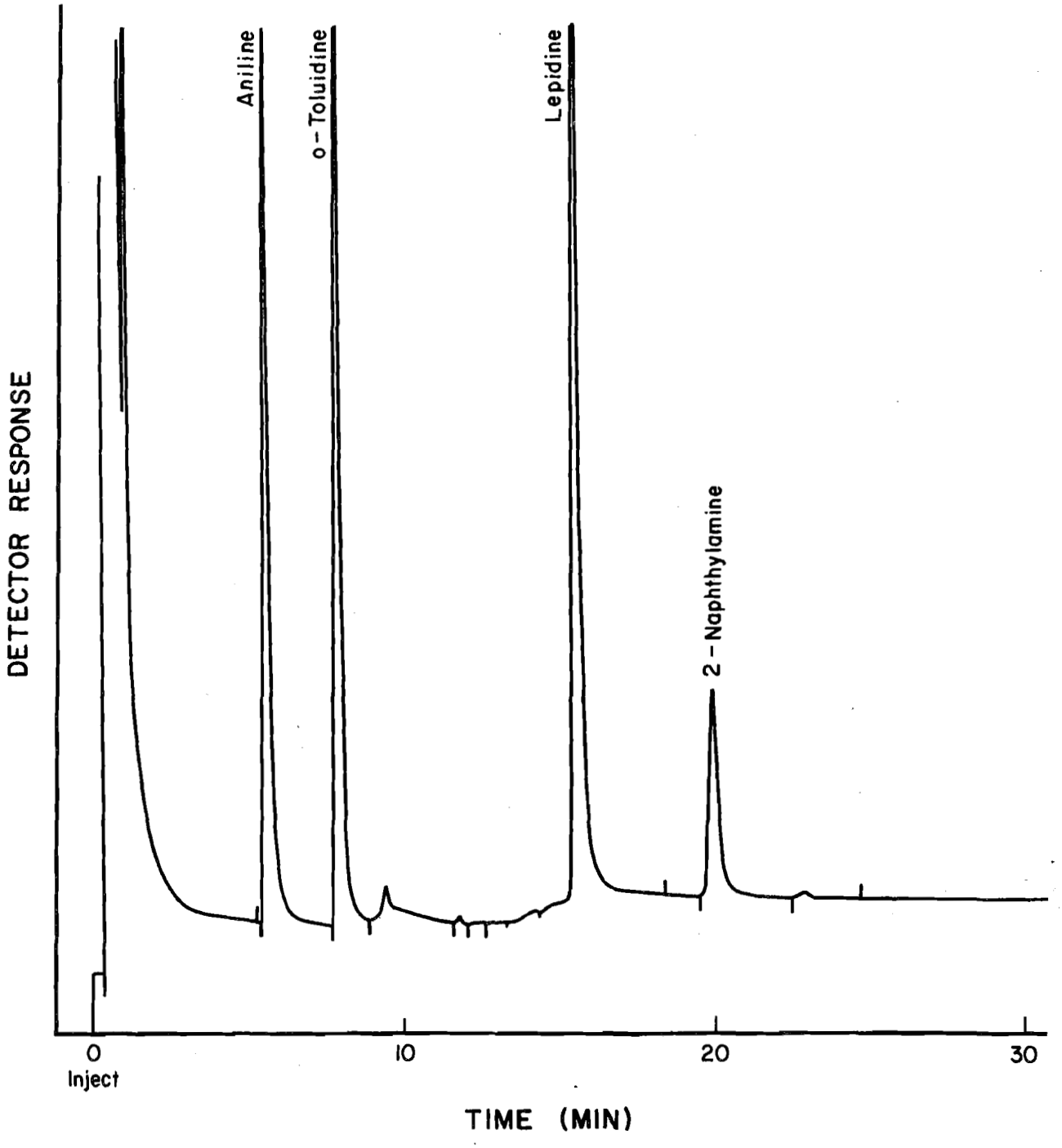

Figure 7. Separation of standard amine mixture on 10% Apiezon L + 2% KOH on 80% Chromasorb W AW.

Temperature 1: 100°C. Time 1: 2 min. Rate: 8°C/min.
Temperature 2: 200°C. Nitrogen as carrier gas: 20 ml/min.
Detection system: flame ionization.

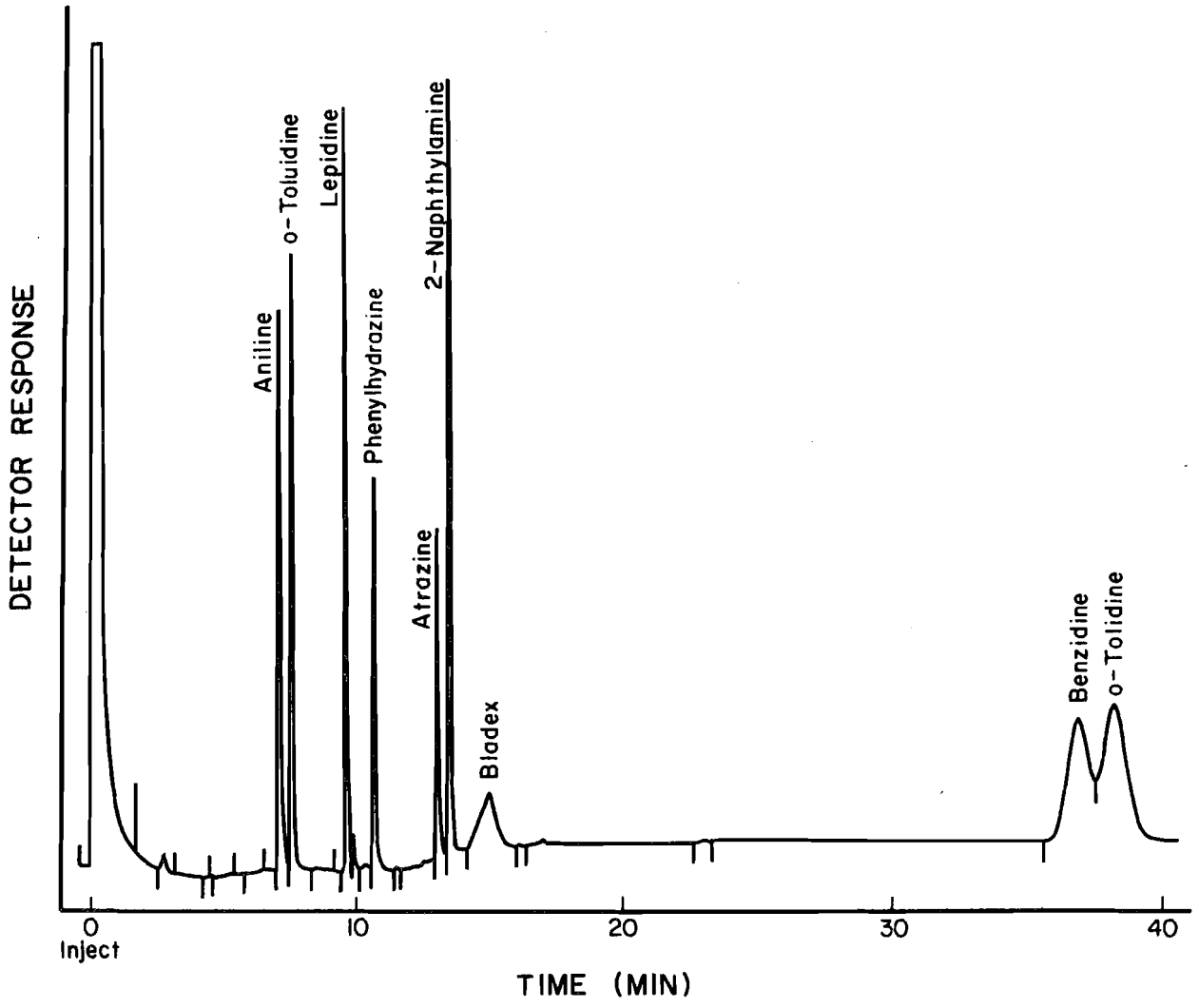

Figure 8. Separation of standard amine mixture on 3% SP-2340 on 100/120 Supelcoport.

Temperature 1: 125°C. Time 1: 4 min. Rate: 16°C/min.
Temperature 2: 270°C. Nitrogen as carrier gas: 20 ml/min.
Detection system: flame ionization.

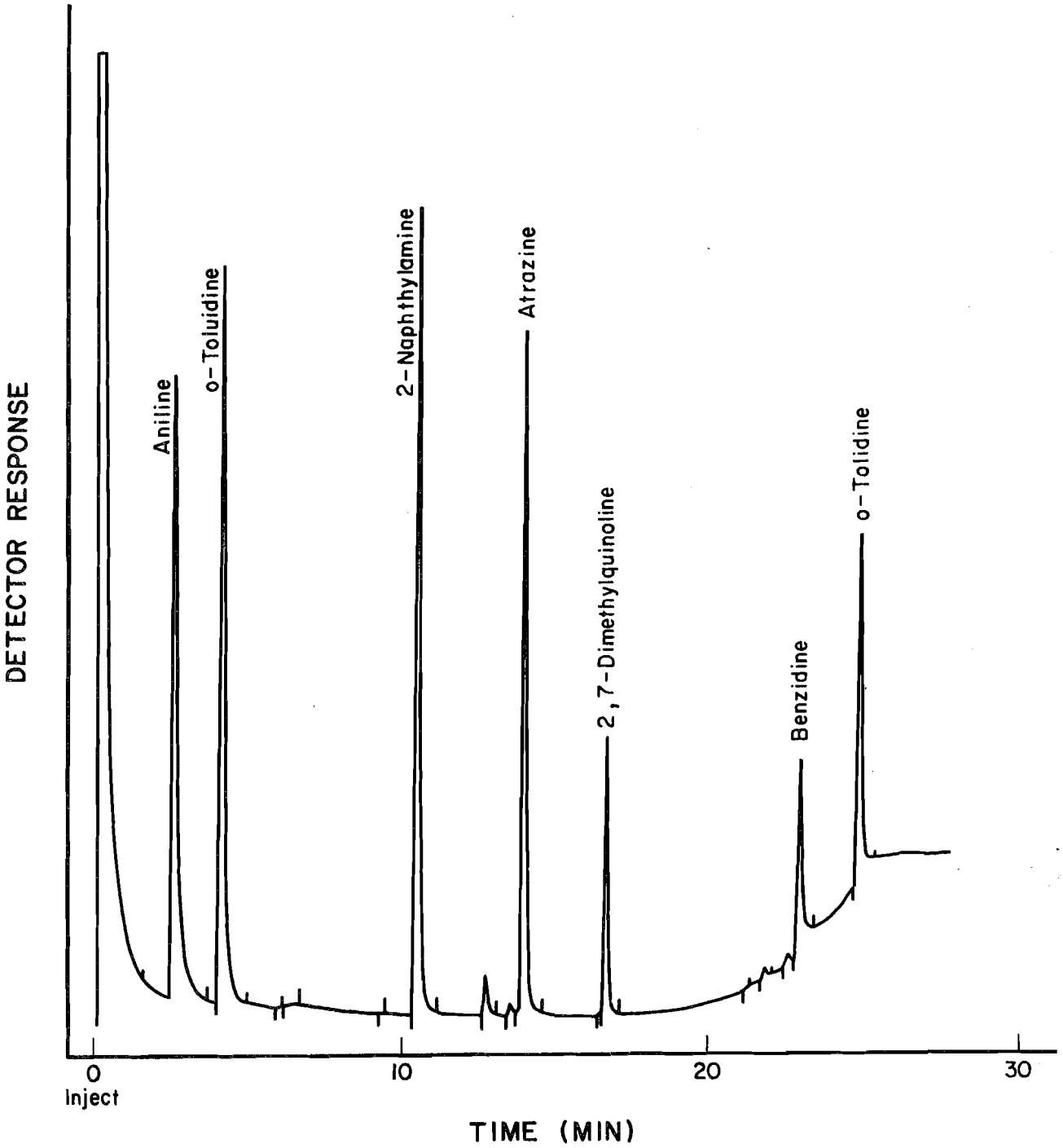

Figure 9. Separation of standard amine mixture on 3% SP-2250 DB on 100/120 Supelcoport.

Temperature 1: 90°C. Time 1: 2 min. Rate: 8°C/min.
Temperature 2: 275°C. Nitrogen as carrier gas: 20 ml/min.
Detection system: flame ionization.

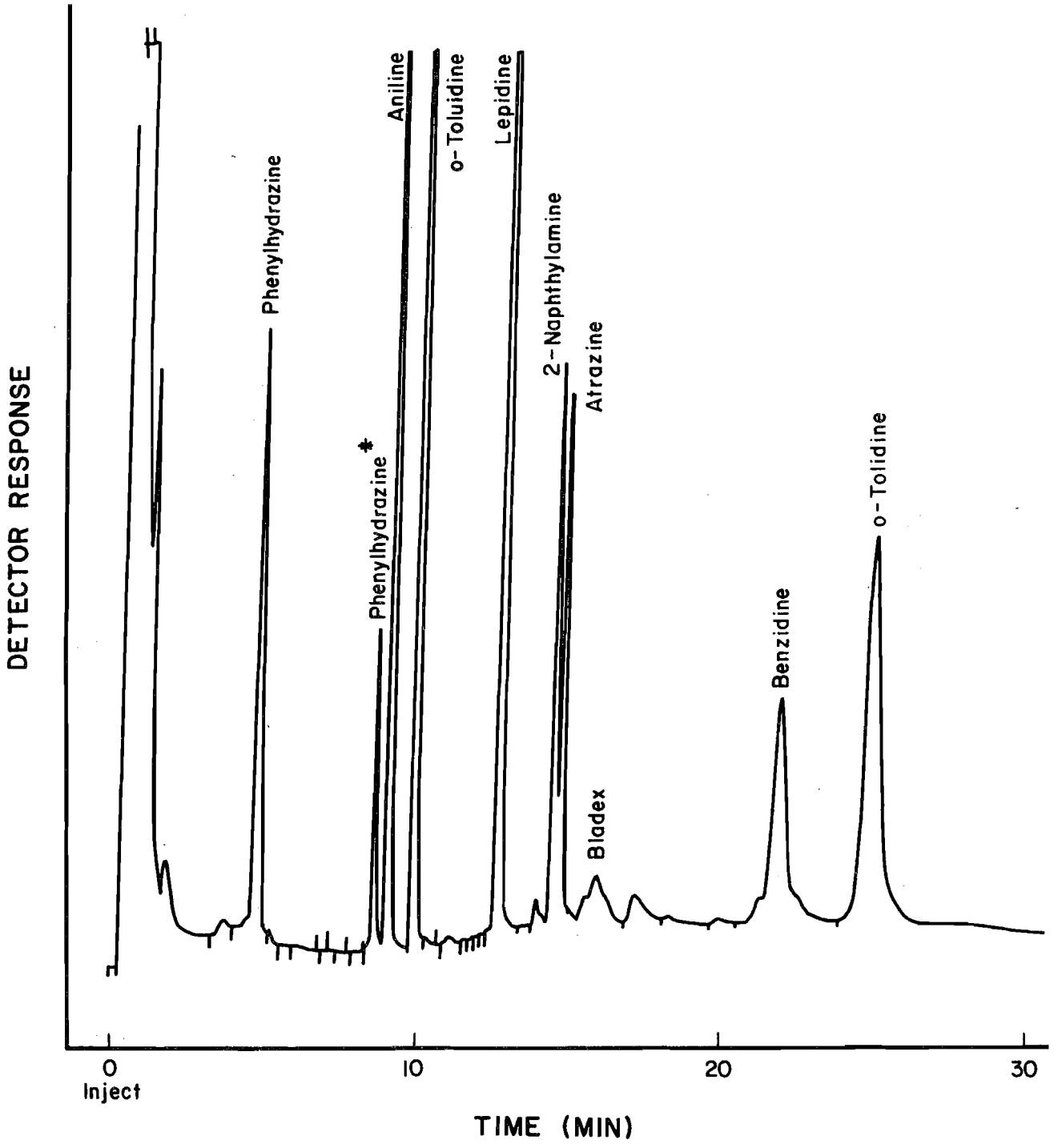

Figure 10. Separation of standard amine mixture on Tenax GC 60/80 mesh.

Temperature 1: 130°C. Time 1: 2 min. Rate: 16°C/min.
Temperature 2: 330°C. Nitrogen as carrier gas: 20 ml/min.
Detection system: flame ionization.

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* This peak probably represents a decomposition product of phenylhydrazine.

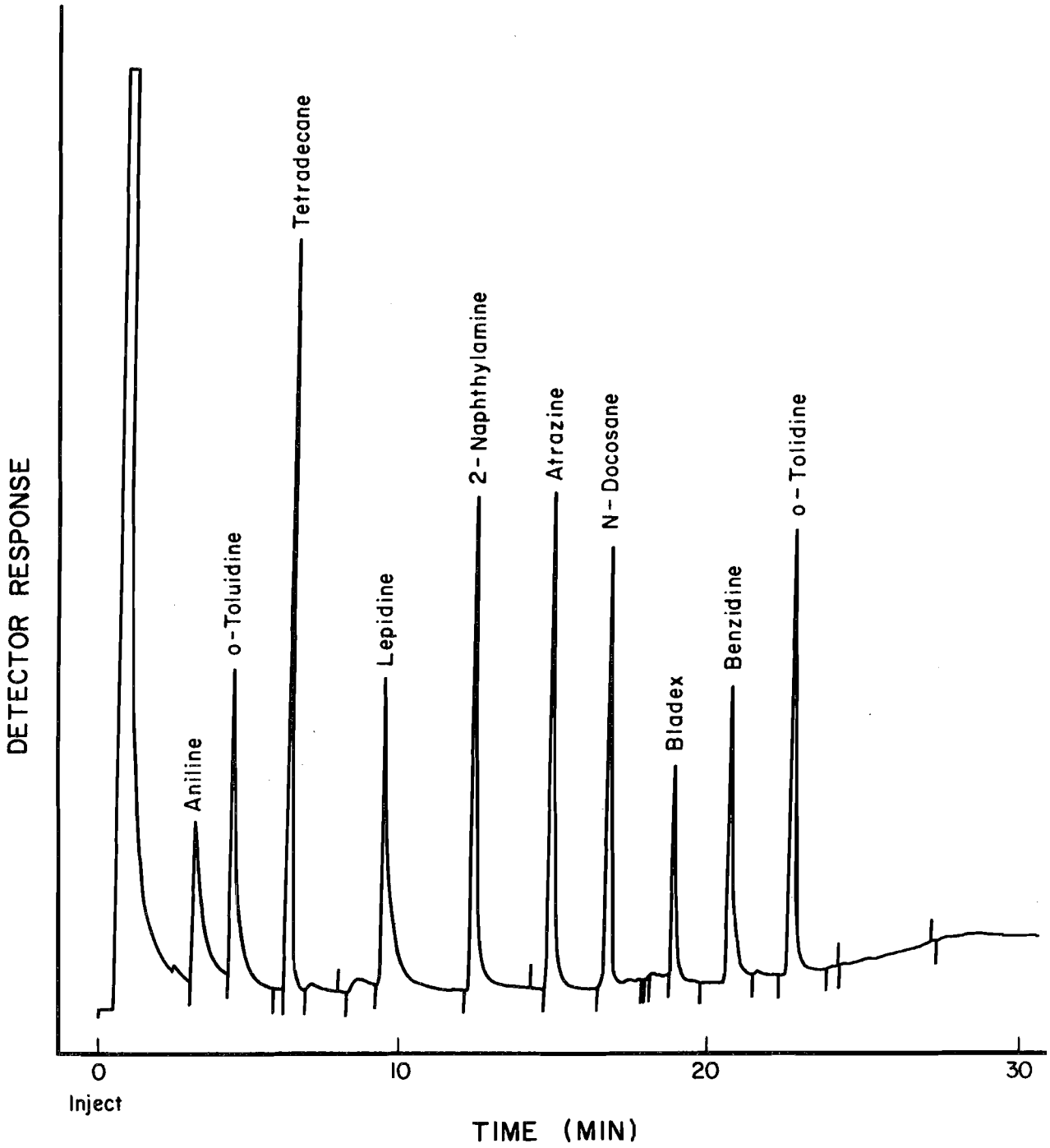

Figure 11. Separation of standard amine mixture on 3% OV 17 on 80/100 Supelcoport.

Temperature 1: 100°C. Time 1: 2 min. Rate: 8°C/min.
Temperature 2: 300°C. Nitrogen as carrier gas: 20 ml/min.
Detection system: flame ionization.

were more stable at higher temperatures than were the packings previously evaluated. Tenax GC required higher column temperatures to accomplish elutions comparable to 3% OV 17; atrazine and 2-naphthylamine appeared as an unresolved doublet, and phenylhydrazine exhibited two separate peaks.* Because of the above reasons, Tenax GC was dropped in favor of 3% OV 17, which was employed to calculate detector responses of various standard amines and to determine the sensitivity or limits of detection.

Detector Response of Standard Amines on a Flame Ionization Detector

It was observed that Simazine, one of the selected standard amines, was not appreciably soluble in methanol in desired concentrations; it was therefore dropped from the study. It was also found that phenylhydrazine, another selected standard amine, was unstable at lower concentrations in methanol. In freshly prepared solutions, phenylhydrazine was resolved as a sharp and distinct peak upon analysis by gas chromatography; but, with subsequent injections from the same vial, the peak area decreased and ultimately disappeared. The detector responses of each standard amine were calculated compared to an internal standard (2,7-dimethylquinoline) by the use of flame ionization detection (Table 9). It was found that aniline, *o*-toluidine, and 2-naphthylamine have relative responses close to the internal standard. Atrazine exhibited the lowest detector response of all the standard amines tested.

Linearity of Detector Response Over a Range of Sample Sizes on a Flame Ionization Detector

Sensitivity of the method was determined by injecting 10-20 nanograms of standard amines into the column. Although the standards were detected at these low levels by the flame ionization detector, the responses were not found to be linear below 50 ng for aniline, atrazine, and 2,7-dimethylquinoline and below 100 ng for *o*-toluidine, 2-naphthylamine, benzidine, and *o*-tolidine. All the standard amines evaluated gave a linear response up to 500 ng as shown in Fig. 12 and Fig. 13.

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*The first peak was more prominent when a fresh solution was injected; for subsequent injections with the same solution, the second peak became more prominent—then both peaks decreased in height and finally disappeared. The first peak is probably the true phenylhydrazine peak, and the second one probably represents a decomposition product.

TABLE 9

Detector Response for Selected Standard Amines
Using Flame Ionization Detector

Standard Amine	Amount Injected (ng)	Response/ng (area units)	Relative Response
Aniline	500	7.00×10^2	0.934
o-Toluidine	500	7.46×10^2	0.942
2-Naphthylamine	500	7.62×10^2	0.962
Atrazine	1000	9.70×10^1	0.245
Benzidine	500	4.56×10^2	0.576
o-Tolidine	500	5.74×10^2	0.725
2,7-Dimethylquinoline	500	7.92×10^2	1.000

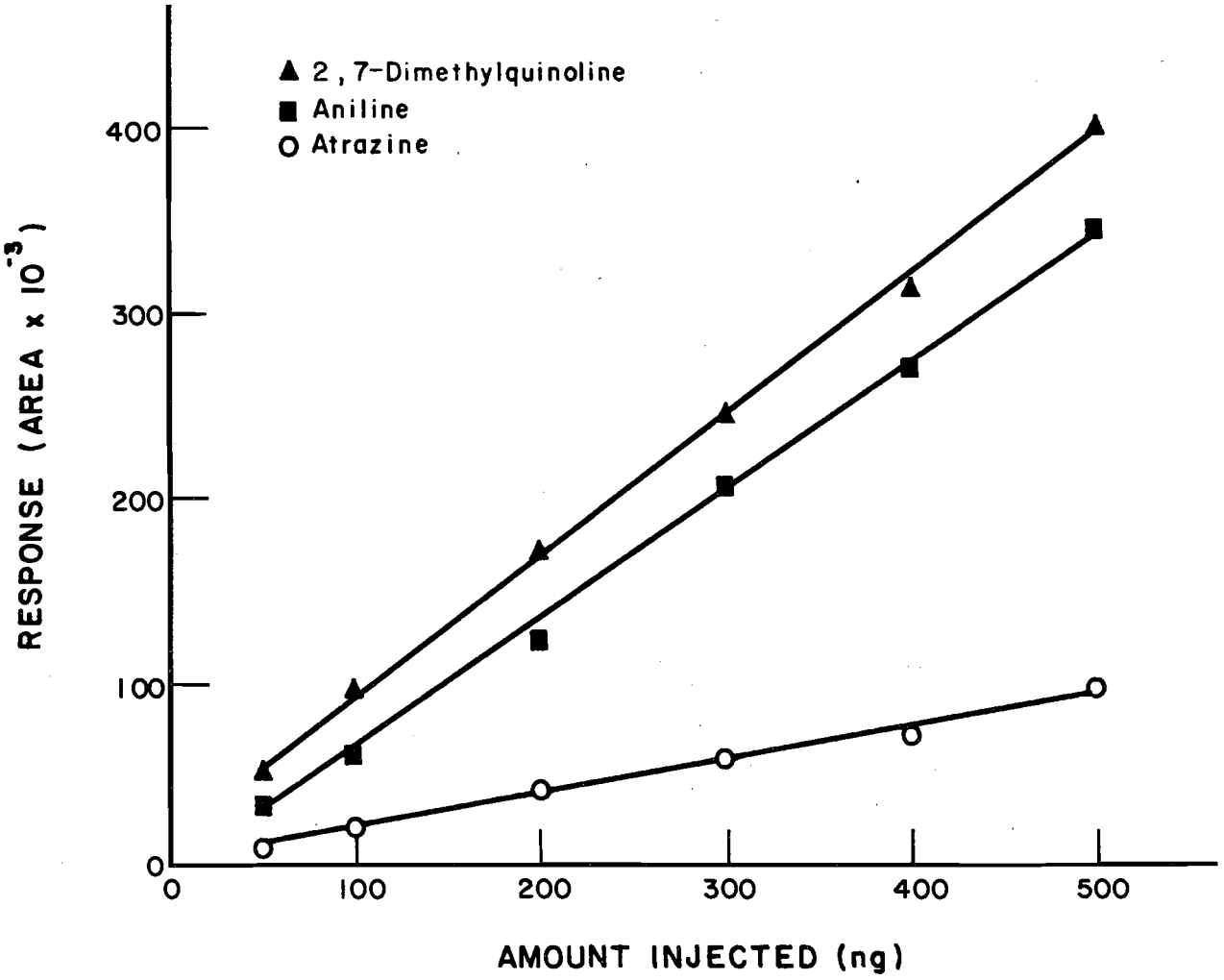

Figure 12. Plot of area responses of 2,7-dimethylquinoline, aniline, and atrazine versus amounts injected (ng) as calculated from analysis by flame ionization detection.

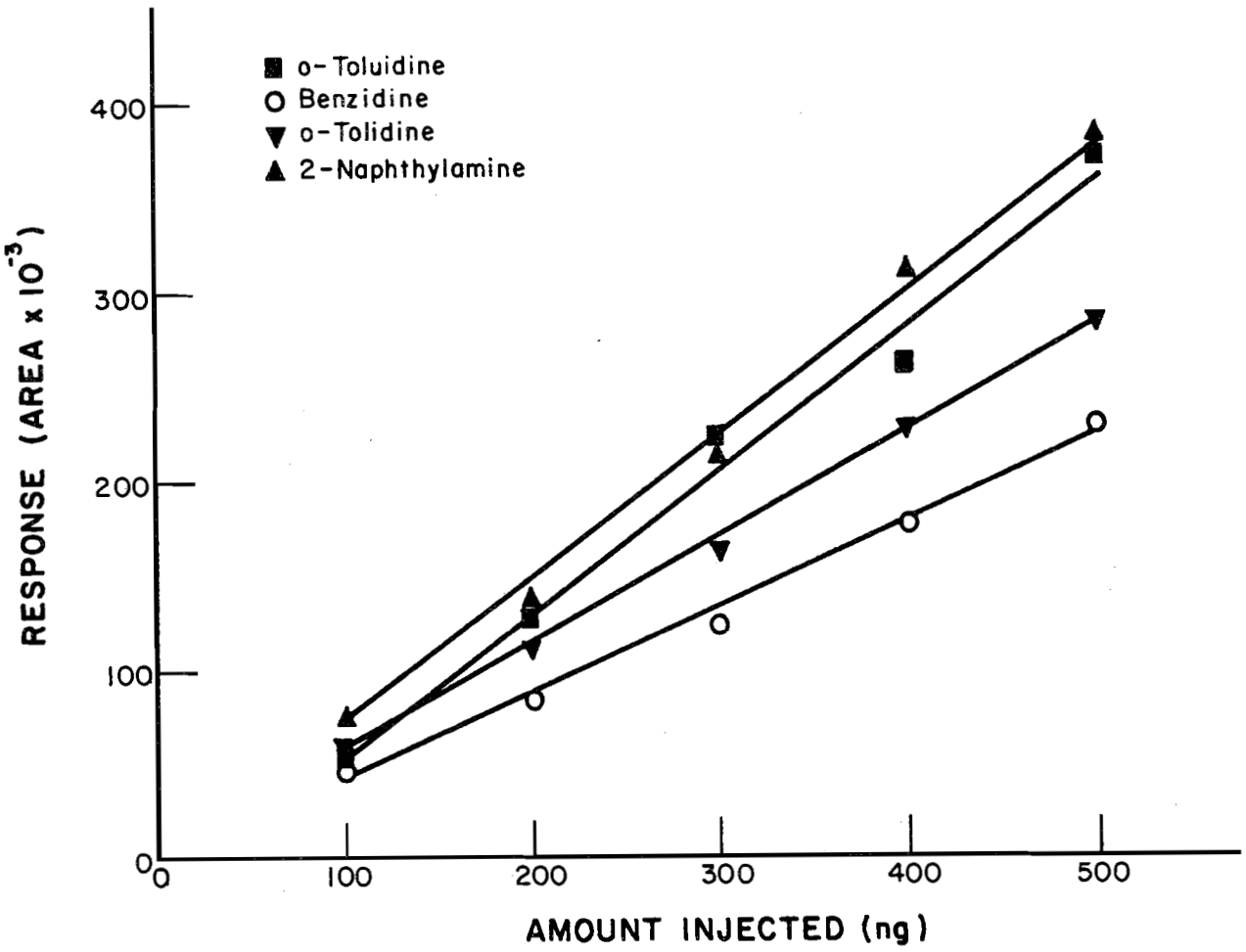

Figure 13. Plot of area responses of *o*-toluidine, benzidine, *o*-tolidine, and 2-naphthylamine versus amounts injected (ng) as calculated from analysis by flame ionization detection.

Extraction of Standard Amines from Distilled Water

The extraction studies were carried out by spiking the water at two levels, 500 ppb and 100 ppb, for most of the standard amines. The standard amines were analyzed by a flame ionization detector, and the recoveries were calculated as percentages (Table 10). Recovered standard amines ranged from 27.86% for aniline to 90.74% for *o*-tolidine, at the higher level of spike. At the lower level of spike, 100 ppb, the recoveries for most of the amines were lower except for aniline. Recoveries ranged from 28.06% for aniline to 85.06% for *o*-tolidine.

Detector Response of Standard Amines to Electron Capture Detection

The heptafluorobutyric derivatives of aromatic amines were evaluated in an attempt to obtain higher sensitivity with electron capture gas chromatography. At 100-pg levels, aniline and *o*-toluidine were separated as sharp peaks; but baseline drifts, even at a low rate of temperature programming, were excessive. The pen deflected to full scale after a 15-20^{o} temperature increase. Because of the above results, calculations of detector responses by electron capture were dropped in favor of the nitrogen specific detector.

Detector Response of Standard Amines to the Nitrogen Specific Detector

The usefulness and applicability of nitrogen specific detection were investigated for the standard carcinogenic amines. The detector responses calculated for various standard amines are presented in Table 11. Table 11 shows that various amines exhibited responses ranging from 0.532 to 3.803, relative to 1.000 for the internal standard, lepidine. From the comparisons of area responses of standard amines on a nitrogen specific detector as compared to a flame ionization detector, it seems that the nitrogen specific detector gives at least a 9-times-better response for aniline and *o*-toluidine, whereas the response was more than 400 times better for atrazine. Moreover, elimination of interfering solvent peaks and peaks due to compounds not containing nitrogen and phosphorus improves the quantitation and reproducibility of the analyses.

TABLE 10

Percent Recoveries of Standard Carcinogenic Amines from Distilled Water Using Flame Ionization Detector

Standard Amine	Percent Recovered (level A)[1]	Percent Recovered (level B)[2]
Aniline	27.86	28.06
o-Toluidine	51.82	40.00
2,7-Dimethylquinoline	90.06	79.22
2-Naphthylamine	86.65	72.28
Atrazine	87.50	83.12
Benzidine	90.03	84.44
o-Tolidine	90.74	85.06

[1]Standard amines spiked at 0.5 ppm level, except atrazine at 1 ppm level

[2]Standard amines spiked at 100 ppb levels, except atrazine at 200 ppb level

TABLE 11

Detector Response for Selected Standard Amines
Using Nitrogen Specific Detector

Standard Amine	Amount Injected (ng)	Response/ng (Area Units)	Relative Response
Aniline	35	6.30×10^3	0.566
o-Toluidine	30	5.93×10^3	0.532
2-Naphthylamine	15	1.34×10^4	1.205
Atrazine	5	4.24×10^4	3.803
Benzidine	7.5	2.76×10^4	2.474
o-Tolidine	7.5	2.81×10^4	2.519
Lepidine	25	1.11×10^4	1.000

Linearity of Detector Response over a Range of Sample Sizes with the Nitrogen Specific Detector

Subnanogram levels of most of the standard amines, individually injected, were detected by nitrogen specific detection. Due to the differences in responses of various standard amines and other factors, quantitation and reproducibility at these low levels of detection could not be achieved. However, a mixture of standard amines, comprised of 5-ng to 35-ng levels of certain amines, was analyzed reproducibly. The ranges of various standard amines detected within linear limits are presented in Figures 14, 15, and 16. Atrazine, which has a greater detector response, was detectable in amounts as low as 5 ng when present in a mixture of amines.

Extraction/Concentration Studies for Analysis by the Nitrogen Specific Detector

Data for various standard amines recovered from distilled water spiked at the 50-ppb level is presented in Table 12. It appeared that the more volatile members of the standard amines, aniline and *o*-tolidine, disappear during the process of extraction. Percent recoveries for the rest of the amines ranged from 29.35 for benzidine to 70.33 for atrazine. It is evident from the above discussion that the lower the amounts of spike, the lower the percent recoveries of standard amines.

Field Trial of Laboratory Methods

A sample of water from the Fox River was screened for the possible presence of carcinogenic amines. No significant amounts of the amines under evaluation were found in the sample. When the water from the Fox River was used as a matrix to spike standard amines, the recoveries of the amines spiked at the 50-ppb level were astonishingly low (Table 12). Aniline and *o*-toluidine were not recovered from this sample. The recoveries of other standard amines were 46% to 56% lower than recoveries from distilled water.

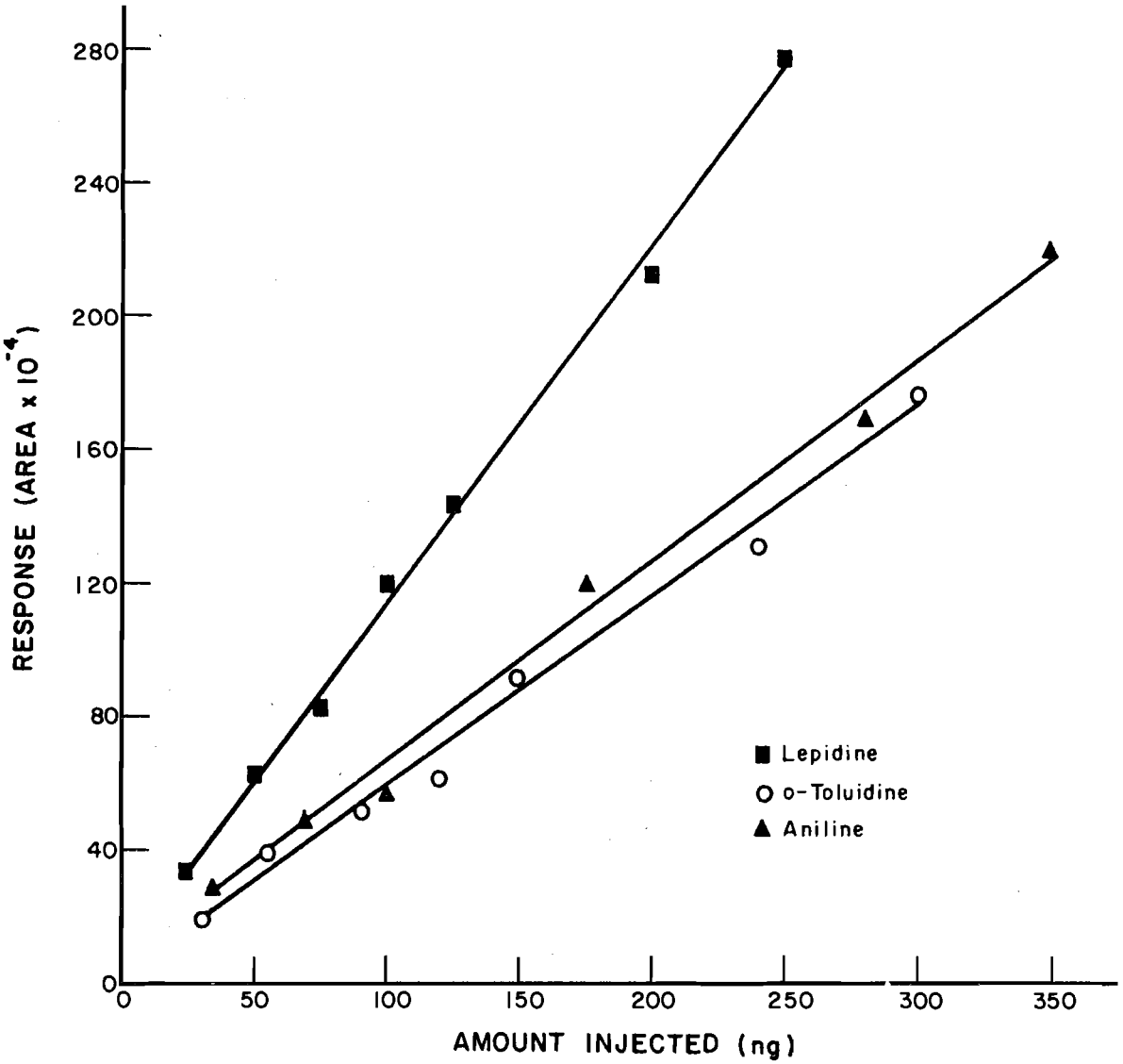

Figure 14. Plot of area responses of lepidine, *o*-toluidine, and aniline versus amounts injected (ng) as calculated from analysis by nitrogen specific detection.

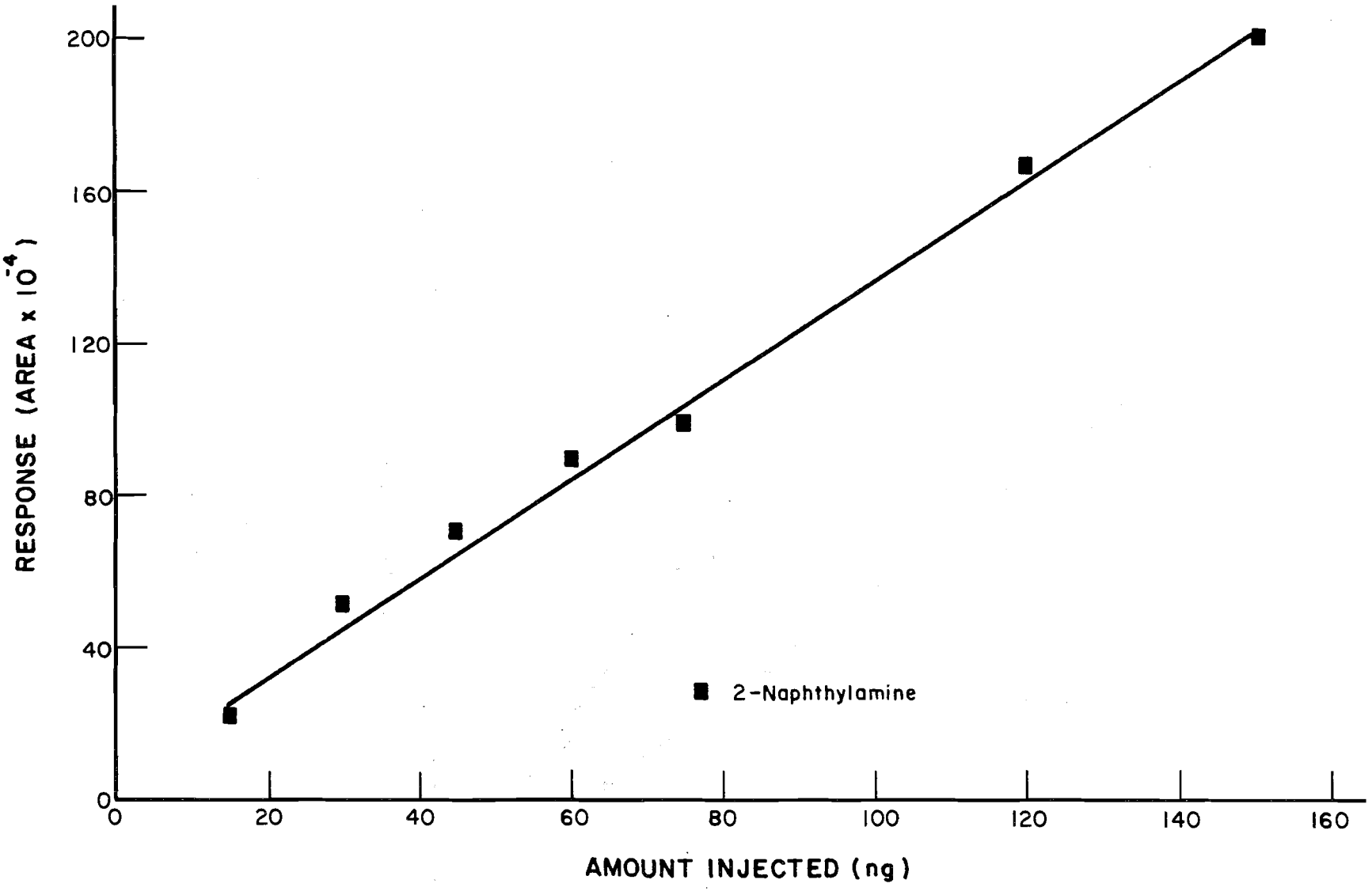

Figure 15. Plot of area responses of 2-naphthylamine versus amounts injected (ng) as calculated from analysis by nitrogen specific detection.

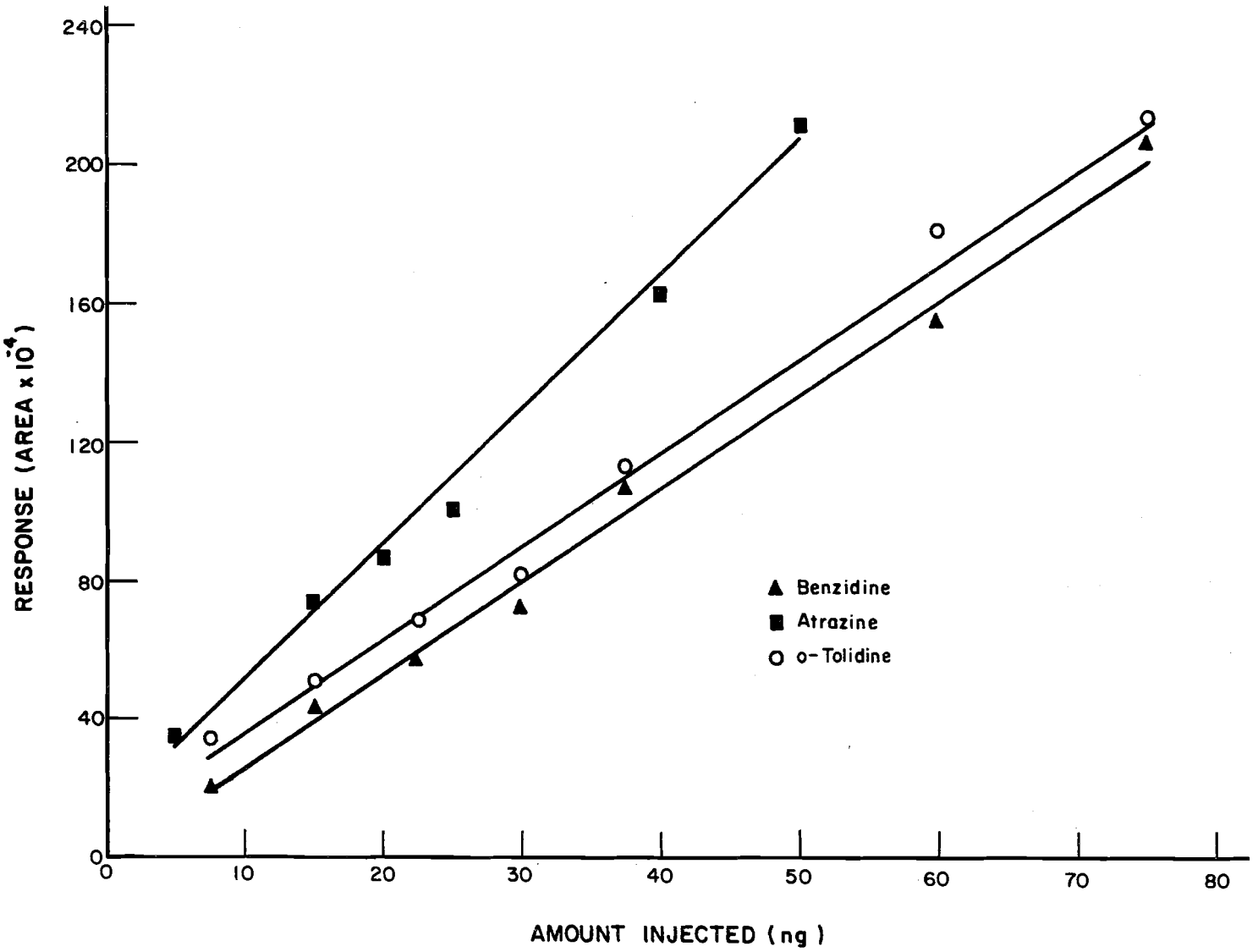

Figure 16. Plot of area responses of benzidine, atrazine, and *o*-tolidine versus amounts injected (ng) as calculated from analysis by nitrogen specific detection.

TABLE 12

Percent Recoveries of Standard Carcinogenic Amines from Water Using Nitrogen Specific Detector

Standard Amine[1]	% Recovery from Distilled Water	% Recovery from Fox River Water
Aniline	----	----
o-Toluidine	----	----
Lepidine	43.69	20.17
2-Naphthylamine	34.48	18.01
Atrazine	70.33	33.66
Bladex	65.94	32.98
Benzidine	29.35	13.24
o-Tolidine	42.41	18.65

[1]Spiked at a level of 50 ppb each

CONCLUSIONS

1. Gas chromatography with flame ionization detection and nitrogen specific detection can successfully be used for the analysis of mixtures of amines with a wide range of volatilization constants, using such fairly nonpolar column packings as OV 17 and Tenax GC.
2. Some of the amines with high volatilities appear to be lost during extraction and concentration; therefore, XAD resins may be better suited for concentration of the amines. Resin columns also eliminate the tedious steps of extraction which affect the recovery of amines.
3. It is evident from the use of Fox River water as a matrix that some of the amines could be bound to the biological and/or inorganic particulate matter present. Procedures should be developed to account for such bound residues.
4. Liquid chromatography using either ultraviolet absorbance or fluorescence detectors can successfully be used for the quantitative analysis of complex mixtures of aromatic and heterocyclic amines. Separation, using gradients of acetonitrile in water and microparticulate reverse-phase columns such as μBondapak C_{18}, yields the highest resolution. Liquid chromatography may offer some advantages over gas chromatography for the analysis of thermally labile compounds or polar metabolites of amines.

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