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NITROGEN-SELECTIVE DETECTOR FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A nitrogen-selective detector for high-performance liquid chromatography (HPLC) is described. The HPLC effluent is introduced into a furnace using a concentric atomizer. Organic nitrogen is converted to ammonia by reaction in hydrogen over a nickel catalyst. The ammonia is collected into water–alcohol and the ammonium ion is measured with a Tracor Hall electrolytic conductivity detector. The limit of detection is 1 ng nitrogen per sec injected onto the chromatographic column. The carbon to nitrogen selectivity ratio is 10,000. Precision is 3.4% relative standard deviation when 0.56 μg nitrogen are injected. The detector is linear over a narrow range. Deviation from linearity is most likely due to incomplete partitioning and ionization of ammonia in the conductivity solution. Applications using both normal and reversed-phase conditions are discussed.

INTRODUCTION

Solvents commonly used in high-performance liquid chromatography (HPLC) make it difficult to develop new detectors. One approach is to modify existing gas chromatographic (GC) detectors to accommodate the HPLC solvent. Additional selectivity against the solvent can be achieved using an element selective detector. In this approach, the element of interest is converted to a unique chemical species that is selectively measured in the presence of other species not containing the element of interest.

Selective detection of nitrogen-containing compounds in HPLC is possible by several means. Fine[1] oxidized bound nitrogen to the nitrosyl radical (NO), reacted that with ozone and measured the subsequent chemiluminescence. Hill and Crist[2] detected nitrogen compounds by reducing organic nitrogen to ammonia. The solvent was removed and eluted components transported by a moving wire interface to a furnace combined with a GC Hall electrolytic conductivity detector (HElCD).

The goal of this work was to develop a nitrogen-selective HPLC detector (NSD) with a submicrogram limit of detection. In this work, the HPLC solvent is introduced directly into a reductive atmosphere pyrolyzer (RAP) where organically

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TABLE I

APPARATUS

Component	*Model number*	*Manufacturer*	*Conditions*
HPLC solvent pump	M-45 & M-6000A	Waters Assoc., Milford, MA, U.S.A.	
Injection valve	7125	Rheodyne, Berkeley, CA, U.S.A.	20-μl sample loop
UV absorption detector	4100–4000	Waters Assoc.	254 nm
Integrator	CDS-111	Varian Instruments, Palo Alto, CA, U.S.A.	
Recorder	HP7132A	Hewlett-Packard, San Diego, CA, U.S.A.	Span = 10 V
Tube furnace (Furnace 1)	55035	Lindberg, Watertown, WI, U.S.A.	925 ± 25°C
Operational amplifier designer	OA-2	E & L Instruments, Derby, CT, U.S.A.	
Quartz tubing	0.25 in. (0.64 cm) O.D. × 15.7 in. (40 cm)	Quartz Scientific, Fairport Harbor, OH, U.S.A.	
Flow controller for Hydrogen 1	8944	Brooks, Hartfield, PA, U.S.A.	Typically 0.9 l/min
Flow controller for Hydrogen 2	VCD-1000	Porter Instruments Co., Hatfield, PA, U.S.A.	Typically 0.1 l/min
Nickel catalyst for main furnace		Procter & Gamble, Cincinnati, OH, U.S.A.	
Hall electrolytic conductivity detector	700	Tracor Instruments, Austin, TX, U.S.A.	

bound nitrogen is catalytically converted to ammonia. The resulting ammonia is measured with an HElCD.

EXPERIMENTAL

Detector design

The HElCD was modified and interfaced to an HPLC using a RAP designed with readily available parts. The necessary components and equipment are summarized in Table I.

A schematic design of the detector is shown in Fig. 1. The HPLC effluent was split prior to its introduction into the RAP when the flow-rate exceeded 0.2 ml/min. The liquid flow splitter was built using a zero dead volume "tee union" (Valco Instruments) and a precision needle valve (Series M, Nupro) to adjust the split ratio. Peak broadening in the transfer line between the liquid flow splitter and the atomizer of the RAP was minimized by using transfer tubing [0.02 in. (0.5 mm) I.D.].

The atomizer was designed to deliver solvent into the hot zone of the RAP to cause rapid vaporization and pyrolysis of the HPLC effluent. Industrial grade hydrogen was used for all experiments and was flowed around the transfer line (Hydrogen 1) containing the HPLC effluent to prevent premature solvent vaporization and plug-

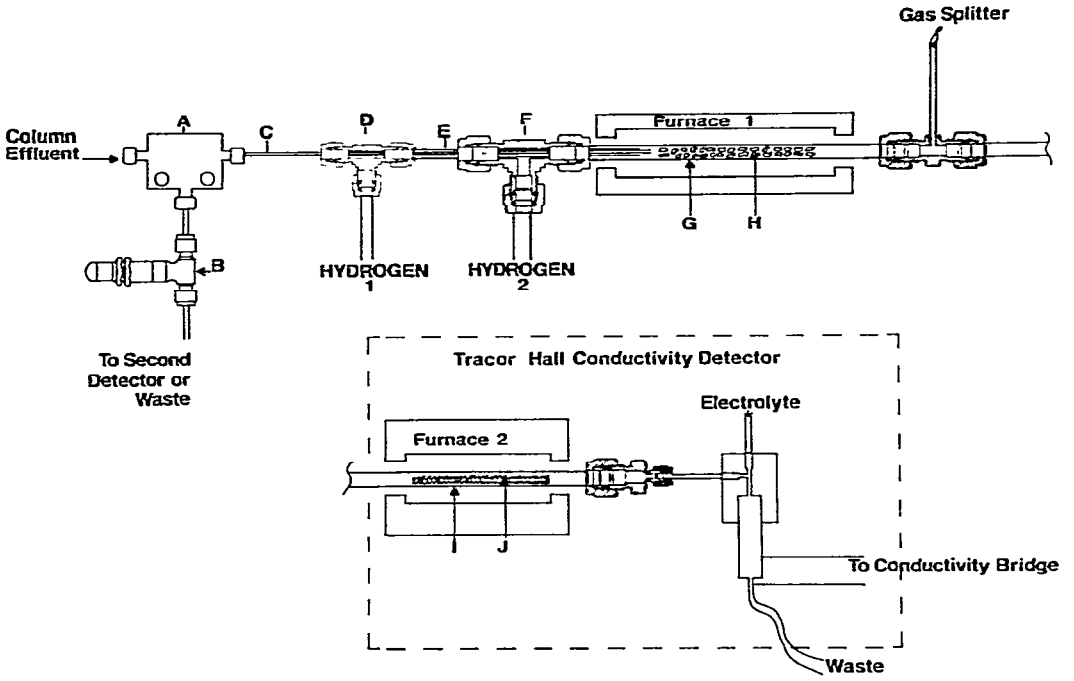

Fig. 1. Schematic diagram of nitrogen-selective HPLC detector. A = Zero dead volume tee; B = precision needle valve; C = 1/16 in. (0.16 cm) O.D. × 0.02 in. (0.05 cm) I.D. tubing; D = 1/8-in. (0.32-cm) tee union; E = 1/8-in. (0.32-cm) tubing; F = 1/4-in. (0.64-cm) tee union; G and I = 1/4-in. (0.64-cm) O.D. quartz tube; H = nickel catalyst (lathe turnings) and J = nickel catalyst (wire).

ging. Hydrogen 2 flow prevents back diffusion of the vaporized HPLC effluent which would result in peak broadening. A gas-tight connection between the atomizer and the quartz tube was made using 40% graphite–60% vespel·ferrules (Alltech, Arlington Heights, IL, U.S.A.).

The RAP catalyst was approximately 6 g of nickel lathe turnings cut to about 0.4-cm lengths, cleaned in hexane and placed inside the quartz tube. The nickel catalyst was positioned in the center of the quartz tube and held in place on each end with 1-cm quartz wool plugs (Perkin-Elmer, Norwalk, CT, U.S.A.). The quartz tube was positioned concentrically inside the tube furnace with the ends of the furnace covered to prevent convective cooling.

The RAP quartz tube was connected to the reaction furnace of the HElCD. A 1/4 in. (0.64 cm)–1/8 in. (0.32 cm) adaptor (Swagelok) and 40% graphite–60% vespel ferrules were used. The 1/16-in. (0.16-cm) tube of the HElCD conventionally used to introduce hydrogen into the furnace was used to provide a gas flow splitter. Typically, the gas flow-rate to the conductivity cell was 100–200 ml/min. The HElCD furnace was operated at 900°C with the nickel wire catalyst centered in the oven. A nickel catalyst was also used in the HElCD to maximize conversion of organically bound nitrogen to ammonia. When necessary, acidic components, such as hydrogen chloride, were removed from the gas stream with quartz wool coated with potassium hydroxide (Tracor, Austin, TX, U.S.A.). The solution used for measuring conduc-

tivity was 40% (v/v) *n*-propanol (Burdick & Jackson Labs., Muskegon, MI, U.S.A.) in distilled, deionized water and was delivered from a bottle (Model 100 PFB, Omnifit) pressurized with helium to 10 p.s.i. (Pressure-Stat, Model 123PSA, Omnifit). The flow-rate was typically set to 0.33 ml/min using a precision needle valve (Series M, Nupro). The water–alcohol solution flowed from the pressurized bottle through the needle valve and ion exchanger (Tracor) to the conductivity cell. To overcome the potential difficulties associated with recycling the water–alcohol solution as discussed by Goo *et al.*[3], it was used only once and then discarded.

An active filter (time constant 0.3 sec) was breadboarded with operational amplifiers (741, Analog Devices) and was used between the 10-V computer output of the HElCD and recorder to reduce noise.

RAP operating characteristics

The decomposition products of pentane and methanol were determined by GC to measure their degree of pyrolysis in the RAP. These solvents were individually introduced to the RAP at 0.1 ml/min with the furnace temperatures varied between 600°C and 1000°C. A gas-tight syringe (Precision Sampling, Baton Rouge, LA, U.S.A.) was used to sample and inject 25 μl of the RAP effluent into a Varian 1440 gas chromatograph. The latter was equipped with a flame ionization detector and a stainless-steel column [6.6 ft. (2.0 m) × 1/8 in. (0.32 cm)] packed with Chromosorb 102 (60–80 mesh) (Supelco, Bellefonte, PA, U.S.A.). The carrier gas was helium at 40 ml/min. The injection port, column oven and detector temperatures were 190°C, 130°C and 200°C, respectively.

The efficiency of converting organically bound nitrogen to ammonia was determined as a function of the total hydrogen flow-rate through the NSD. Equal weights of nitrogen were sequentially injected as caffeine and aqueous ammonia. To prevent potential errors caused by on-column adsorption or tailing of the compounds, no chromatographic column was used. Methanol was used to transport the sample to the NSD without liquid splitting. The total hydrogen flow-rate was varied between 0.79 l/min and 2.45 l/min. Because the gas split ratio changed with total hydrogen flow-rate, both caffeine and ammonia were injected at every flow-rate.

Detector performance

The relative efficiency of reductively pyrolyzing organically bound nitrogen to ammonia was determined for several nitrogen functionalities. The compounds listed in Table II were prepared in methanol to a nitrogen concentration of 3 μg N per 20 μl. These standards were introduced into the NSD with methanol at a flow-rate of 0.1 ml/min. The total solvent flow was introduced into the NSD without splitting. Again, the HPLC column was omitted to avoid adsorption-related problems.

The carbon to nitrogen selectivity ratio was measured by comparing the NSD response to nitrogen contained in caffeine to carbon in acetylsalicylic acid (Aspirin). Precision of the NSD was determined using caffeine. Multiple injections of 580 ng nitrogen as caffeine were made onto a Partisil 5 ODS column (Whatman, Clifton, N.J., U.S.A.) using 50% methanol in distilled water as the mobile phase. A calibration curve was generated with caffeine standards chromatographed on a μBondapak C_{18} column (Waters) using 60% methanol in distilled water as the mobile phase.

TABLE II

LIST OF COMPOUNDS USED TO DETERMINE THE RESPONSE FACTORS OF THE NSD TO ORGANIC NITROGEN

n-Butylamine	Di-*n*-pentylamine	N,N-Diethylaniline
n-Pentylamine	Di-*n*-hexylamine	Benzylamine
n-Hexylamine	Triethylamine	Dibenzylamine
n-Heptylamine	Tri-*n*-butylamine	Piperidine
n-Octylamine	Aniline	*o*-Toluidine
n-Nonylamine	N-Methylaniline	Tetramethylurea
n-Decylamine	N-Ethylaniline	Pyridine
Di-*n*-butylamine	N,N-Dimethylaniline	Caffeine

RESULTS

RAP operating characteristics

Pentane, when used as a solvent, was not detected in the gas exiting from the RAP at furnace temperatures above 850°C. The hydrocarbons produced with furnace temperatures between 900 and 950°C, in decreasing order of abundance, are ethane, methane and propane. Carbon in methanol was quantitatively converted to methane at furnace temperatures above 950°C. At temperatures below 850°C, the conversion of the HPLC solvent to low-molecular-weight hydrocarbons is incomplete.

The ratio of NSD response to equal weights of nitrogen individually injected as caffeine and ammonia as a function of total RAP hydrogen flow is shown in Fig. 2. Increasing the total hydrogen flow-rate results in a shorter residence time in the RAP, and a decrease in the response to nitrogen in caffeine relative to nitrogen in ammonia.

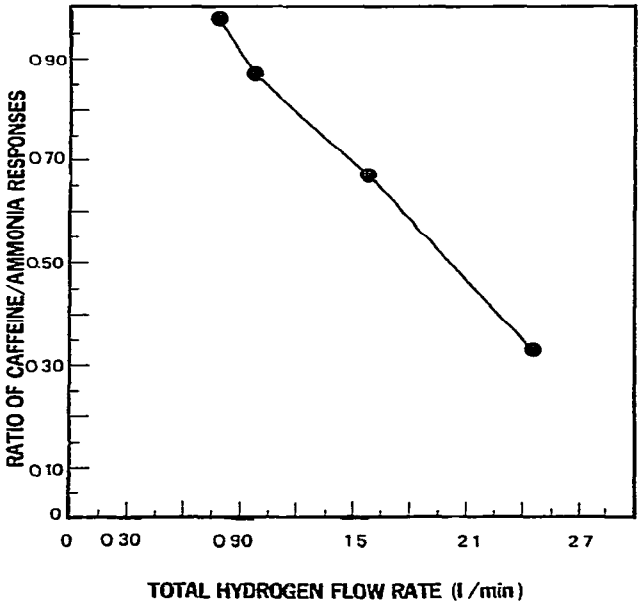

Fig. 2. Effect of total hydrogen flow-rate through RAP on conversion of nitrogen in caffeine to ammonia.

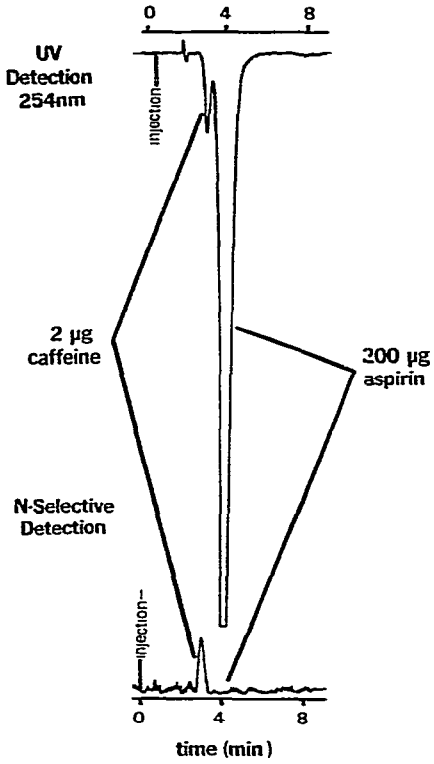

Fig. 3. Chromatogram of caffeine and Aspirin with simultaneous UV and nitrogen-selective detection. HPLC conditions: µBondapak C_{18} column; solvent 0.6% acetic acid in water–methanol (60:40); flow-rate 2 ml/min.

Detector performance

The relative efficiency of converting different nitrogen functional groups to ammonia was measured and expressed in terms of the observed NSD sensitivities (peak height response per µg N). The sensitivities were constant to within 6% relative standard deviation for all compounds listed in Table II. Since the peak widths were identical for all compounds, peak height measurements were used throughout.

The selectivity ratio, defined as the weight of carbon required to produce a detector response equal to that produced by a unit weight of nitrogen, is a minimum of 1×10^4. This selectivity ratio does not include the carbon contribution of the solvent (50% methanol in distilled water). Fig. 3 shows a chromatogram of Aspirin and caffeine purposely using HPLC conditions causing only partial resolution. There is no discernible detector response during the elution of Aspirin. The precision observed for multiple injections of caffeine was better than 4% relative standard deviation for injections of 2 µg caffeine (0.6 µg nitrogen).

A calibration curve is shown in Fig. 4 for standards containing between 0.144 and 28.9 µg nitrogen injected as caffeine. The response of the NSD is linear only over a very narrow range. The limit of detection (signal-to-noise ratio, 2) is 1 ng nitrogen per sec injected onto the chromatographic column with the split ratios used.

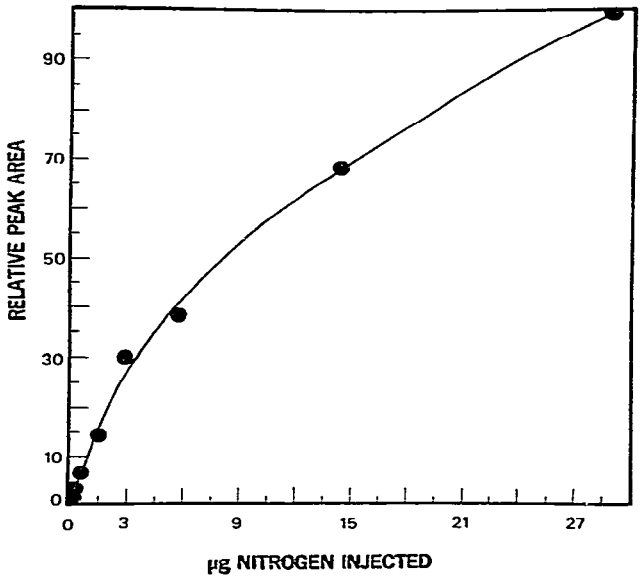

Fig. 4. Nitrogen-selective detector response as a function of nitrogen injected as caffeine.

DISCUSSION

Detector design

The RAP was designed to facilitate the interfacing of an HPLC to an HElCD. The design of the atomizer for introducing the liquid into the RAP is important. Cross flow nebulizers were not effective for aqueous mobile phases within the desirable range of hydrogen flow-rates. The concentric atomizer design shown in Fig. 1 works equally well with both aqueous and organic mobile phases because the hydrogen gas sheath prevents premature mobile phase vaporization.

A 1/16-in. (0.16-cm) liquid introduction tube without the 1/8-in. (0.32-cm) hydrogen sheath tube was often plugged by carbon deposits caused by the premature vaporization of the solvent in the tube.

There was virtually no conversion of organic nitrogen to ammonia when the nickel catalysts were removed. Thus, selective detection of organohalide compounds (which form the corresponding hydrogen halides) is possible using the NSD without the catalyst. Since ammonia does not ionize to an appreciable extent in neat 1-propanol, it can be used as the conductivity solution to provide additional halogen selectivity. When pure distilled water was used to measure the conductivity, the background noise was too high for ammonia measurements.

RAP operating characteristics

The HPLC solvent and eluted peaks are reductively pyrolyzed in the first furnace of the NSD. The first furnace must completely pyrolyze the solvent to low-molecular-weight hydrocarbons and water to prevent HPLC solvent interference in the conductivity measurement. Acetic acid was present at the level of 0.6% in the solvent used to separate aspirin and caffeine (Fig. 3), but there was no measurable

increase in the background conductivity from acetic acid. Therefore, the RAP effectively decomposes acetic acid permitting its use in the solvent.

There are limitations to the composition of the mobile phase. Nitrogen-containing solvents cannot be used since these would also be converted to ammonia and detected. Organohalide solvents, such as chloroform, produce hydrogen halides and result in an unacceptably high background conductivity. Since the HPLC solvent is vaporized, inorganic salts cannot be used in the solvents since they would accumulate in the furnace causing plugging of the quartz tube. Permissible solvents include alcohols, esters, hydrocarbons, organic acids and water.

Since the efficiency of converting organically bound nitrogen to ammonia in the RAP changes with the hydrogen flow-rate (Fig. 2), the hydrogen flow-rate must be optimized for the individual furnace geometries, furnace temperatures and nickel catalysts.

Detector performance

The sensitivity of the NSD for a compound can be estimated knowing its percent nitrogen composition because the relative sensitivities and observed conversion efficiencies were constant for the nitrogen compounds studied. Nitrogen in pyridine and *n*-butylamine is converted to ammonia with equal efficiency. Decomposition of pyridine was expected to be significantly more difficult due to its resonance stabilization. Therefore, it is expected that the NSD sensitivity will not vary with other nitrogen functional groups. Because of the high nitrogen to carbon selectivity, there is no measurable NSD response to aspirin and no distortion of the back side of the caffeine peak (Fig. 3) from the partially co-eluting aspirin peak. Conversion of nitrogen to ammonia is not affected by the increased carbon level during peak elution.

The negative deviation from linearity, shown in Fig. 4, is most likely caused by incomplete partitioning of gaseous ammonia into the water and *n*-propanol solution used to measure conductivity and by incomplete ionization of dissolved ammonia when high levels of organic nitrogen are injected. Standards of ammonia injected over the same nitrogen calibration range exhibit the same negative deviation from linearity at the higher concentrations. Therefore, the negative deviation is not caused by reduced conversion efficiency of organic nitrogen to ammonia at high concentrations. If non-linearity at high concentrations is not tolerable, the use of the neutralization reaction between ammonia and hydrochloric acid, as reported by Jones and Nickless[4], may increase linearity. Also, higher conductivity electrolyte flow-rates should increase the fraction of ammonia ionized. However, this may result in a poorer limit of detection since HElCD sensitivity varies inversely with solvent flow through the conductivity cell. Using the NSD operating conditions discussed, the limit of detection is about 100 ng of a compound (containing approximately 25% nitrogen) injected with the split ratios used.

Detector applications

The detector's sensitivity and high nitrogen to carbon selectivity make possible its application to many HPLC separations using both reversed-phase and normal-phase conditions. Fig. 5 shows the separation and nitrogen-selective detection of adenosine and adenine. The liquid inlet design is important when detecting these and other non-volatile compounds that have high carbon to hydrogen ratios. If these are

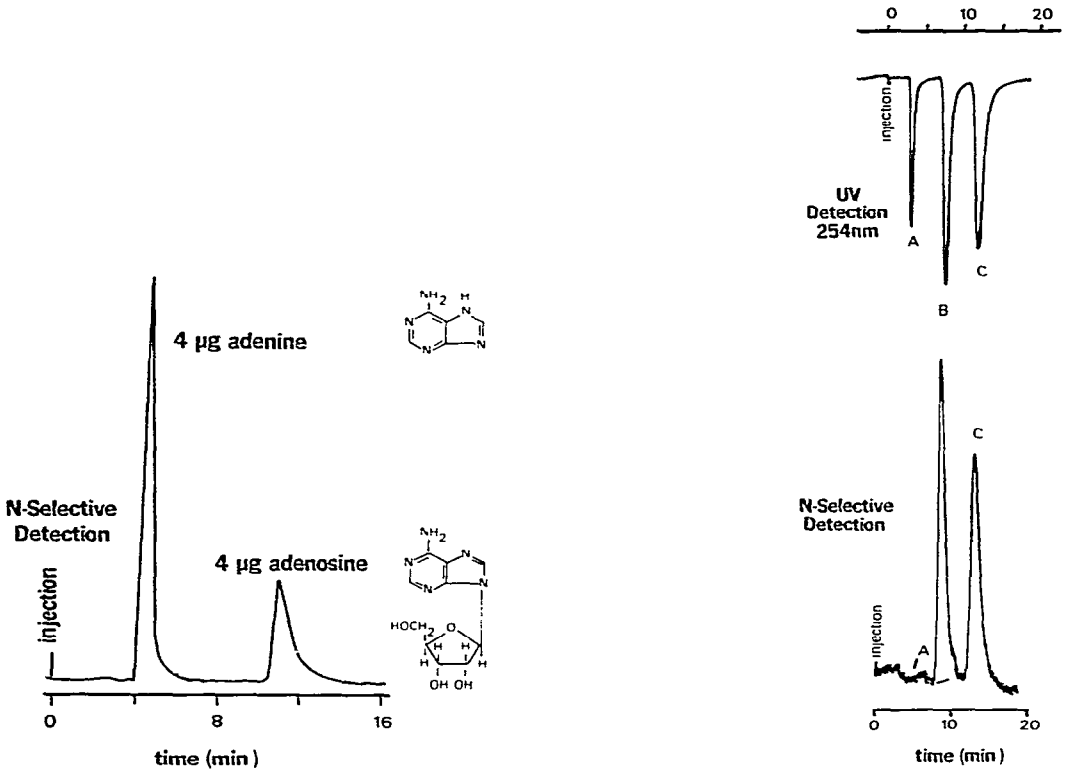

Fig. 5. Nitrogen-selective chromatogram of adenine and adenosine on a Whatman Partisil 5/25 ODS column. Solvent: 0.05% acetic acid in water–methanol (10:90); flow-rate 1 ml/min.

Fig. 6. Chromatography of 200 µg xylenes (A), 20 µg *o*-nitroaniline (B) and 20 µg 3-nitro-4-methylaniline (C) with UV detection (upper) and nitrogen-selective detection (lower). HPLC conditions: Whatman Partisil PAC column; solvent 10% isopropanol in hexane, flow-rate 1.5 ml/min.

decomposed in the 1/16-in. (0.16-cm) O.D. inlet tube, there is insufficient hydrogen present to form C_1–C_3 hydrocarbons. The result is the deposition of pyrolytic carbon which may cause plugging of the liquid inlet tube.

The normal-phase separation of xylenes, *o*-nitroaniline and 3-nitro-4-methylaniline is shown in Fig. 6. UV detection and nitrogen-selective detection were made in series with the UV measurement first. The peaks observed with the NSD are slightly broader than those for the UV detector because band spreading occurred in the UV detector and transfer line to the NSD. There was no measurable NSD response during the elution of the xylenes. The background NSD noise observed is slightly higher for normal-phase conditions than for reversed-phase conditions.

The introduction of mobile phase primarily composed of hexane causes a slight buildup of pyrolytic carbon on the quartz tube. This carbon deposit is readily removed by introducing oxygen with the furnaces heated to about 900°C. An important cautionary note is all hydrogen must be purged from the detector with helium prior to introducing oxygen and *vice versa*. Otherwise a violent hydrogen–oxygen reaction may occur. Typically the quartz tube needs oxygen cleaning daily when the NSD is operated with normal phase separations.

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