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SPECIFIC DETECTION IN GAS–LIQUID CHROMATOGRAPHIC EFFLUENTS WITH ON-LINE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC OPTICAL DETECTORS

ARTHUR KARMEN*, STANLEY LAM, GRACE CUMMINGS and FRANCIS CHOW
Department of Laboratory Medicine, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461 (U.S.A.)

SUMMARY

High-performance liquid chromatographic (HPLC) optical detector technology has been adapted for use with gas–liquid chromatography (GLC). Compounds in the effluent of packed GLC columns were scrubbed continuously, with minimal loss of resolution, into a liquid stream flowing at a nominal rate of 1 ml/min. This was done through the use of a capillary tube that simulated an open-tubular GLC column in which the liquid phase was continuously replaced. The liquid stream was then debubbled and drawn through the flow cell of the appropriate HPLC detector.

Mixing the liquid effluent with *o*-phthalaldehyde reagent led to the formation of derivatives of primary amines and ammonia that were detected with high sensitivity and specificity by their fluorescence. Scrubbing the effluent into methanol followed by on-line monitoring of fluorescence or UV absorbance permitted UV absorbing and fluorescent compounds such as aromatic and heterocyclic compounds to be measured in gasoline with minimal interference from other compounds. The results demonstrated the feasibility of using the high specificity and sensitivity of HPLC detector technology with GLC. This should prove useful in trace analyses in which GLC is the preferred separation technique, but there are interfering quantities of compounds with similar chromatographic properties in the mixture.

INTRODUCTION

In the work described here we studied the adaptation of high-performance liquid chromatography (HPLC) detector technology for use with gas–liquid chromatography (GLC).

HPLC detectors are generally quite sensitive. They respond to the concentration of the analyte in the liquid stream rather than to its gross quantity. The use of low-volume flow cells and low flow-rates of solvent therefore permits small quantities to be detected. They also tend to be quite specific. Even the widely applicable UV monitors are hardly affected by the presence of compounds that do not absorb at the wavelength measured. Fluorometric detectors have even greater selective sensitivity, and both can be used in conjunction with highly specific pre- or post-column

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derivatization reactions. Because of this specificity, their useful sensitivity may be higher than that offered by a more absolutely sensitive GLC ionization detector, particularly when the sample contains compounds with chromatographic behavior similar to that of the analyte and which can interfere when a non-specific detector is used. Since this is so often the case in biological trace analyses, part of the rapid growth in the popularity of HPLC for these applications may be due to the specificity of these detectors. This hypothesis stirred our interest in finding ways of using them with GLC.

Since HPLC detectors characteristically operate as flow-through devices on a liquid phase, we reasoned that adapting them to GLC would premarily require that the analytes be scrubbed from the GLC effluent into a suitable liquid stream without excessive loss of resolution. This approach had been used before in connection with other GLC detection methods. In the first description of GLC in 1952, James and Martin[1] scrubbed fatty acids into an aqueous solution which they titrated continuously with alkali. Labeled compounds have been scrubbed into liquid scintillation fluid for measurement of radioactivity[2]. The column effluent has also been reacted with hydrogen at high temperature and then scrubbed for detecting halogens or nitrogen by coulometry or conductimetry[3].

Several years ago, in devising a method for measuring radioactivity in GLC effluents, we found it feasible to scrub ^{14}C-labeled carbon dioxide quantitatively from a flowing gas stream into a small volume of liquid[4]. We did this by delivering the gas stream to a capillary tube that was simultaneously supplied with approximately 1 ml/min of an alkaline liquid. We considered this tube to be the analogue of an open-tubular GLC column, in which the CO_2 was effectively brought into close contact with the liquid film, could dissolve in it and be retained. The gas was quantitatively trapped in a relatively short length of capillary and there was minimal loss of resolution. We subsequently used the same approach to scrub ammonia from the effluent of a post-column hydrogen reactor[5]. In the work described here, we used it to scrub intact organic compounds from a GLC effluent. We then detected: (1) primary amines specifically by reacting them with *o*-phthalaldehyde and monitoring fluorescence; (2) aromatic compounds in gasoline by monitoring UV absorbance; and (3) fluorescent compounds in the same gasoline by monitoring fluorescence.

Apparatus

GLC was performed in Shimadzu Model GC-3BF and Perkin-Elmer Model 3920 gas chromatographs, both of which are equipped with flame-ionization detectors (FIDs). The columns used were 6 ft. × 1/8 in. O.D. stainless steel. For analysis of free amines, columns were packed with 3% OV-17 on 80–100 mesh Chromosorb W HP (Supelco, Bellefonte, PA, U.S.A.). For analysis of hydrocarbons, columns packed with 2% OV-1 on Chromosorb W were used and the temperature was programmed to optimize the time of the analysis. The effluent of the column was delivered to a 1/16-in. stainless-steel Swagelok tee connector secured to the wall of the column oven. The scrubbing solvent was delivered to a second arm of the tee with either a Milton-Roy Model 396 "Minipump" (LDC, Riviera Beach, FL, U.S.A.), or a Technicon AutoAnalyzer I peristaltic pump. The combined liquid–gas stream emerging from the third arm of the tee was delivered to a "mixing coil" consisting of a 10 cm × 0.7 mm I.D. polyethylene tubing. The stream then entered a debubbler

made from the funnel-shaped end of a truncated Pasteur pipette which was modified by blowing a small overflow hole near the bottom of the funnel. For detecting amines and for measuring fluorescence, the solution was drawn from the debubbler through the 70-μl flow cell of an Aminco Fluoromonitor, Model J4-7461 (American Instruments Company, Silver Spring, MD, U.S.A.), fitted with a Corning-60 excitation filter and a Wratten 8 emission filter by a Buchler "Polystaltic" pump at 0.6–1.0 ml/min. For detecting aromatics in gasoline, the liquid was drawn through the 125-μl "preparative" flow cell of a Tracor Model 100 UV detector operated at the fixed wavelength of 254 nm.

Reagents

1,2-Benzyl-dicarboxaldehyde (OPA) was bought from Aldrich (Milwaukee, WI, U.S.A.). Buffered borate was prepared by dissolving 24 g of boric acid in 1 l of distilled water and adjusting the pH to 9.5 with potassium hydroxide. OPA was dissolved in ethanol (600 mg/20 ml) and filtered into the buffer with stirring. Two ml of mercaptoethanol were then added. The effluent was either scrubbed directly into the OPA reagent solution or was scrubbed into 0.3 ml/min of ethylene glycol to which 0.7 ml/min of OPA was added. For UV absorbance and for fluorescence detection, the effluent was scrubbed into methanol.

RESULTS

Specific amine detection by fluorescence

The feasibility of the concept was tested in analyses of simple mixtures of straight-chain amines. These compounds could be resolved isothermally at 120°C in 6 min with moderate to minimal tailing with the column used, as determined by the record of the hydrogen FID. In these assays, the fluorescence detector did not respond to the injection solvent (Fig. 1). The pentyl amine, buried in the solvent peak in the record of the hydrogen FID, was detected as a well resolved peak by the fluorometer. Comparison of the analytical record with that yielded by the FID showed very little peak broadening, although there was some increase in apparent retention time (Fig. 2). Ammonia in the same mixture, which is undetectable by the FID, was detected by fluorometry, although the response, as reported previously, was less than those for the primary amines.

As can be seen, there was a minimal difference in peak width between the records of the fluorometer and those of the FID. The response of the fluorometer, in peak height, was related to the concentration of amine.

Concentrations as low as 1 nmole of amine were conveniently detected with acceptable signal to noise ratios.

There was minimal decrease in the resolution of the chromatography. Doubling the length of the scrubbing tubing did not increase either peak height or area. Use of a somewhat larger diameter tube increased the delay between the detection of a compound by the FID and detection by the fluorometer and caused a minimal degree of peak broadening.

Detection of aromatics in gasoline by monitoring UV absorbance

As expected, the 254 nm UV absorbance detector responded to aromatics,

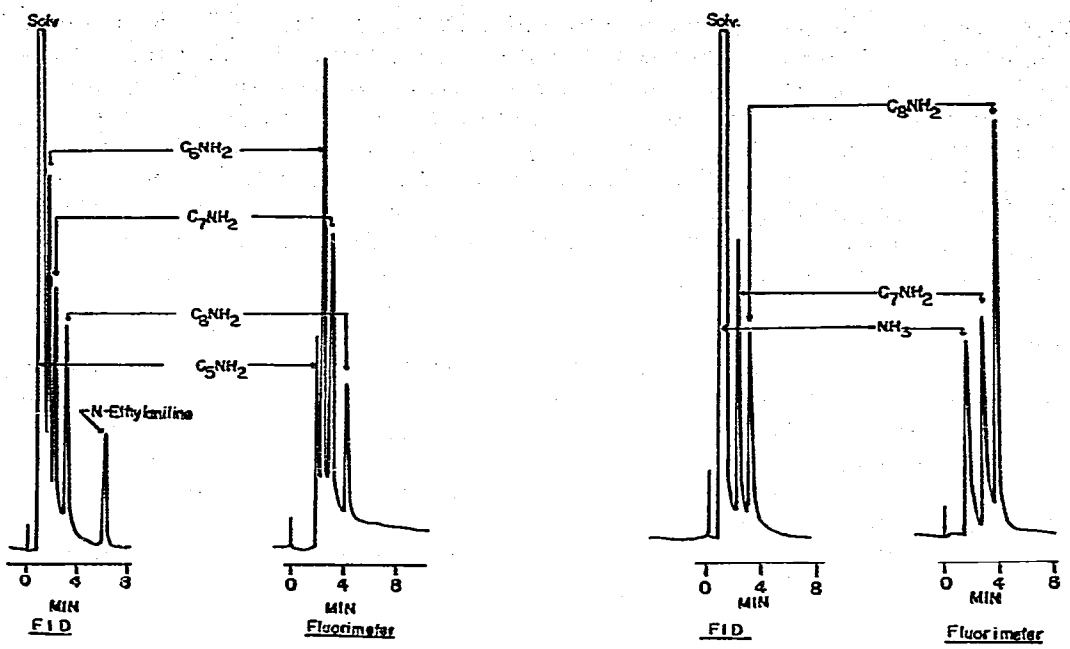


Fig. 1. Detection of aliphatic amines with FID and fluorescence detection. Column: 3% OV-17 6 ft. × 1/8 in. O.D. S.S. Temperature: 120°C. Sample size: 1 μg.

Fig. 2. Separation of aliphatic amines and ammonia. Conditions as in Fig. 3.

but not to the straight-chain hydrocarbons (Fig. 3). The response of the UV absorbance detector to repeated injections of the same mixture of aromatic hydrocarbons demonstrated good reproducibility.

Detection of hydrocarbons in the complex gasoline sample by the FID and the ultraviolet absorbance detector showed that, as expected, a much smaller number of compounds were detected by the UV detector. (Figs. 4 and 5).

Scrubbing efficiency was affected by the solvent used. When water was used in the hydrocarbon assay, less of the aromatics was detected. Methanol and hexane were apparently equally effective.

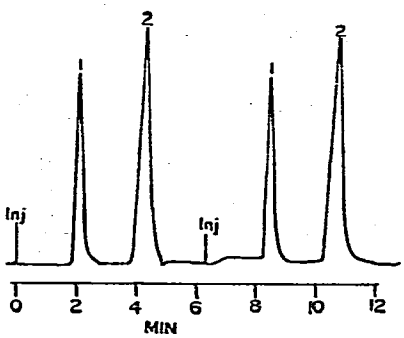
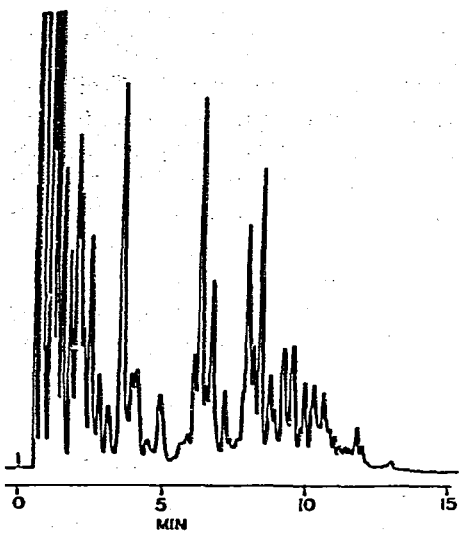


Fig. 3. Detection of benzene and toluene in a GLC effluent by their absorbance at 254 nm. Peaks: 1 = benzene; 2 = toluene.



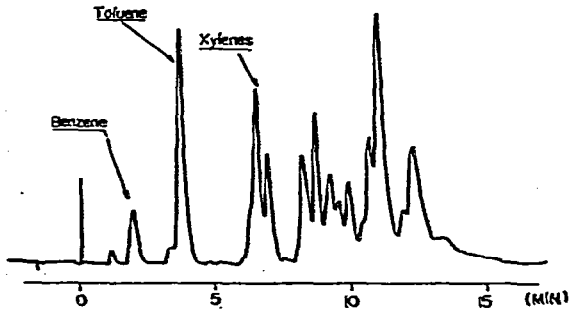


Fig. 4. Detection of hydrocarbons in 1 microliter of gasoline with the FID.

Fig. 5. Detection of the same mixture of hydrocarbons as in Fig. 4 by UV absorbance at 254 nm.

Detection of naturally fluorescent materials

The fluorometer responded to many compounds in the same hydrocarbon mixture (Fig. 6) using the same excitation and emission wavelengths as for amine detection. We made no attempt to identify the fluorescent compounds, or to measure fluorescence at other wavelengths.

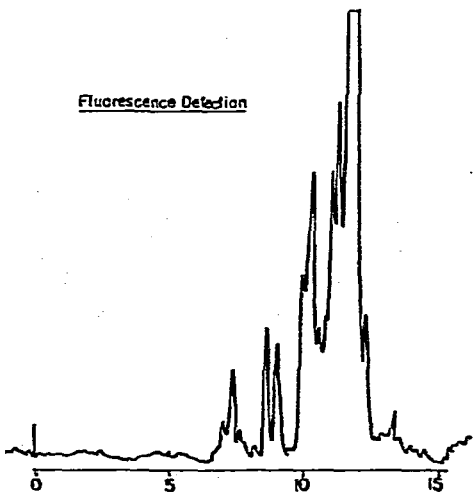


Fig. 6. Detection of fluorescent compounds in the same mixture as in Fig. 4.

DISCUSSION

This study demonstrated the feasibility of detecting amines in a GLC effluent specifically by their fluorogenic reaction with OPA, of detecting compounds that absorb in the UV in the presence of others that do not, and of detecting fluorescent compounds by their fluorescence. Although we analyzed only artificial mixtures of amines, it is reasonable to assume that the approach can be extended to more complicated and interesting analyses.

The specific reactivity of *o*-phthalaldehyde with primary amines is fairly well known. As expected, there was no response to dimethylaniline, used as an example of a substituted amine, or to fatty acids or hydrocarbons, and apparently complete insensitivity to the solvent used for injecting the sample.

Although advances in column technology have permitted some free amines to be analyzed directly without derivatization, in most analyses adsorption on the column is still discernible to some extent. This may limit detection sensitivity and accuracy of quantification. For this reason, amide or trimethylsylyl derivatives of free amines are generally preferred for analysis. Preliminary experiments demonstrated the feasibility of detecting the amines as trimethylsilyl (TMS) derivatives by taking advantage of the susceptibility of these compounds to hydrolysis by water. This should extend the applicability of the technique to compounds with primary amino groups that can be analyzed by GLC as TMS derivatives. On-line hydrolysis of amide derivatives should also be feasible.

Scrubbing high boiling compounds from GLC effluents is somewhat more difficult than scrubbing more soluble as well as more volatile low boiling compounds. Experience with detecting radioactive compounds taught that the column effluent must be kept hot up to the point at which it joins the scrubbing solvent in order to avoid premature condensation and loss of resolution. Aerosol formation must also be avoided. In some of our experiments, we delivered ethylene glycol (b.p. 197°C) to a tee within the heated detector oven of the chromatograph to begin the scrubbing process and added the OPA reagent to the second tee outside the column oven. This maneuver had no apparent deleterious effect on either sensitivity or resolution.

In all these analyses, the most important source of loss of resolution was the debubbler, in which some mixing of the stream occurs. The turnover of liquid in this section of the apparatus is proportional to the volume and inversely proportional to the flow-rate of liquid through it. Since the sensitivity of detection with any given flow cell volume is proportional to the concentration of the solution, we sought to minimize the volume of the debubbler rather than to increase the flow-rate. We limited the volume by providing a small overflow vent at the side of the funnel or, in other experiments, inserted a small diameter suction tube down into the funnel to the level desired.

The volume of the flow cell was also somewhat critical. A sample could be drawn through a 70-μl flow cell at 0.5 to 1.0 ml/min by suction. Flows of this magnitude were not possible in 10-μl flow cells without causing noise in the fluorometer, presumably because of bubble formation.

The technology for converting nitrogen-containing compounds to ammonia by reaction with hydrogen at high temperature has been well worked out for use with other detectors. The ammonia reacts with *o*-phthalaldehyde and yields a fluor-

escent product that can be detected with the same equipment described here. The system can then compete with other nitrogen detectors in sensitivity and selectivity.

We have so far found the sensitivity of the system to be not quite so high as has been reported for the same detection system in HPLC assays of amino acids. We believe that the somewhat higher noise level we have observed is attributable to our less than complete success in eliminating all bubbles from the detector. Some degree of bubble formation may be inherent when reduced pressure is applied to the cell to draw the effluent through it.

UV detection should prove useful in assays of complex biological mixtures, such as those analyzed in "metabolic profiles", by aiding in identifying and classifying compounds as well as by providing somewhat simpler analytical records.

The fluorometer should also prove useful in this kind of application. It may also provide somewhat more reliable quantification than other GLC detectors when the analyte is fluorescent, and emerges from the column incompletely resolved from non-fluorescent compounds. In these analyses, the ability to make measurements at different wavelengths should be helpful.

All these detection schemes should be applicable in trace analyses in which specific compounds are to be assayed in the presence of much larger quantities of other unrelated compounds. In these assays the almost complete insensitivity of the detectors to compounds other than those that are detected should simplify and reduce the number and complexity of the cleanup procedures required prior to GLC.

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