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Detection of alcohols in gas chromatographic effluent by laser-light scattering

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Abstract

A laser-light-scattering detector that is sensitive to alcohols has been developed for gas chromatography. The detector consists of a miniature concentric nebulizer that uses a cold atomization gas and an Ar^+ laser. Calibration curves for the alcohols exhibit characteristic sigmoidal shapes. Signal-to-noise ratios were optimized by examining the photomultiplier tube temperature, collection wavelength and detection scheme (*i.e.*, photon counting *vs.* direct current detection). Limits of detection for five test alcohols were in the 2–8 μg/s range.

1. Introduction

Evaporative laser-light-scattering detection (ELSD) has been shown to be useful for detecting analytes in liquid chromatographic effluent [1–6]. The basic operational principles of ELSD involve the generation of an analyte aerosol free of the mobile phase and the detection of the aerosol by light scattering. To these ends, nebulizers coupled with heated drift tubes are necessary in order to produce the analyte aerosol and minimize or eliminate the mobile phase. Of course, a laser beam and an adequate detection system are required in order to detect the aerosol.

Since its inception, ELSD has been applied exclusively to liquid chromatographic techniques, *e.g.*, high-performance liquid chromatography [1–4], gel permeation chromatography [5], supercritical fluid chromatography [6], etc. Surprisingly, there is no published report of an application of laser-light-scattering detection (LSD) to gas chromatography (GC). In theory, the principle of LSD should be equally applicable to GC. In fact, a heated drift tube would not be required to remove the mobile phase since it is a permanent gas. Therefore, cooling the chromatographic effluent before or during nebulization should allow the detection of the aerosols of the separated analytes by laser-light scattering.

This paper is a preliminary report on the development of a LSD system for GC. The detector described in this paper has shown sensitivity to alcohols. Other compounds, such as benzene, toluene, pentane and chloroform, produce no response from this detector in its current configuration. In order to understand, and ultimately expand, the selectivity of LSD, several optimization studies were conducted. These studies included: the selection of detection wavelength, detector temperature and nebulizer gas temperature. Application of LSD to GC is advantageous from the standpoint of species-

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specific (*i.e.*, alcohol) detection. Specifically, the ability to selectively detect alcoholic compounds in complex matrices (*e.g.*, alcohols in fuel hydrocarbons) would greatly simplify the chromatography since no interference from the hydrocarbons would exist. The applications of LSD to GC will be the subject of a future communication.

2. Experimental

2.1. Apparatus

Fig. 1 shows a schematic diagram of the GC–LSD system. A Coherent Innova Series 70 argon ion laser (Palo Alto, CA, USA) lazing at 514.5 nm with a maximum operating output power of 450 mW served as the excitation source. A Continental Optical (Hauppauge, NY, USA) Pellin-Broca prism was installed near the front of the laser head, which serves as a plasma line filter. At the location where the sample exits the nebulizer, the measured laser power was 80 mW due to scattering losses from the prism and mirrors.

The scattered radiation from the analytes in the GC effluent is collected via a Nikon camera lens (collection throughput of f/3.9) and focused onto the entrance slit of a scanning 1/4-m

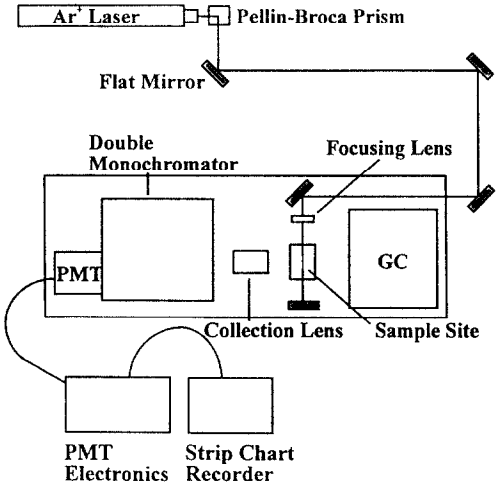

Fig. 1. Schematic diagram of the LSD system.

Digikrom DK 242 double monochromator (CVI, Albuquerque, NM, USA). The double monochromator is fitted with two 1800-g/mm holographic diffraction gratings and employs unilaterally adjustable slits. Unless otherwise noted, the monochromator slits were fixed at widths of 10 μm and the monochromator detection wavelength was fixed at 514.73 nm.

The detection system consisted of a red-sensitive Hamamatsu (Hamamatsu City, Japan) R928 photomultiplier tube (PMT) operating at −1000 V and held 40°C below ambient temperature (final temperature −17°C) by a Thorn/EMI (Fairfield, NJ, USA) Model WCTS-02 thermoelectric cooler. A Thorn/EMI C10 photon counter was employed for the photon-counting measurements. Although the integration time of the photon counter was fixed at 0.1 s for all data acquisitions, its analog output employs a 1-s integration time. The direct current (d.c.) experiments utilized an Analog Modules Model 341-3 current–voltage converter (Longwood, FL, USA) with an adjustable gain of 10–10000 V/A and an output time constant of 1 s. For all the data presented in this paper, the gain of the current–voltage converter was fixed at 10000 V/A. The data from either experiment (*i.e.*, photon counting or d.c. detection) was displayed on an analog strip-chart recorder (Omni-Scribe, Model 5211-12, Bellaire, TX, USA).

A Varian Aerograph Model 90P-3 gas chromatograph (Walnut Creek, CA, USA) was used for all experiments and was fitted with a 0.2% Carbowax 1500 column (180 × 0.20 cm I.D.), 150–175 μm Carbopack C) (Supelco, Bellefonte, CA, USA). The outlet of the column was connected to the nebulizer input capillary (see above) with the use of a 24 cm × 0.20 cm O.D. stainless-steel transfer tube fed through a hole in the side of the GC oven. The transfer tube was then reduced with the use of a voidless reducing fitting to a 0.16 cm O.D. stainless-steel capillary which served as the nebulizer input tube. Thermal tape (Model L-03105-40; Cole-Parmer, Chicago, IL, USA), set at 160°C, was wrapped around the transfer tube in order to prevent effluent condensation. Helium was used as the carrier gas and was held at a constant flow-rate of 30 ml/min.

A miniature concentric nebulizer was constructed in this laboratory and evaluated for its ability to form aerosols from the analytes in the GC effluent. The nebulizer consists of two sections, the head and the body, and is shown schematically in Fig. 2. The nebulizer body was made from a solid block of aluminum 3 3/4 in. (1 in. = 2.54 cm) on each side. The nebulizer head was machined from a 1 in. long × 1 in. diameter aluminum rod and a 0.094-cm hole was drilled through the center. As shown in Fig. 2, the nebulizer head was mounted on the body by boring a 1.85-cm diameter hole at one end of the aluminum block. A 0.094-cm diameter hole was drilled through the center of the aluminum block to accommodate the capillary transfer tube which passes the GC effluent to the nebulizer head. The capillary tube was admitted into the bottom of the body through a standard 1/4-in. nominal pipe thread (NPT)-to-Swagelok connector. A septum, placed into the Swage end of the connector and tightened with a 1/4-in. nut, prevented leaking of the nebulizer air.

The nebulizer capillary tubing was placed into the center of the 0.094-cm hole (2 mm from the outlet), and the head inserted into the body and sealed via an O-ring. The orifice where the capillary tube enters the nebulizer head is sealed with silicon sealant (part 64-2314B; Archer, Fort Worth, TX, USA) in order to restrict the nebulizer gas from passing into the cavity of the nebulizer body. Air was used as the nebulizer gas and was introduced into the head through a standard 1/4-in. NPT fitting tapped into its side. Standard 1/16-in. copper tubing connected the air-tank regulator output and the 1/4-in. NPT fitting. A type K thermocouple purchased from Omega (Stamford, CT, USA) was used to monitor both the temperature of the capillary transfer tube and the temperature of the nebulizer gas.

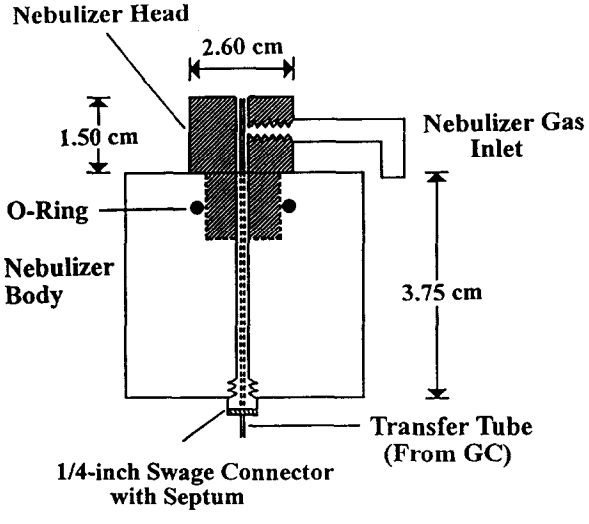

Fig. 2. Schematic diagram of the miniature cross-flow nebulizer.

2.2. Reagents

All chemicals were of reagent grade and were obtained from Fisher Scientific (Itasca, IL, USA) and were used as purchased. Helium as the carrier gas for the GC system and compressed air for the nebulizer were obtained from local sources and were used as purchased.

2.3. Procedure

Prior to data acquisition, the GC and spectrometer system were allowed to warm up for 1 h. The GC column temperature was fixed at 130°C, the injector temperature was held at 150°C and the temperature of the heat tape surrounding the transfer tubing was held at 160°C. Calibration curves for the alcohol test compounds were obtained by plotting the average peak area (triplicate measurements) *vs.* concentration. Limits of detection were obtained by injecting successively smaller amounts of a compound until its corresponding signal measured $2N_{\text{peak-to-peak}}$ (where N = noise) of the GC system.

3. Results and discussion

3.1. Instrumental considerations

The optical design of this GC detector, shown in Fig. 1, is modeled from a conventional fluorescence spectrometer with a 90° scattering geometry. Analytes exiting the chromatographic column pass through the heated transfer tube and

are aspirated into the path of the laser beam by a miniature concentric nebulizer. The aerosol of the separated analytes is generated simultaneously with nebulization by employing a cold nebulizer gas (see below). The resulting scattered radiation from the aerosol of the eluting species is collected by the lens and focused onto the entrance slit of the double monochromator. The signal generated by the PMT is measured via the photon counter and displayed on the chart recorder.

The nebulizer assembly is mounted on a three-direction translation stage and is shown schematically in Fig. 3. The x–y–z translation stage provides sufficient flexibility for aligning the outlet of the transfer tube directly over the path of the laser beam. The inverted geometrical arrangement of the nebulizer (as opposed to upright) was found to be useful in facilitating the generation of the aerosol.

The GC system used in these experiments, because of its age, did not have temperature-programming features. In addition, capillary columns were incompatible with the oven due to size and plumbing requirements. Thus, a packed column with a fairly polar stationary phase was recommended by its manufacturer (Supelco) for the separation of alcohols.

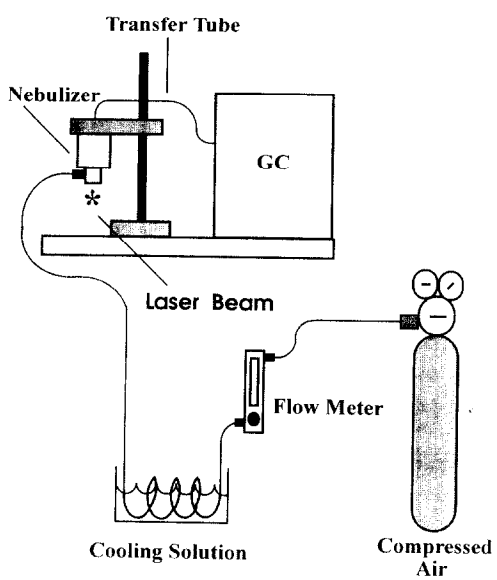

Fig. 3. Diagram of the nebulizer geometry relative to the outlet of the GC system.

3.2. Gas chromatograph interface

The essential design consideration of the ELSD system is in the generation of an aerosol with a high concentration of analyte and a relatively low concentration of mobile phase. In contrast, the major concern in the application of LSD to GC is in the generation of an aerosol from the gas-phase analyte. Since the mobile phase is a gas with an extremely low boiling point, separation of the analyte from the mobile phase is unnecessary. However, the formation of the analyte aerosol is of primary concern because the analytes exiting the chromatographic column must be cooled before or during nebulization in order to provide the nebulizer with a liquid. In this detector, cooling is accomplished simultaneously with nebulization by employing a cold nebulizer gas. As the analytes exit the transfer tube they come in contact with the cooled stream of air and condense to form an aerosol.

The nebulizer gas is cooled by submersing a portion of the nebulizer supply tubing (180 × 0.62 cm I.D. copper tubing wound into a coil) into either a solution of dry ice–acetone or liquid nitrogen. The cooling solution lowers the temperature of the nebulizer gas by an amount corresponding to the length (*i.e.*, the number of copper coils) submersed in the cooling solution and the flow-rate of the nebulizer gas. With a nebulizer-gas flow-rate of 19.4 l/min (the maximum flow-rate examined in this study), the dry ice–acetone bath and liquid nitrogen bath allowed temperatures as low as +10°C and −10°C to be attained, respectively. It should be pointed out that temperatures higher than about 0°C could not be obtained with the liquid nitrogen bath. In addition, temperatures below −10°C could not be obtained with either bath due to the freezing of trace amounts of water in the nebulizer supply gas.

Since the formation of the analyte aerosol depends on the temperature of the nebulizer gas, the effect of cooling the nebulizer gas on the

signal-to-noise ratio (S/N) was examined. Ethanol was chosen as a representative analyte, and the temperature of the coolant gas was adjusted in the range of −10 to +25°C using either the dry ice–acetone mixture or the liquid nitrogen. The critical parameter in cooling the nebulizer gas was maintaining the nebulizer flow-rate at 19.4 l/min (see below). To achieve both cooling and proper nebulizer gas flow-rate, individual coils of the copper tubing were submersed in the cooling solution. This approach allowed different temperatures to be obtained while maintaining the flow at a desired rate.

The results from this temperature study show that the optimum S/N for ethanol is found at a cooling temperature of +10°C. As the temperature of the nebulizer gas is decreased from +25°C to −10°C, the S/N for ethanol begins to increase and reaches a maximum at +10°C. At temperatures below +10°C, the ethanol freezes at the tip of the transfer tube and causes the nebulizer to sputter. Thus, the gaseous ethanol eluting from the gas chromatograph is not converted into the liquid phase until the nebulizer gas is sufficiently cooled below ambient temperature. Therefore, all the data discussed in the proceeding sections were obtained by maintaining the nebulizer gas at a temperature of +10°C.

The optimum flow-rate of the nebulizer gas for this capillary nebulizer was determined to be 19.4 l/min. This flow-rate is needed to ensure complete expulsion of small sample quantities from the transfer tube. As the flow-rate is decreased, the ability to properly aspirate the separated analytes begins to decrease until the flow-rate is so slow that essentially no aerosol is generated. On the other hand, if the flow-rate of the nebulizer gas is too high, the droplet size becomes too small and decreases the amount of scattered radiation. It should be noted that previous work by Stolyhwo *et al.* [7] showed that ELSD in liquid chromatography require significant flow-rates of nebulizer gas as well. Of course, the high nebulizer flow-rate may be minimized by decreasing the diameter of the nebulizer tip. A smaller diameter would effectively give rise to the same nebulization effect but at a lower nebulizer gas flow-rate.

3.3. Detection wavelength

Although several studies have examined the effect of the excitation wavelength on the detection signal, little work has been undertaken in examining the effect of the choice of detection wavelength [8,9]. A collection wavelength study was performed over the wavelength interval of 514.14–514.90 nm. Triplicate 0.5-μl aliquots of ethanol were injected onto the column, and the corresponding baseline noise and signal of each injection was recorded at each of the specified wavelengths. The average values of the signals and the peak-to-peak noises were tabulated, and plots of wavelength *vs.* relative signal and wavelength *vs.* relative noise are shown in Fig. 4. It should be noted that the magnitudes of the noises and signals shown in Fig. 4 are in relative logarithmic intensity units.

It can be concluded from Fig. 4 that the signal begins to rise appreciably near 514.25 nm. The signal then plateaus from 514.40–514.75 nm and then drastically decreases. The noise recorded at each wavelength appears to follow the same general trend as the signal except in the range of 514.60–514.90 nm. Within this range, the magnitude of the noise is significantly low and is due to the dark-current noise of the PMT. At 514.73 nm, the optimum collection wavelength, the

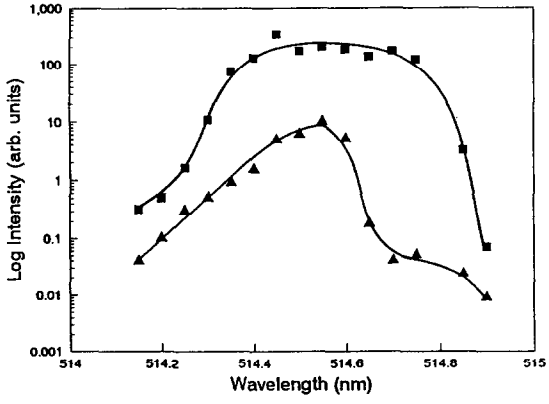

Fig. 4. Logarithmic intensity (arbitrary units) of the signal and the noise from triplicate 0.5-μl injections of ethanol as a function of collection wavelength (nm). The squares represent the signal and the triangles represent the noise at each of the wavelength intervals.

noise is 200-fold lower than observed at the Rayleigh line (514.54 nm).

This experimental result may have direct application in the ELSD systems more commonly used in high-performance liquid chromatography. These systems detect radiation at the wavelength of the excitation source (in fact, wavelength selection devices are not generally employed). The increase in the noise at the Rayleigh line is attributed to extraneous scattering from the mirrors and atmospheric particles (*i.e.*, dust). A spectrum of the Rayleigh line, a scan from 514.30–514.90 nm, shows that the intensity of scattering due to the atmosphere and the mirrors is approximately 5-fold greater at the Rayleigh line than at 514.73 nm. Thus, the collection wavelength throughout this study was fixed at 514.73 nm, which yielded an S/N for ethanol that was 200-fold greater than that observed at the Rayleigh line.

3.4. Photon counting vs. d.c. detection

To date, all LSD systems employ PMT circuits that utilize d.c. detection. Specifically, the current output of the PMT detectors is converted to a voltage signal with the use of a suitable preamplifier. It is well-known that under some circumstances, *e.g.*, detector-noise limited measurements, photon counting can improve S/N by factors of 3–10 [10]. These improvements are made possible with the use of discrimination electronics to effectively reduce dark-current counts.

Photon-counting detection was compared to d.c. detection by injecting triplicate 0.5-μl aliquots of ethanol onto the packed column. In order to compare the results obtained by photon counting, the same experiment was conducted with the exception of the replacement of the photon-counting electronics with a current-to-voltage converter. The average S/N obtained from the 0.5-μl injections for photon-counting detection and d.c. detection were 567 and 20, respectively, at a sample laser power of 80 mW. This result shows that there is a significant improvement in the S/N (28-fold) when utilizing photon-counting *vs.* d.c. detection.

In d.c. detection, the signal and noise of the PMT are measured simultaneously. If the observed signal is quite intense, then the dark current noise of the PMT becomes insignificant. However, the signals observed in this detector are very weak (on the order of Raman scattering signals). Therefore, the noise of the PMT becomes more predominant as the strength of the signal decreases, which results in the poor S/N values observed for d.c. detection. Photon counting discriminates between the signal and the noise, therefore making photon counting more useful in low-light-level detection.

3.5. Detector cooling

It is a common practice in many "low"-light-level techniques, such as Raman and fluorescence spectroscopies, to cool the detector below ambient temperature in order to improve the S/N by reducing the dark-current noise [11]. Consequently, an experiment was conducted to determine if a significant difference exists in the S/N values of chromatograms obtained with a non-cooled PMT (ambient temperature, 23°C) *vs.* that obtained using a cooled PMT (−17°C). Triplicate 1.0-μl injections of ethanol were introduced onto the column and the corresponding average S/N values were calculated for both the cooled and non-cooled PMT.

It was found that the S/N obtained for the cooled PMT was twofold greater than the S/N for the ambient-temperature PMT. The principal reason for the decrease in the S/N for the ambient-temperature PMT was the increase in the dark current. The dark current increased from 350–400 counts/s (cooled tube) to 700–800 counts/s (ambient tube). Since the dark-current noise in a PMT is proportional to the square root of the dark current, an improvement in the S/N on the order of 50% would be expected. Therefore, all chromatographic data presented in this paper utilized the cooled PMT.

3.6. Analytical calibration data

Fig. 5 shows calibration curves for methanol, ethanol, isopropanol, propanol and butanol.

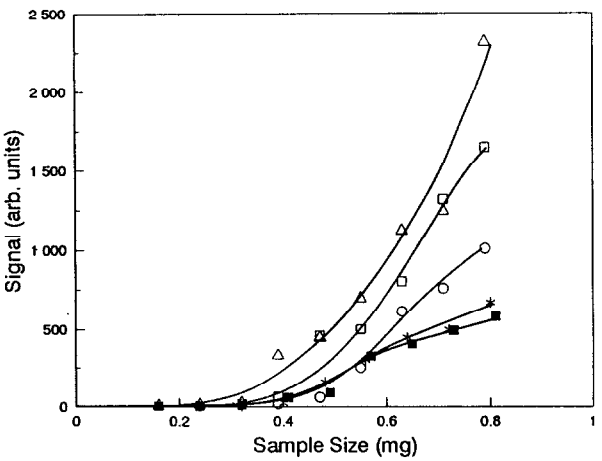

Fig. 5. Calibration curves for methanol (□), ethanol (△), isopropanol (*), propanol (○) and butanol (■).

Each calibration curve was obtained by injecting successively larger amounts of the pure compound onto the column. As can be seen in this figure, the calibration curves are sigmoidal-shaped over the entire concentration range.

It has been well demonstrated that sigmoidal calibration curves are obtained from light-scattering detectors employed in liquid chromatographic methods [5,7,9,12–14]. These sigmoidal curves have been determined to arise from the change of the average aerosol-particle diameter as the amount of the analyte is varied. Since the concentrations of the analytes in this gas chromatographic experiment are less than $2 \cdot 10^{-4}$ g/ml (assuming an average retention volume of 4.0 ml at a carrier gas flow-rate of 30 ml/min), exponential/sigmoidal-shaped calibration curves would be predicted [5]. Thus, the shapes of these calibration curves (the lower portions of the sigmoids) are in agreement with the prior work on the application of LSD to liquid chromatography.

It should be noted that the deviation from linearity of the calibration curves is attributed to particle size effects and not to non-linearity of the photon counter. The upper limit of linearity for the photon counter employed in these experiments is 10^6–10^7, according to the manufacturer's specifications [15]. Typically, the highest photon counts observed in 1.0 s was $6 \cdot 10^5$ for a 1.0-μl injection of ethanol, which is well within the linearity range of the photon counter.

After characterization of the operating parameters of the LSD system with respect to the five alcohols, the LSD responses to various compounds were examined. It was immediately observed that compounds such as toluene, benzene, pentane, tetrahydrofuran, ethylene glycol and 1,2-dichloroethane yielded no appreciable signal. However, all alcohols used in the study yielded significant signals. The apparent sensitivity of this system to alcohols has been tentatively appointed to the surface tension of the nebulized droplets. Hydrogen bonding in the alcohols is much greater than that for the other compounds tested. This hydrogen bonding is thought to enhance the formation of droplets when the liquid effluent is nebulized. Further work is underway in this laboratory to gain insight into this phenomenon.

The limits of detection of the five test alcohols were determined by making triplicate injections at decreasing injection sizes until the signals were buried within the noise of the system. Table 1 presents a compilation of the limits of detection for the five test alcohols obtained under identical operating conditions. In addition, Table 1 lists the reproducibilities of each of the triplicate injections. As can be seen from Table 1, the limits of detection range from 7.54 to 2.46 μg/s and increase approximately as the boiling points of the alcohols decrease. However, the series of the five examples is interrupted by ethanol exhibiting a limit of detection similar to that obtained for butanol.

Table 1
LSD calibration data for five alcohols

Alcohol	Limit of detection[a] (μg/s)	R.S.D.[b] (%)
Methanol	7.54	7.0
Ethanol	2.65	7.4
Isopropanol	4.49	7.4
Propanol	4.06	7.1
Butanol	2.46	6.5

[a] Measured at a signal intensity equal to $2N_{\text{peak-to-peak}}$.
[b] Determined from quadruplicate 1.0-μl injections.

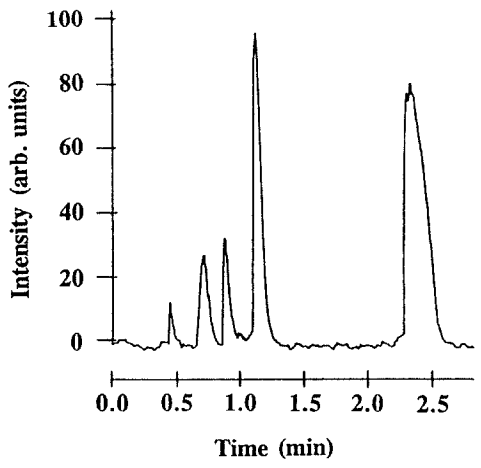

Fig. 6. A chromatogram of a 3.0-μl injection of methanol, ethanol, isopropanol, propanol and butanol (in order of elution). Operating parameters: injector temperature 150°C, column temperature 130°C, carrier gas flow-rate 30 ml/min, nebulizer flow-rate 19.4 l/min, nebulizer gas temperature 10°C.

3.7. Chromatographic application

A typical chromatogram obtained with this LSD system is shown in Fig. 6. Although all the peaks in the chromatogram of Fig. 6 are baseline resolved, they are somewhat asymmetrical and exhibit tailing in their trailing edges. This slight asymmetry is most likely due to poor chromatography. It should be noted that butanol, which is the last compound to elute from the column, appears in the chromatogram as slightly "split". This splitting is presumably due to a slight sputtering of the cold butanol as its aerosol is being generated. Sputtering can be eliminated by warming the nebulizer gas slightly, but at the expense of a poorer detection limit.

4. Conclusions

LSD can be applied to GC provided the analytes in the GC effluent can be sufficiently converted to the liquid state and nebulized. However, the LSD selectivity is apparently limited to alcohols. The results of various S/N studies show that the S/N ratio is increased 200-fold by detecting the scattered light at 514.73 nm rather than at the Rayleigh line, increased twofold by cooling the PMT to −17°C, and increased 28-fold by using photon counting instead of d.c. detection. These improvements result in an overall S/N enhancement greater than four orders of magnitude.

5. Acknowledgement

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