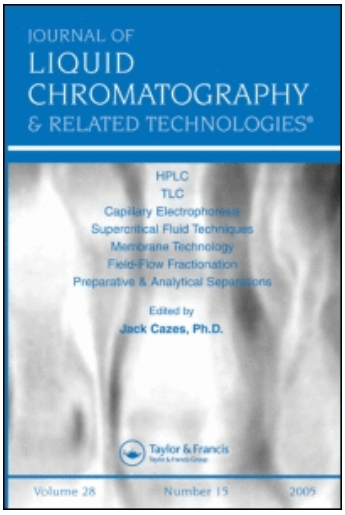

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Haleem J. Issaq[a]
[a] Chemical Synthesis and Analysis Laboratory, NCI-Frederick Cancer Research Facility, Frederick, Maryland

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A COMBINED THIN-LAYER CHROMATOGRAPHY/MICRO INFRARED METHOD

Haleem J. Issaq
Chemical Synthesis and Analysis Laboratory
NCI-Frederick Cancer Research Facility
P.O. Box B
Frederick, Maryland 21701

ABSTRACT

A method is described for the automatic elution of chromatographed compounds on thin-layer chromatography plates and their subsequent identification by micro infrared spectroscopy.

The method is simple, easy to perform in a few minutes, and requires 5 μg of material, 3 mg of KBr powder, and 150 μl of solvent.

INTRODUCTION

The use of combined techniques for chemical analysis is not only useful but in most cases time saving. The combination of chromatography, which gives a pure sample, and spectrometry, which provides structural information, supply the analyst with valuable data.

Numerous gas chromatography/mass spectrometry (GC/MS) (1-5), liquid chromatography/mass spectrometry (LC/MS) (6-10), and recently thin-layer chromatography/mass (TLC/MS) (11) coupling systems have been reported. The combination of TLC with flameless atomic absorption spectrometry for the identification of inorganic ions and organometallic complexes has

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been described, and that of TLC with infrared spectroscopy (TLC/IR) was summarized by Szekely (13). However, most of the methods described require scraping and subsequent elution of the sample from the adsorbent which leaves adsorbent particles in the eluate. These particles cannot be removed by filtration and, as a result, interference signals are observed. These aforementioned methods require a large amount of solvent, compared with the sample, for spot elution. They are time consuming and in certain cases, the recoveries are not quantitative.

This paper presents a combined TLC/IR method which, unlike the other methods (13), uses a novel elution technique requiring approximately 150 µl of solvent for quantitative elution. The sample obtained is free from the adsorbent and ready for analysis. Six samples can be eluted from the plate simultaneously. The amount of sample required is approximately 5 ug.

EXPERIMENTAL

Reagents and Materials

Silica gel 60 precoated TLC plates (EM Labs, Inc., Elmsford, NY) were used after activation at 110°C for 2 hrs. All solvents were previously distilled in glass (Burdick and Jackson, Muskegon, MI). Benzo(a)pyrene (B(a)P), and dimethylbenzanthracene (DMBA) (Aldrich Chemical Co., Milwaukee, WI) and pyrene Chem Service, Inc., West Chester, PA) were used without purification. Sample solutions were freshly prepared in chloroform. Benzene: hexane (10:90) was used as a developing solvent, and methanol as an eluant.

Apparatus

IR. Perkin-Elmer, (Norwalk, Conn.) Model 180 IR spectrometer with a 6X beam condenser attachment was used. KBr pellets were prepared using a Perkin-Elmer ultra-micro die kit and the AgCl mini-cell windows (Wilks Scientific, Norwalk, Conn.) were supported on a Perkin-Elmer demountable micro cell mount.

TLC. The Eluchrom Automatic Elution System by Camag, Inc. was used to elude the spots from the plate. Standard TLC developing tanks were used. Samples weere spotted and eluted under subdued light in an inert atmosphere (nitrogen) to prevent sample decomposition.

Method

10 µl of freshly prepared solution (1 µg/µl) were spotted on silica gel plates which were then developed in benzene:hexane (10:90). Following developement the plates were dried under nitrogen at room temperature. The plate was then covered with a clean glass plate and spots were located under UV radiation. A circle perimeter of 2.5 cm was scored through the plate adsorbent coating and centrally about each spot to be eluted, using a special milling device which is provided as an accessory to the Eluchrom System. After a plate was scored, it was placed on the Eluchrom unit and an elution head was placed over each scored circle. The elution heads were tightly clamped on the plate, forming a teflon (elution head) - glass (TLC plate) seal. Eultion was carried out at the slowest flow setting (0.1 ml/min) and the spot eluant was collected into a clean test tube. 150 µl of methanol was required to quantitatively elute each spot. The sample was eluted into a 0.5 ml reaction tube (Supelco, Inc., Bellefonte, PA), containing approximately 3 mg of KBr powder (Harshaw Chemical Co., Solon, OH). After elution the reaction tube containing the sample was placed overnight in a vacuum oven at 35°C to assure complete dryness (2 hours are an adequate time). The dry KBr powder with the sample was formed into a 1.5-mm pellet of which the spectra was recorded. A background spectrum was obtained for a blank which was similarly prepared from part of the plate where no samples were spotted.

Alternatively the eluant was collected in a clean 1 ml screw cap septum vial in which the solvent was evaporated to 10 µl. The sample was then deposited in the center of a silver chloride (AgCl) mini-cell

window using a 10 µl syringe. The sample deposit was built up as a circle with a diameter of about 2-mm, by carefully transferring the solution to the AgCl window in 2 µl portions and evaporating the solvent with nitrogen before further addition. A demountable micro cell mount was used to support the AgCl window. A 2-mm metal disc pellet holder was sandwiched between the AgCl window and the base-plate in order to mask off an area of the crystal so that only radiation passing through the sample was allowed to reach the detector. The spectra were then recorded using a Perkin Elmer X6 beam condensor and a reference beam attenuator. Using a blank AgCl window, a transmittance reading of 48% was obtained at 1,000 cm^{-1} while under similar conditions a blank 1.5-mm KBr pellet transmitted ~ 10% of incident radiation. The better energy transmittance offers advantages in terms of spectral recording accuracy and scan time saving.

DISCUSSION

The use of combined TLC/IR is not new (13), however, almost all of these methods require (a) eluting the sample from the adsorbent after scraping the spot and (b) redissolving the concentrated extract in a minimum amount of solvent or forming a micro-KBr pellet. The disadvantages of scraping and subsequent elution were recently discussed (14). The most prominent disadvantage and the one that affects the results most is that the extremely fine adsorbent particles cannot be removed from the eluate by filtration or centrifugation and leads to interference signals. For example, silica gel produced strong signal at about 9 µ.

Much better results are obtained by those methods in which the substance is transferred with a little solvent from the spot into the KBr powder. Some of the techniques used for obtaining a sample free from the adsorbent are (a) the wick-stick method (15) which utilizes a KBr triangle (25 x 8 x 2 mm) (Harshaw Chemical Co.). The triangle is placed

in a small beaker. The adsorbent, which contains the separated substance, is transferred into the beaker, a small amount of solvent is added, the substance is extracted from the sorbent, the solution rising in the wick-stick, and the solvent evaporates in the upper region where the compound accumulates at the tip of the triangle. The tip is cut, dried and used to obtain the spectrum. The advantage of this method is that it enables the spectra to be obtained free from background interferences. Good IR spectra are obtained for 10 μg of component, however, the recovery is 50-80%; (b) the direct transfer method (16) requires 20 μg of KBr and works as follows: after the spot is located on the plate, the adsorbent around the spot is scraped to form a "tear-drop" shape. The KBr powder is then placed on the plate in the form of a rod 6-8 mm long and 2 mm wide. The KBr is in contact with the adsorbent. The substrate is then eluted from the adsorbent to the KBr by adding solvent from a syringe to the adsorbent. The nearer half of KBr which is in contact with the adsorbent is discarded. Spectra is obtained by using the other half of the KBr. The disadvantages of the method are (i) 20 mg of KBr are used, this is too large an amount for micro sample; (ii) too rapid an addition of solvent can lead to a loss of substance; and (iii) not all the sample is eluted. In a modification of the above method De Klein (17) formed a half-moon arrangement from the KBr. A good spectra was obtained but severe contamination effects were obvserved due to the presence of adsorbent material. (c) Another microtransfer procedure was developed (18) in which the TLC spot is scraped off, collected and placed on top of a small amount of KBr powder, tamped down the cone joint of an 18-gauge hypodermic needle. A 1-ml glass syringe is filled with pure acetone, connected to the needle and solute is eluted drop by drop into 10 mg KBr. Each drop is allowed to evaporate completely before the addition of the next drop. About twenty drops are needed. A 1.5 mm disc is formed. 5-30 μg of sample is needed. (d) The plate transfer method (19) uses

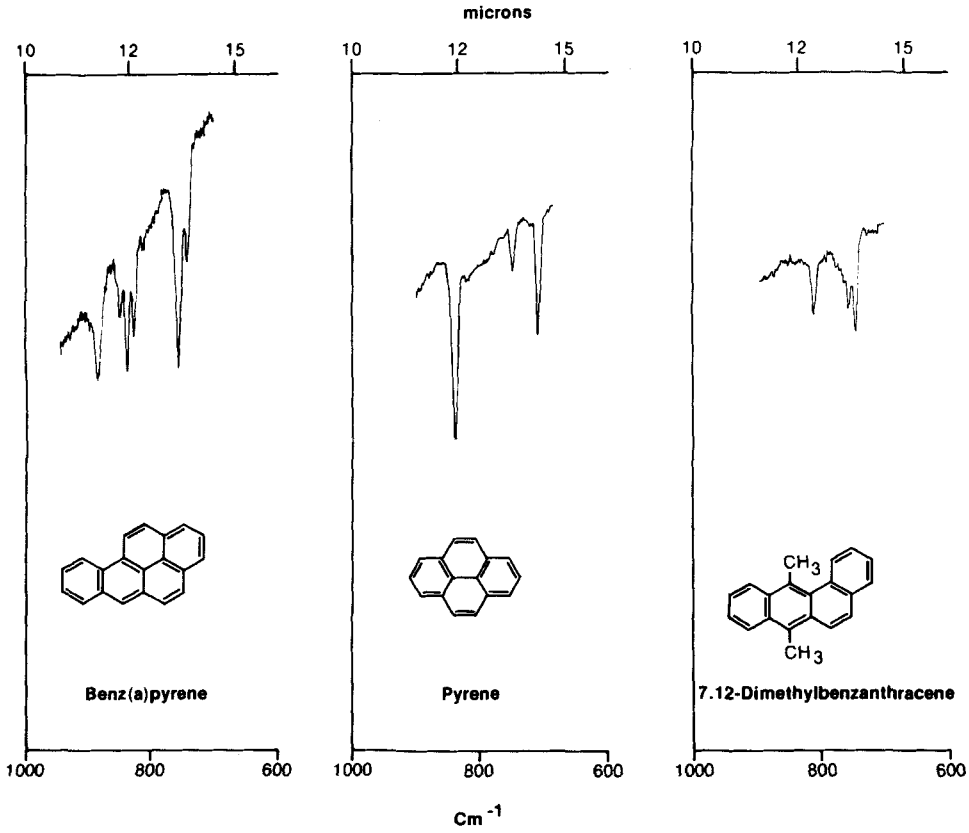

FIGURE 1

two plates, one coated with an adsorbent, the other with KBr, connected layer against layer the sample is spotted on the adsorbent layer after separation spots are eluted from the separating layer into the KBr layer. The size of sample required for identification is 50 µg.

All the above transfer methods have a common weakness, namely quantitative transfer of the sample from the plate into the KBr free of adsorbent. This was easily achieved in our laboratory by employing a unique elution system, the Eluchrom automatic elution system. This unit is capable of eluting six samples simultaneously. The amount of solvent

required for elution of each sample is approximate 150 µl. The sample is eluted directly into 3 mg KBr, placed in a 0.5 ml reaction vessel, after which the sample is evaporated and a 1.5 mm KBr pellet is prepared from which the spectra (shown in Figure 1) was recorded. The advantages of this method are:

1. A sample free of adsorbent is obtained (no silica gel bands were observed).

2. Only 150 µl of methanol was required to elute each of pyrene, benzo(a)pyrene, and dimethylbenzanthracene quantitatively with better than 98% recovery.

3. Six samples can be eluted simultaneously in approximately two minutes.

4. No loss of sample due to scraping and transferring, since these steps are eliminated by using the Eluchrom.

The amount of sample needed to produce good quality IR spectra ranges between 1-10 µg, depending on the sample. 5 µg were needed to produce the spectra shown in Figure 1.

Compared to the other TLC-IR techniques, the method presented here is superior, it is quantitative, sensitive, and time saving.

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