

Separation and Identification of Multicomponent Mixtures Using Centri-Chromatography/Mass Spectrometry

Francis W. Karasek and Paul W. Rasmussen

University of Waterloo, Waterloo, Ontario

THE TECHNIQUE OF Centri-Chromatography involves the application of centrifugal force to accelerate the migration and separation by solid-liquid chromatography of samples through columns of microparticulate silica. Based upon the findings reported by others (1-3), the technique has been developed by Ribí (4) and recently reviewed by Karasek (5). The method has been applied successfully to the separation of steroid hormones (6), lipids (7, 8) and cannabinoids (9).

The Centri-Chromatography apparatus (Centri-Chrom) consists of a chromatographic unit which fits into the rotating buckets of a standard centrifuge. Details of the instrumentation and its operation have been reported previously (5, 6). Up to a milligram of a sample mixture can be separated without loss of resolution (7) and as little as 2 micrograms of a component in the mixture can be detected using conventional thin layer chromatography detection methods (6).

Although the method permits rapid and very selective separations of complex mixtures in the 2-100 microgram range, the lack of a detector at the column outlet requires the use of conventional methods of identification of the component bands separated on the column. Component identification by retention time comparisons is a time consuming and inaccurate method. Since these component concentrations are suitable for mass spectrometric analysis, this investigation was undertaken to explore the qualitative capabilities of the mass spectrometer with its potential for rapid and positive identification.

EXPERIMENTAL

Apparatus. A prototype of the Centri-Chromatography apparatus (Centri-Chrom, Ivan Sorvall Inc., Norwalk, Conn.) was used. The mass spectrometer was a Hitachi Perkin-Elmer RMU-6E single focusing instrument. Sections from the chromatography columns were easily introduced into the mass spectrometer through the standard liquid inlet system. Comparable results could be obtained using the solid inlet system, if small amounts or involatile components were present.

Reagents. The column packing used in these experiments was microparticulate silica (Quso G-32, 100-200 Å size, Philadelphia Quartz Co., Philadelphia, Pa.), packed and developed with hexane-benzene mixtures.

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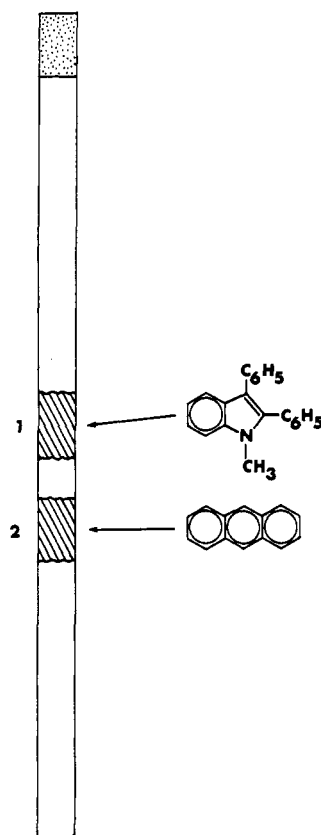


Figure 1. Centri-Chromatographic separation of *N*-methyl-2,3-diphenylindole and anthracene

Procedure. In each experiment, 3 grams of microparticulate silica and 80 ml of solvent were shaken together for 1 hour; 3 ml of the resulting slurry were then packed in the 3-mm i.d. glass column by centrifuging at 2700 rpm for 7 minutes. The test solution (5-10 microliters) was then placed on the polyethylene sample holder at the top of the column and developed, with fresh solvent in the reservoir, at 2700 rpm for various times. The silica column was then extruded, dried, and examined under ultraviolet light. Components could be detected either by their own fluorescence, or by the addition of Luminous Pigment ZS Super (Brinkmann Instruments, Limited, Rexdale, Ontario) to the silica slurry before packing, which results in detectable component bands.

RESULTS AND DISCUSSION

Anthracene (15.5 mg) and *N*-methyl-2,3-diphenylindole (21.0 mg) were dissolved in 6 ml of 1:1 hexane-benzene. Five microliters of this solution were then placed on a silica column and developed by centrifuging at 2700 rpm for 3 minutes with 1:1 hexane-benzene in the reservoir. After extruding and drying (60 °C for 1 minute) the column, examination under ultraviolet light revealed the two blue-fluorescing bands shown in Figure 1. Each band was cut out and heated to 150 °C in

Table I. Comparison of Mass Spectra of *N*-Methyl-2,3-diphenylindole and Band 1, Figure 1.

<i>m/e</i>	Relative abundance, %		Deviation
	Reference spectrum	Spectrum of extruded band	
284	25	24	1
283	100	100	0
282	8	8	0
267	19	17	2
266	6	6	0
141.5	9	7	2
140.5	7	6	1
134	9	7	2
133.5	10	9	1
132.5	5	4	1
Total deviation			10

Table II. Comparison of Mass Spectra of Anthracene and Band 2, Figure 1

<i>m/e</i>	Relative abundance, %		Deviation
	Reference spectrum	Spectrum of extruded band	
179	18	16	2
178	100	100	0
177	19	17	2
176	33	24	9
152	12	13	1
151	12	11	1
150	8	7	1
89	29	31	2
88	18	13	5
76	22	24	2
Total deviation			25

the liquid inlet system of the mass spectrometer. In Tables I and II, the resulting mass spectra are compared with spectra obtained for the pure materials on the same instrument. As the data indicate, good agreement is obtained between the mass spectra of extruded column bands and the reference spectra. A total deviation value is obtained by summation of absolute deviations of each of the ten relative peak heights for a given compound. A total deviation of less than 50 has been used as a positive correlation in an established mass spectrometer-computer matching system (10).

To determine the capabilities of this method, a more complex, seven-component mixture was made up. The mixture contained α -methyl stilbene, *N*-methyl-2,3-diphenylindole, coumarin, 2,5-diphenylthiadiazole, 2,5-diphenyloxadiazole, *N*-benzoylbenzophenone imine, and dicyclopropyl ketone ozine in about equal concentrations of about 7 mg each in 1.5 ml of hexane-benzene (7:3) solution. The separation column was prepared by using a slurry of 3.33 grams of silica in 80 ml of 1:1 hexane-benzene to which 31.4 mg of Luminous Pigment ZS Super was added. This latter substance imparts a green luminescence to the silica when exposed to ultraviolet light. The slurry was then used to pack columns as before. A 7.5 microliter sample of the seven-component mixture was introduced to the silica column and chromatographed for 3 minutes at 2700 rpm using a 1:1 hexane-benzene carrier solvent. When the column was extruded, dried, and examined under ultraviolet light, four bands were detected as seen in

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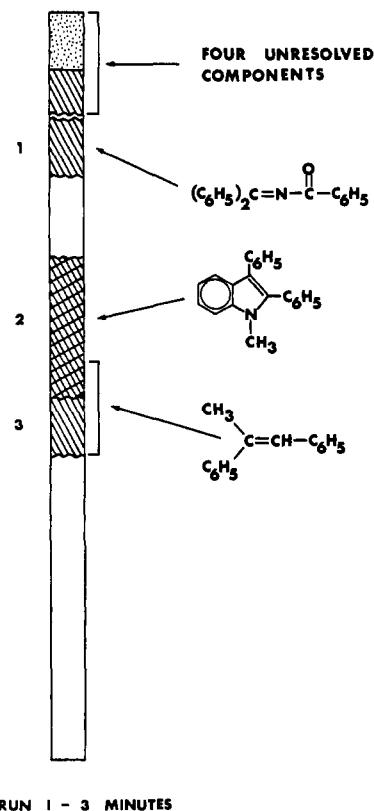
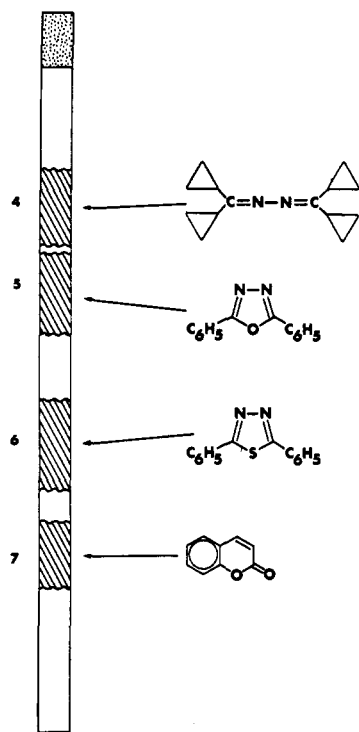


Figure 2a. Partial separation of seven component mixture

Figure 2a. The two faster moving bands were identified by a comparison of their mass spectra to reference spectra. When another 7.5 microliter sample of this mixture was eluted for a longer time (40 min at 2700 rpm) with the same carrier solvent, the three faster moving components were completely eluted from the column, leaving the four compounds indicated in Figure 2b separated on the column. These four total bands are identified by comparison of their mass spectra. The total deviation between reference spectra and those of the separated bands in all cases was less than 50.

In the case of the slowest moving of the three separated components shown in Figure 2 (*N*-benzoylbenzophenone imine band), a positive correlation was not obtained. When this band from the column was introduced into the liquid inlet system of the mass spectrometer and heated to about 150 °C, the molecular ion peak (*m/e* 285) was of very low intensity, whereas the spectrum of pure *N*-benzoylbenzophenone imine obtained in the same instrument at an inlet temperature about 100 °C showed a molecular ion peak intensity 94% of the *m/e* 105 base peak. To check whether this behavior was due to a decomposition catalyzed by the presence of the silica, two control experiments were carried out. Pure *N*-benzoylbenzophenone imine was slurried with some microparticulate silica in 1:1 hexane-benzene, dried, and introduced into the liquid inlet system. The molecular ion peak gradually decreased in intensity relative to the base peak at *m/e* 105 as the inlet system was heated. When pure *N*-benzoylbenzophenone imine was run in the absence of silica, approximately the same behavior was observed on heating. Thus, in this case, it appears that the instability is probably due to the heating above 100 °C in the sample inlet of the mass spectrometer rather than the presence of the silica.

Both 2,5-diphenylthiadiazole and 2,5-diphenyloxadiazole were also found to be sensitive to strong heating. However,



RUN 11 - 40 MINUTES

Figure 2b. Separation of the four unresolved components of Figure 2a

the data obtained in these experiments indicated they produce reproducible spectra if the inlet system is held below 150 °C.

The combination of centrifugal microparticulate bed chromatography and mass spectrometry provides a fast accurate method of separating and identifying components in a mixture without the necessity of isolation and purification after chromatography. No difficulty is encountered in running mass spectra of the compounds studied here in the presence of silica. Several of the compounds used in this study were found to be sensitive to heat and special care had to be taken when running mass spectra of these compounds. Special

consideration should be given compounds strongly adsorbed by silica, such as alcohols and amines, that require a high volatilization temperature. Since the solid inlet system of the RMU-6E mass spectrometer introduces the sample directly into the high vacuum of the ionization chamber, less heat is generally required to volatilize a sample introduced in this manner than in the liquid inlet system. Preliminary experiments indicate that introduction of the extruded column bands via the solid inlet gives results comparable to those reported here. When the solid inlet was opened to the ionization chamber, the sudden decrease in pressure often causes much of the silica to blow out of the sample dipper into the chamber. After the spectrum was obtained, the ionization chamber was heated to 150 °C for a short time and then the next sample could be run. During the course of these experiments, the slight accumulation of silica in the chamber had no noticeable adverse effects on subsequent spectra.

The silica used in this work adsorbed water very rapidly from the atmosphere. Thus all spectra run on bands from an extruded column showed strong peaks at m/e 18. A specially prepared 3-gram controlled moisture silica is now available for use in the Centri-Chromatography system (11). If the water content of the silica is kept small by minimizing contact with the air, the strong peak at m/e 18 does not interfere with the interpretation of the spectra. The presence of too much water causes the separating efficiency of the Centri-Chromatograph to fall off drastically, and its presence should be minimized for that reason.

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Tetraphenylboric Acid Salts as Stationary Phases in Gas Chromatography

George E. Baiulescu

Department of Analytical Chemistry, University of Bucharest, Romania

Vasile A. Ilie

Institute of Chemical Researches, Bucharest, Romania

SELECTIVE SEPARATION using metallic salts as liquid or solid stationary phases in gas chromatography is well known (1). Recently, bis(2-ethylhexyl)phosphoric acid and its lithium, sodium, potassium, thorium, zirconium, uranium(VI), and $[\text{Co(en)}_3]^{8+}$ salts (2, 3) were studied as stationary phases, and

lithium and sodium bis(2-ethylhexyl)phosphates were proposed as stationary phases for the rapid separation in an isothermal system of chlorinated derivatives of benzene (4).

Until recently, adsorbents used in gas-solid chromatography have included coal, silica gel, alumina, and molecular sieves (5). Using silver nitrate as the adsorbant, the separa-

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