Gas chromatographic-mass spectrometric (GC-MS) identification of compounds in concentrates of Puerto Rican rums[1]

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ABSTRACT

A procedure is described for the improved identification of compounds in rums. It includes the preparation of rum concentrates using a Kuderna concentrator (KC), and the profiles of the resultant products using a gas chromatographic (GC) system, where the separation with a packed column is compared with that of a capillary column. The packed column was connected in the chromatograph to the flame ionization detector (FID) whereas the capillary column was interfaced, in the same chromatograph, with the mass spectrometry (MS) probe. These techniques assessed simultaneously, provided alternative methods for compound profiles, one using the capillary column only for identification purpose with the GC-MS, and the other with a high performance gas chromatograpy-flame ionization detector (HPGC-FID) with a .column. The HPGC-FID offered the best combination for the direct quantitative identification of compounds in rum concentrates. As expected, the number of compounds separated by the two procedures was the same, although some signals in the chromatogram of the packed column were too broad and unresolved. This is the case when highly polar compounds are separated in a packed column with an unmodified liquid phase such as Carbowax 20M. Twenty-two compounds were identified in one rum concentrate, 16 with a probability of 70% or higher of being correctly identified. The present procedure resulted in about a 250 to 500-fold increase in the concentration of compounds, as compared with the concentration of compounds in the original rum sample. The vast majority of these compounds were found at a concentration of 20 ppb or less.

RESUMEN

Cromatografía de gases y espectrometría de masa para identificar compuestos en rones de Puerto Rico concentrados

Se describe un procedimiento para identificar los compuestos en rones. El mismo incluye la preparación de concentrados de rones con un concentrador tipo Kuderna y el perfil de los productos obtenidos con un sistema cromatográfico, en el que la separación con una columna de empaque se compara con una columna capilar. La columna de empaque fue conectada en el cromatógrafo detector de gas de ionización de flama (CG-DIF), mientras la columna capilar se conectó al espectrómetro de masa (EM). Las técnicas evaluadas simultáneamente proveyeron métodos alternos para

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obtener el perfil de concentrados de rones, uno usando la columna capilar con el objetivo de obtener la identificación de los compuestos y la otra con la columna de empaque para la determinación cuantitativa en los mismos concentrados. La técnica de columna de empaque de alta eficiencia ofrece la mejor combinación para la determinación cuantitativa de los compuestos en los concentrados. Como se esperaba, el número de compuestos separados con los dos sistemas de columnas resultó ser parecido, aunque algunas señales del cromatograma de la columna de empaque resultaron bastante amplias y con poca separación entre sí. Eso es así cuando compuestos demasiado polares son separados en una fase líquida no modificada como Carbowax 20M. El trabajo capilar con el cromatógrafo de gas-espectrómetro de masa (CG-EM) result en la identificación de 20 compuestos en uno de los concentrados de un ron oro, 16 de los cuales tienen una probabilidad de 70% o mejor de ser identificados correctamente.

La técnica desarrollada resultó en un aumento de la concentración de los compuestos de los concentrados de 250 a 500 veces la concentración de la muestra original. La gran mayoría de los compuestos presentes se encuentran a una concentración de 20 ppb o menors.

INTRODUCTION

The gas-chromatograph-mass spectrometry (GC-MS) analysis of distilled alcoholic beverages is one of the most demanding problems in analytical chemistry because these beverages contain a large number of compounds with only a few at sufficiently high concentrations to be detected directly by mass spectrometry. Whenever concentration is done by traditional techniques it fails because of difficulties in the separation of compounds having characteristics identical to those of the ethanol matrix. Techniques must be developed to prepare suitable concentrates: (a) solvent extraction using liquid-liquid techniques, followed by concentration under a flow of an inert gas or in a rotatory vacuum evaporator; (b) separation in a preparative column and analysis of the fraction in analytical GC columns; and (c) adsorption procedures that use selected solid adsorbents with sample recovery using appropriate solvents or by heat desorption. Rapid and direct methods for accomplishing these procedures for rum analysis with gas chromatography-flame ionization detector (GC-FID), GC-MS, and high pressure liquid chromatography-mass spectrometer (HPLC-MS) without sample contamination are still required to provide suitable data for better quality determination (4,8,12).

The flavor of alcoholic beverages is composed of many different volatile and non volatile compounds which give a beverage its typical aroma and taste. Nearly 1,000 compounds have been identified in different beverages (9,10,,2,15,6,17,18). Fusel alcohols, fatty acids, and esters form the largest groups of compounds in the volatile aroma fraction of alcoholic beverages. Other components, such as phenols, carbonyl, nitrogen and sulfur compounds, are in minor quantities, but their contribution to the

overall quality of the beverages is significant (15,17,18). In many imported foreign rums the number of compounds identified is more restricted than in other beverages, such as whiskeys, but about 400 compounds have been identified (13,14). In Puerto Rican rums the number of components separated are fewer as we have recently reported (1,2,4,6,7).

The composition of rums has been investigated extensively in the Rum Pilot Plant (5,7). Moreover, these methods have been extended for the indirect and direct separations of rum components with a GC-MS utilizing a preparative column coupled to a capillary column either for GC-FID or GC-MS detection (1,2,3,4). A recent publication details recovery data which resulted in about a 1000-fold increase in the concentration of components in their own matrix compared with that of the neat rum (4). The vast majority of these components were found at concentrations as low as 0.01 mg/1 ml with numerous signals identified in the chromatogram not previously observed in Puerto Rican rums.

Although a high degree of concentration was achieved, it was our objective in the research here reported to assess a different simple technique, based on Kuderna concentrator (KC), for concentrating rum, and to identify many components of rum found in extremely smaller amounts, and at the same time, to have on hand a method suitable for routine analysis.

MATERIALS AND METHODS

1. GC-FID and GC-MS Profiles and Kuderna concentrator

A Hewlett Packard 5890A GC-MS was used to conduct capillary column separation for the MS profiles and a packed column for the GC-FID work. This instrument is a dual injector unit with a split/splitless capillary column inlet and a packed column inlet. It has an FID, an HP5970B MSD quadrupole stand-alone GC detector, and an HP59970C Chemstation with a HP5990C computer, hard drive, disk drive and printer. The GC-MS system includes all the necessary software and NBS, NHI, EPA Data Base MS library with mass spectral data for 45,000 compounds.

The capillary column used for the GC-MS work was a Supelcowax™10[4] coated on a 30m x 0.25mm i.d., 0.25μm film thickness fused silica capillary tubing, connected to the MS probe through one of the injection ports of the unit. For the GC-FID work, an analytical packed column (PPR-187), 18 ft x 1/8 in. OD SS containing 5% Carbowax 20M coated on 60/80 Chromosorb W. a/w. was connected to the FID of the GC/MS through the other injection port.

Figure 1 shows the Kuderna concentrator. It has been used to concentrate organic contaminants in water and waste water by stripping the sample of contaminants with an organic solvent denser than water.

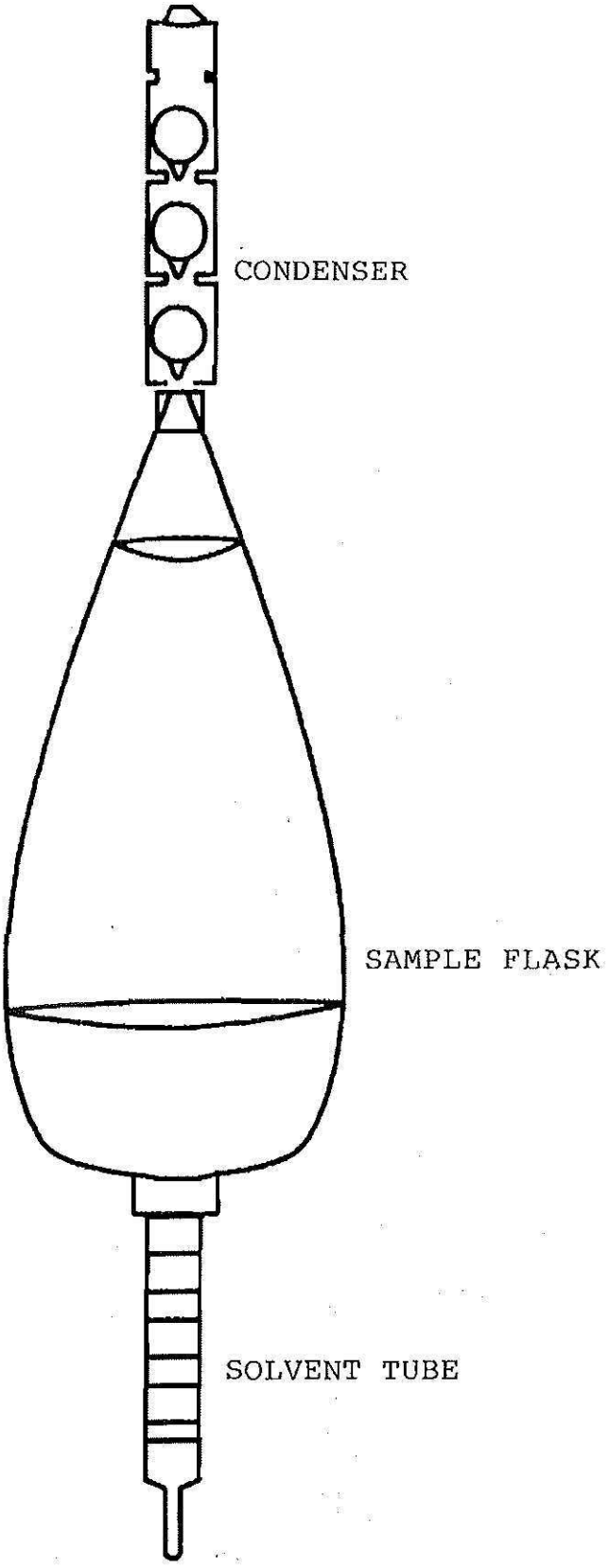


FIG. 1.—Kuderna concentrator.

2. SAMPLE CONCENTRATION AND ANALYSIS

To evaluate the Kuderna concentrator, we placed 500 ml of the appropriate rum in the middle sample flask (fig. 1) and charged the concentrator with a given volume of spectral grade dichloromethane, which fell to the concentrating tube below. Similar samples were extracted with boiling 10, 15, 30, 50, or 100 ml portions of spectral grade dicloromethane (CH_2Cl_2) at 40°C during a 6-hour period. We found this 6-hour period to be the most adequate for a given extraction after we had treated a 500-ml sample with 100 ml CHl, at different extraction periods 1, 3, 5, 8, and 24 hours. We further concentrated the dichloromethane extracts to a small volume ina 100-ml saperatory funnel connected to a calibrated 5-ml cylinder by blowing pure helium through the sample. The final volume of the reduced extract was difficult to control under these conditions, but about 2 ml was an appropriate volume. For comparison, we reduced an extract to small volume in a rotatory evaporator, but this procedure was tedious and the final volume of the extract was more difficult to control under these conditions.

The untreated (neat) and the concentrated samples were separated both with the capillary and the packed analytical columns at conditions given in table 1. Table 2 shows the GC-MS conditions for the GC-MS profiles:

Samples and extracts

A. GOLD LABEL RUM

R-818 is an 80°P gold label commercial rum.
R-818-1 is an R-818 rum extract, 500-ml sample extracted for 6 hours with 50 ml CH_2Cl_2.
R-818-3 is an R-818-1 extract reduced to 2 ml under a flow of pure helium.
R-830 is an 80°P gold label commercial rum.
R-830-1 is an R-830 rum extract, 500-ml sample extracted for 6 hours with 50 ml CH_2Cl_2.
R-830-2 is an R-830-1 extract reduced to 1 ml under a flow of pure helium.
R-830-3 is an R-830 extract, 500-ml sample extracted for 6 hours with 100 ml CH_2Cl_2.
R-830-4 is an R-830-3 extract reduced to 2 ml under a flow of pure helium.
R-830-5 is an R-830 extract, 500-ml sample extracted for 6 hours with 30 ml CH_2Cl_2, reduced to 2 ml under a flow of helium.
R-830-6 is an R-830-5 extract reduced to 1.9 ml under a flow of pure helium.
R-830-7 is an R-830 extract, 500 ml extracted for 6 hours with 100 ml CH_2Cl_2.
R-830-8 is an R-830-7 reduced to 3.0 ml in a rotatory evaporator (40-50°C at reduced pressure).

B. WHITE LABEL RUM

R-831 is an 80°P white label commercial rum.
R-831-3 is an R-831 rum extract, 500-ml sample extracted for 6 hours with 100 ml CH_2Cl_2.
R-831-5 is an R-831 rum extract, 500-ml sample extracted for 6 hours with 30 ml CH_2Cl_2.
R-831-6 is an R-831-5 extract reduced to 1.6 ml under a flow of pure helium.
R-831-7 is an R-831 extract, 500-ml sample extracted for 6 hours with 100 ml CH_2Cl_2.
R-831-8 is an R-831-7 extract reduced to 3.0 ml in a rotatory evaporator (40-50°C at reduced pressure).

RESULTS AND DISCUSSION

The chromatograms of white- and gold-label rums obtained with packed column PPR-187 (FID Method) are reproduced in figures 2 through 7. Figure 2 is the PPR-187-packed-column chromatogram of 3-μl samples, (A) the neat R-818 sample, (B) R-818-1 dichloromethane rum extract, and (C) R-818-3 reduced dichloromethane extract. Figure 3 is the PPR-187 packed-column chromatogram of 3-μl samples; (A) is the neat sample R-830, (B) R-830-3 dichloromethane rum extract, and (C) R-830-5 reduced dichloromethane extract Figure 4 is the PPR-187-packed-column chromatogram of 3-μl samples; (A) is the neat R-830 sample, (B) R-830-4 reduced dichloromethane extract, and (C) R-830-2 reduced dichloromethane extract). Figure 5 is the PPR-187-packed-column chromatogram of 3-μl samples; (A) is neat R-830 sample, (B) R-830-6 reduced dichloromethane extract, and (C) R-830-8 reduced

TABLE 1.—*Sample and extract conditions for the chromatographic column*

FUNCTION	*PPR-187 COLUMN*	*CAPILLARY COLUMN*
Pressure	60 psi	12 psi
Flow	50 ml/min	1 ml/min
T_i (level 1)	58° C	50° C
T_i (level 2)	–	150° C
T_f (level 3)	140° C	170° C
t_i (level 1)	6 min	6 min
t_i (level 2)	–	2 min
t_f (level 3)	12 min	10 min
Temp. Prog. (level 1)	20 C/min	10° C/min
Temp. Prog. (level 2)	–	10° C/min
Splitter ratio	–	1:30

TABLE 2.—*Conditions for the FC-MS profiles*

SCAN ACQUISITION DATA: CONCRON.A

Solvent	Delay	0.00	eM volts	o relative	Resulting voltage	1800
	Start time	Low mass	High mass	Scan threshold	a/d samples (2N̂)	Scan per second
1	0.00	25.0	103.0	2000	1	10.9
2	3.00	25.0	100.0	2000	1	11.4
3	15.00	25.0	550.0	1000	2	0.82

TEMPERATURE PROGRAM & HEATED ZONES

Run time	30.00	Equilibration	time	0.50	Purge off time	0.00
Level	Initial temp.	Initial time	Rate (°C/min)	Final temp.	Final time	Total time
1	50	6.00	\$0.0	150	2.00	18.00
2			10.0	170	10.00	30.00
			Actual	*Setpoint*		*Limit*
Oven (Standby)			50	50		180
Inj Port B			200	200		200
Detector A			280	280		280
Inj Port A			200	200		250
Transfer Line			280	280		280

RUN TABLE EDITOR

0.00 Valves Divert On
0.75 Valves Divert Off
2.00 Mass Sp Off
5.00 Mass Sp On
30.00 Stop Run

dichloromethane extract. Figure 6 is the PPR-187-packed-column chromatogram of 3-µl samples; (A) is the neat R-831 sample, (B) R-831-7 dichloromethane-rum extract, and (C) R-830-8 reduced dichloromethane extract, and figure 7 is the PPR-187-packed-column chromatogram of 3-µl samples; (A) is neat R-831, (B) R-830-6 reduced dichloromethane extract, and (C) R-831-8 reduced dichloromethane extract.

The GC-MS profiles are shown in figures 8 through 17. Figure 8 is the capillary column GC-MS total ion chromatogram of neat sample R-818, and figure 9 is the corresponding capillary column GC-MS total ion

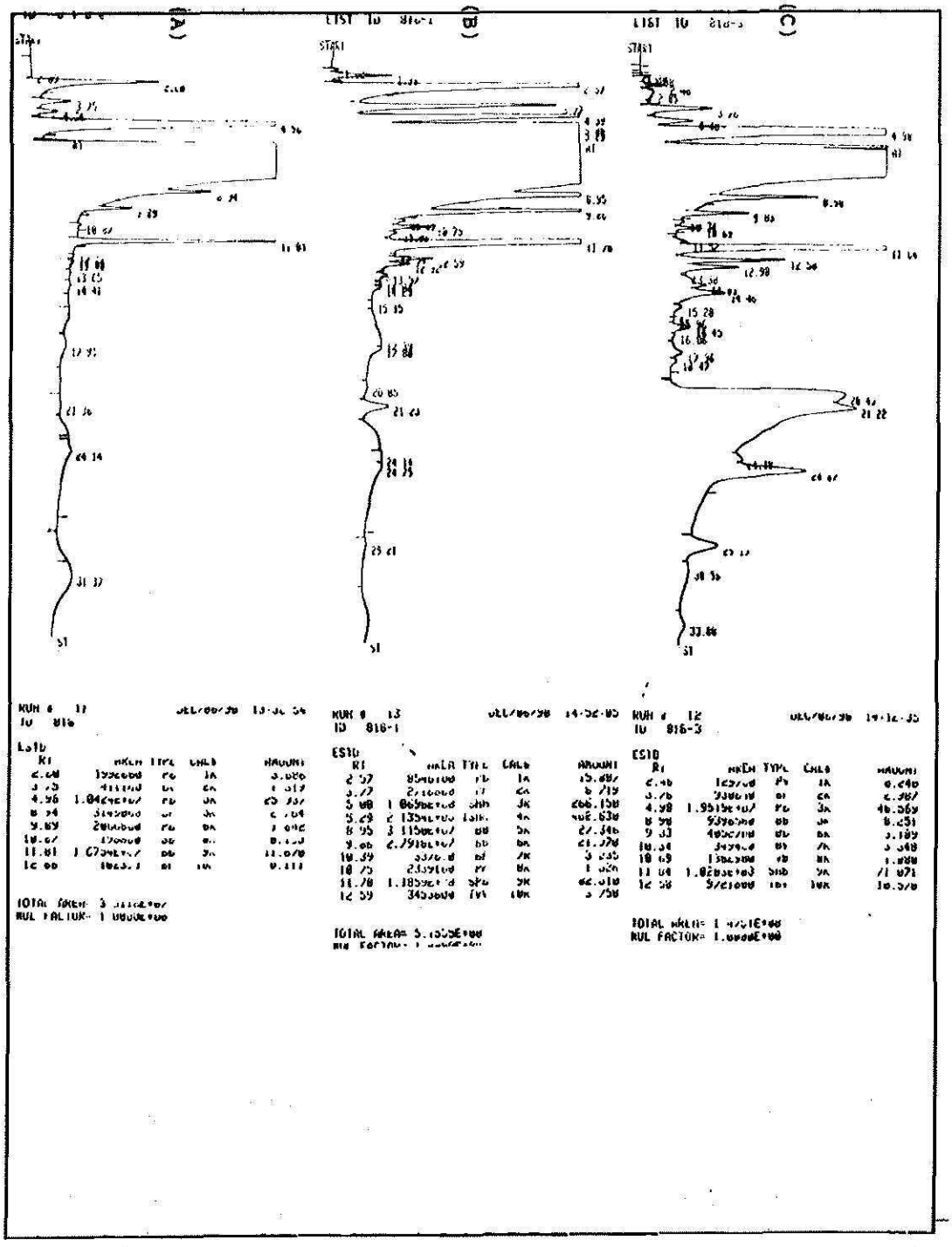


FIG. 2.—Analytical FID chromatogram (PPR-187) of 3ul R-818 samples: (A) neat rum, (B) 500:50 rum: CH_2Cl_2, and (C) 50ml of CH_2Cl_2 (500:50 rum:CH_2Cl_2) reduced to 2ml.

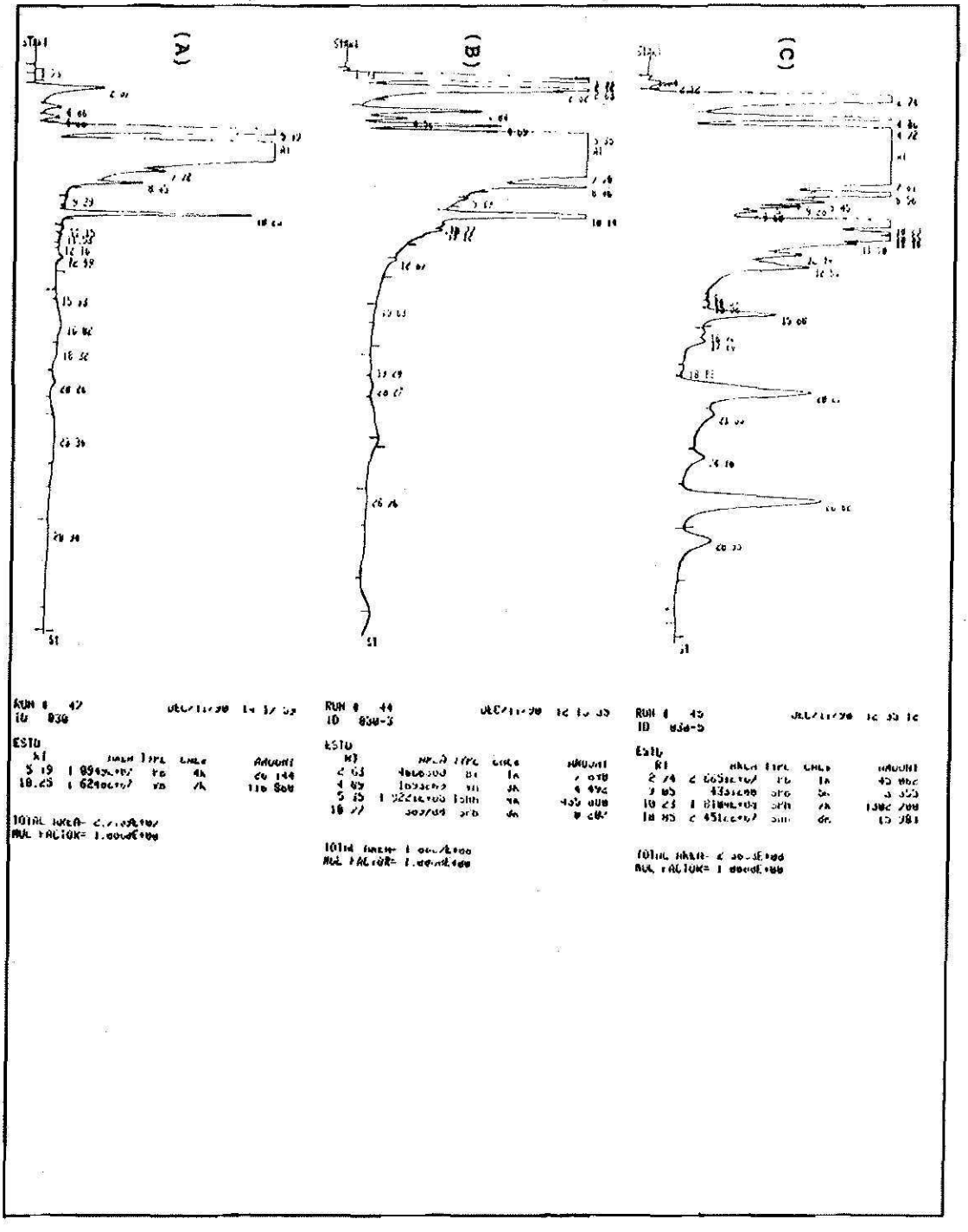


FIG. 3.—Analytical FID chromatogram (PPR-187) of 3ul R-830 samples: (A) neat rum, (B) 500:100 rum: CH_2Cl_2 and (C) 100ml of CH_2Cl_2 (500:100 rum:CH_2Cl_2) reduced to 2ml.

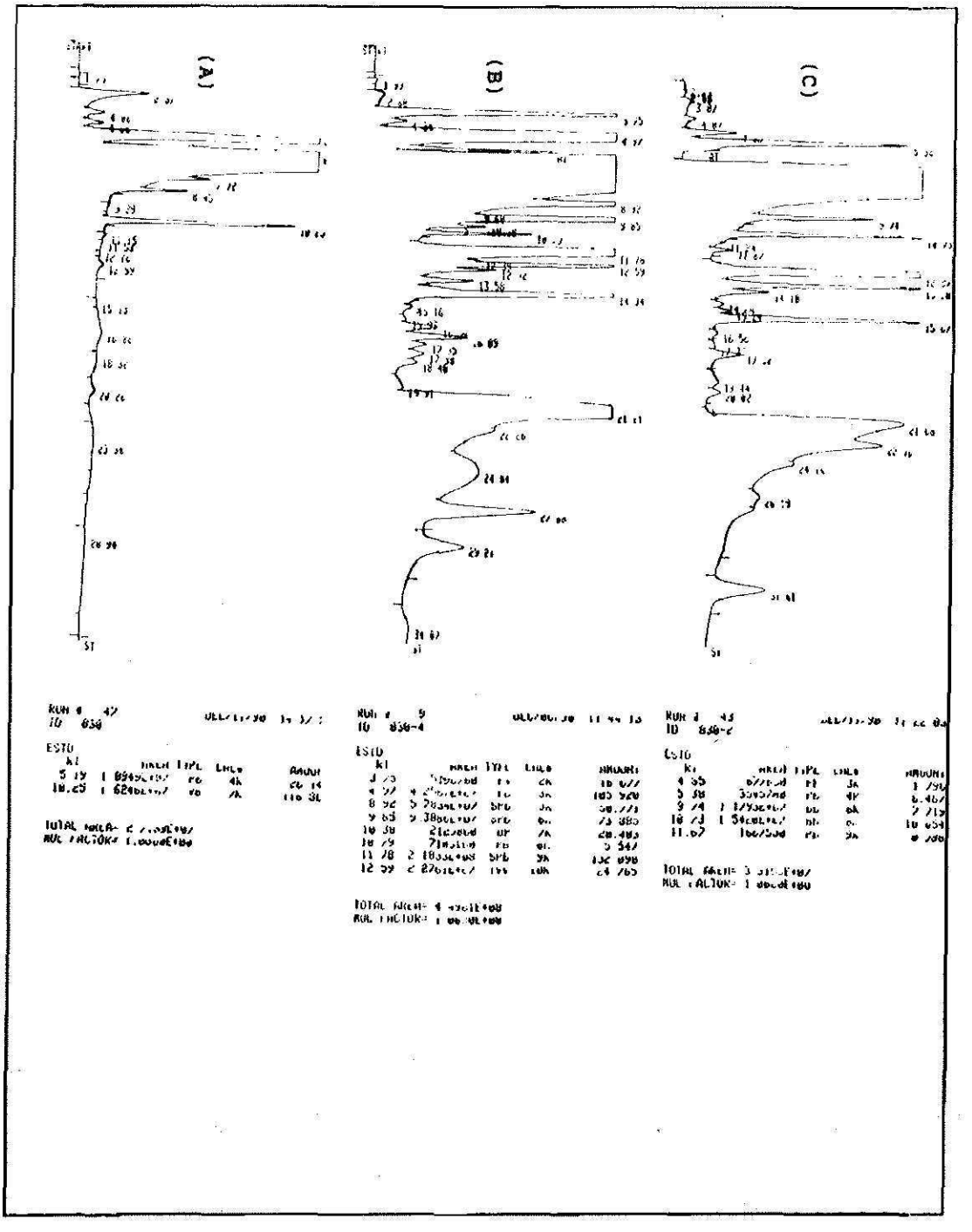


FIG. 4.—Analytical FID chromatogram (PPR-187) of 3ul R-830 samples; (A) neat rum, (B) 100ml of CH_2Cl_2 (500:100 rum:CH_2Cl_2) reduced to 2ml, and (C) 50ml of CH_2Cl_2 (500:50 rum:CH_2Cl_2) reduced to 1ml.

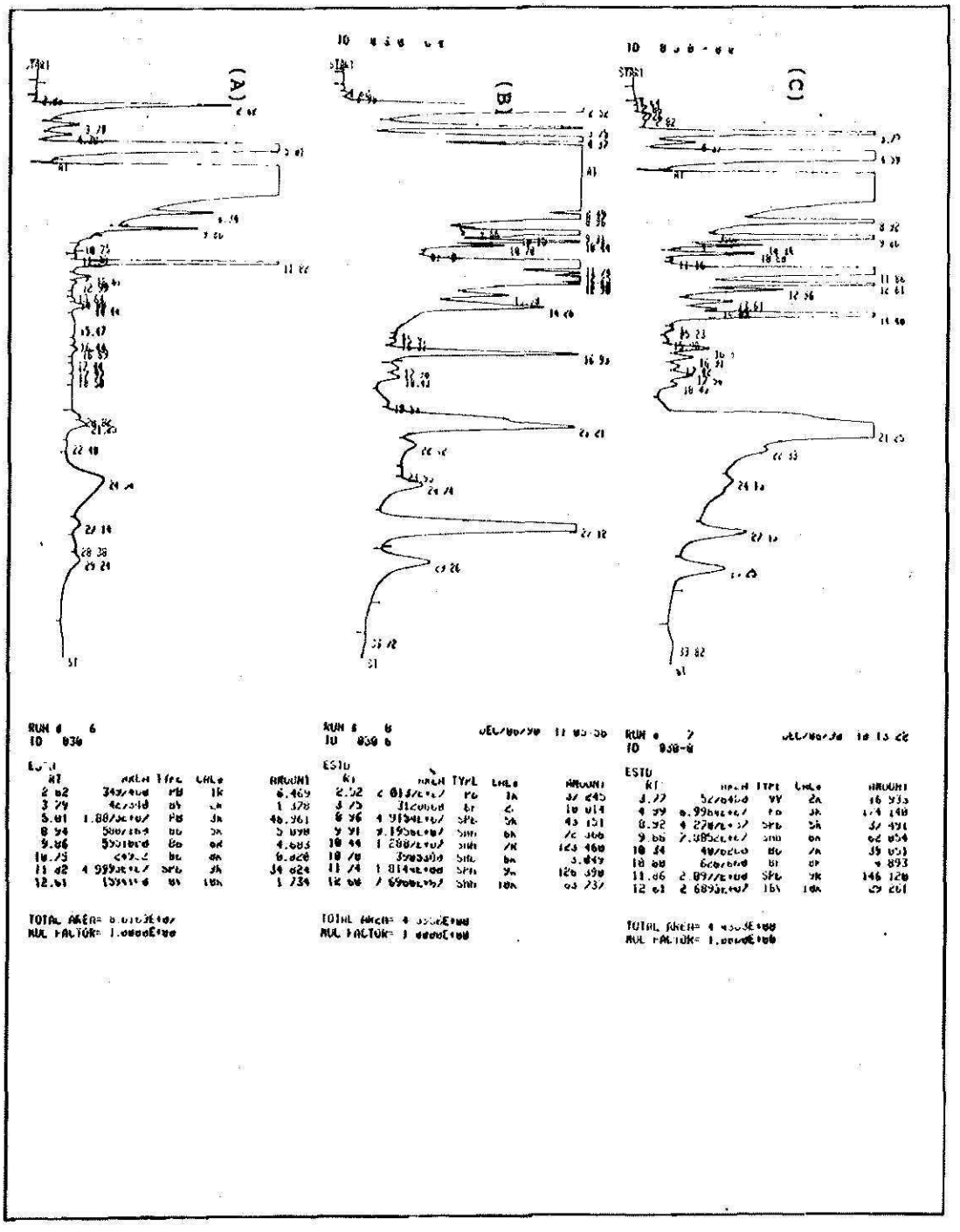


FIG. 5.—Analytical FID chromatogram (PPR-187) of 3ul R-830 samples: (A) neat rum, (B) 100ml of CH_2Cl_2 (500:100 rum:CH_2Cl_2) reduced to 2ml, and (C) 100ml of CH_2Cl_2 500:100 rum:CH_2Cl_2) reduced to 3ml.

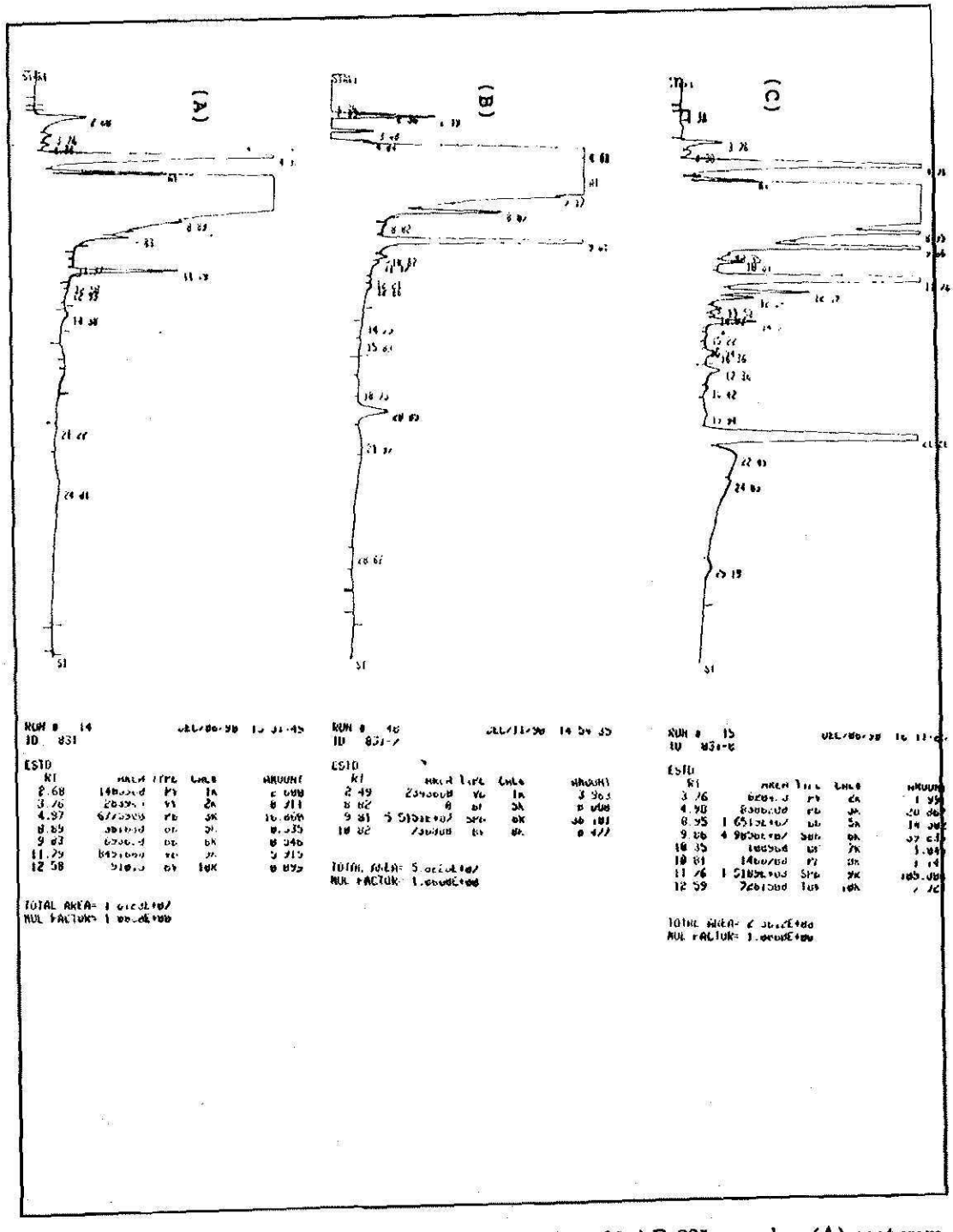


FIG. 6.— Analytical FID chromatogram (PPR-187) of 3ul R-831 samples: (A) neat rum, (B) 500:100 rum:CH_2Cl_2 and (C) 100ml of CH_2Cl_2 (500:100 rum:CH_2Cl_2) reduced to 3ml.

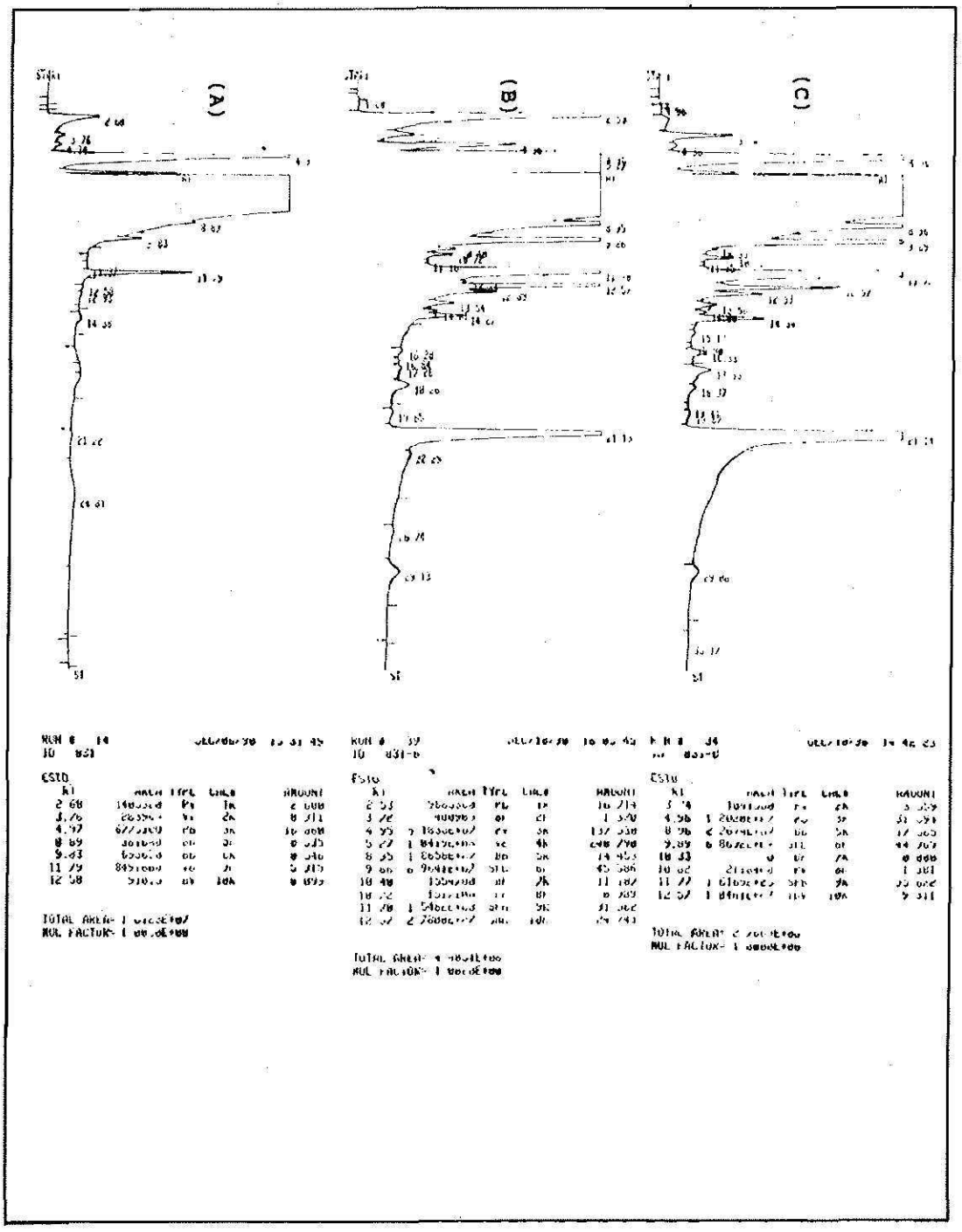
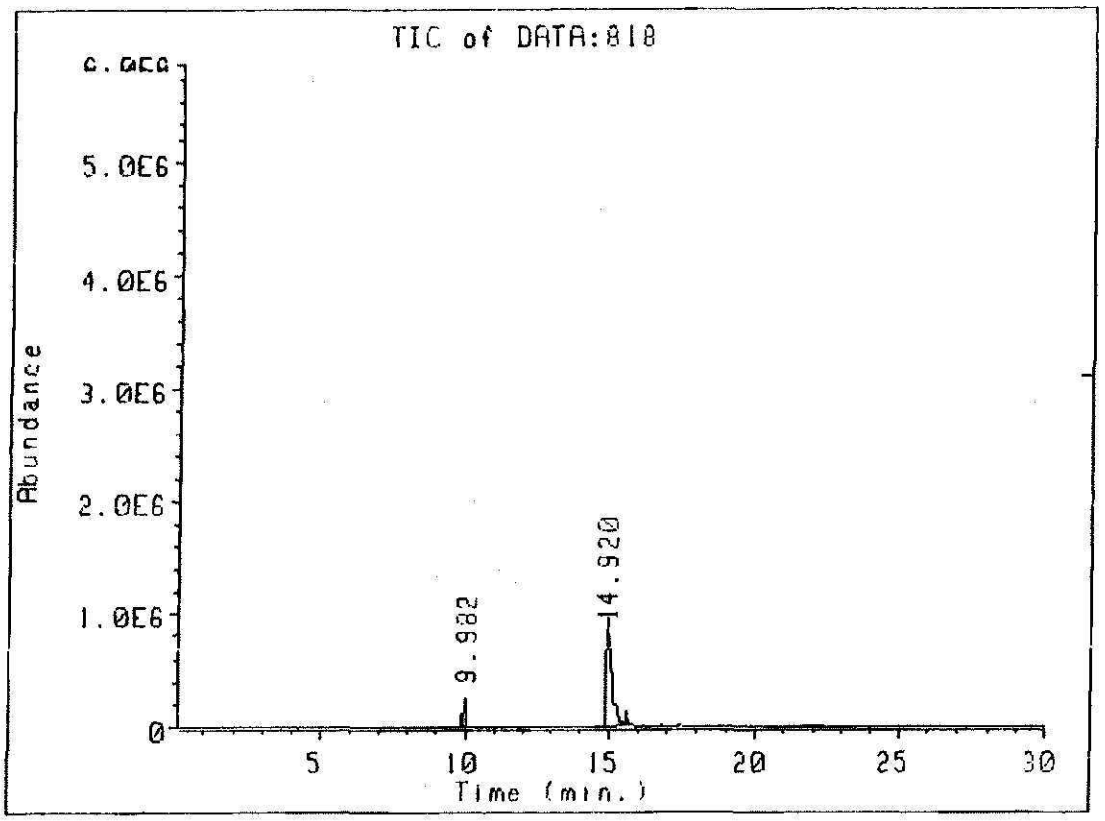


FIG. 7.- Analytical FID chromatogram (PPR-187) of 3ul R-831 samples: (A) neat rum, (B) 100ml of (500-100 rum: CH_2C1_2) reduced to 1.9ml, and (C) 100ml of CH_2C1_2 500:100 rum:CH_2C1_2) reduced to 3ml.



TIC of DATA:818

Peak #	Ret Time	Type	Width	Area	Start Time	End Time
1	9.982	VH	0.059	10391076	9.879	10.030
2	14.920	BV	0.151	116709662	14.854	15.608

FIG. 8.— Capillary column GC-MS total ion chromatogram of neat R-818.

chromatogram of R-818-3 reduced dichloromethane extract. Figure 10 is the capillary column GC-MS total ion chromatogram of neat sample R-830 and figures 11, 12, 13, and 14 are the corresponding capillary column GC-MS total ion chromatograms of the reduced dichloromethane extracts of R-830-2, R-830-4, R-830-6, and R-830-8, respectively. Figure 15 is the capillary column GC-MS total ion chromatogram of neat sample R-831. Figures 16 and 17 are the corresponding capillary column GC-MS total ion chromatograms of the reduced dichloromethane extracts of R-831-6, and R-831-8, respectively.

The application of two different procedures for the separation of the concentrates was necessary in order to have alternative methods available, one with a capillary column just for identification purpose, and a packed column for the quantification of identified components. Since the HPGC-FID combination offers the best choice for quantification, it was

included as an important component in this study. It was not unexpected that the number of compounds separated by the two procedures was the same, although some signals in the chromatogram of the packed column were too broad and unresolved. This is the case when highly polar compounds are separated in a packed column with an unmodified liquid phase such as Carbowax 20M.

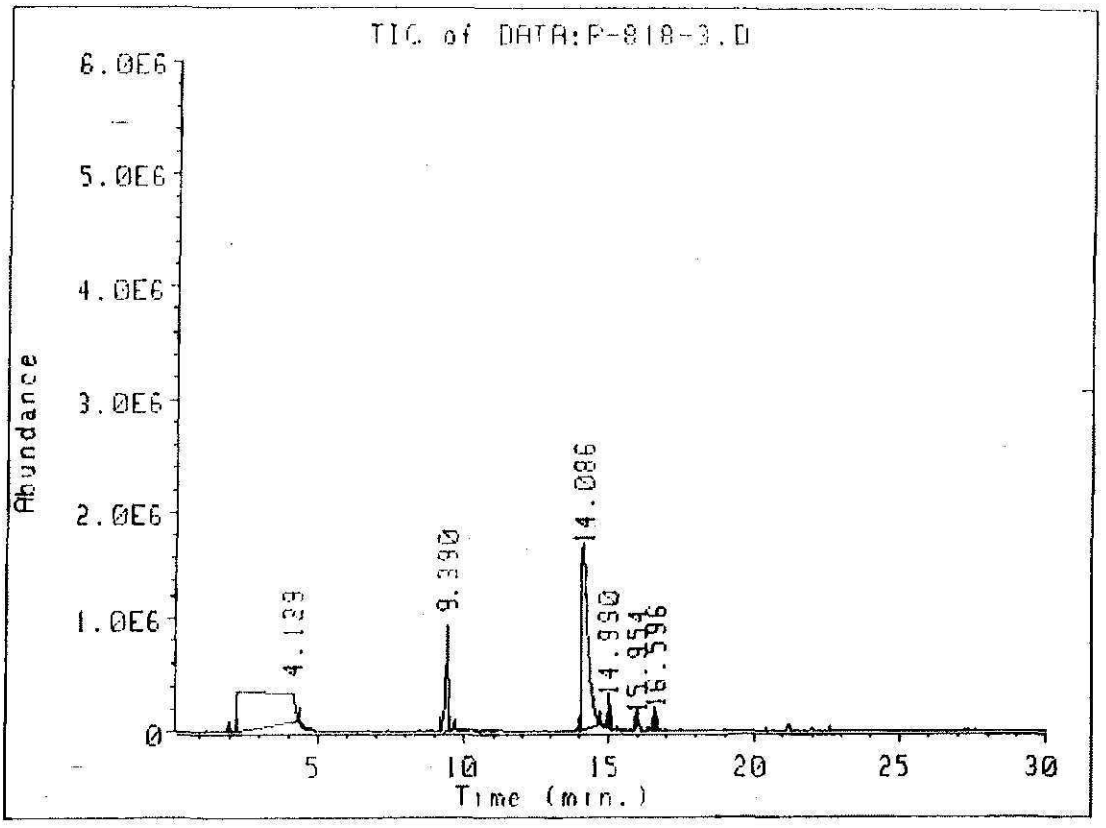
Table 3 presents the list of compounds identified by means of the NBS Library in the chromatogram of the GC-MS profile of the R-830-6 rum extract (fig. 13).

Twenty-two compounds were identified in the rum extract, 16 with a probability of 70% or higher of being correctly identified. According to the HP Manual, PBM Search and Parametric Retrieval Software, a probability of less than 50% represents a significant difference between the unknown signal and the reference data, whereas values higher than 90% will correspond perfectly with the component to be identified. In order

TABLE 3.—*Compounds identified by means of GC-MS NBS Library*

Date:	SEPT. 26, 1990
Sample:	GOLD RUM EXTRACT (R-830-6)
File	830-6

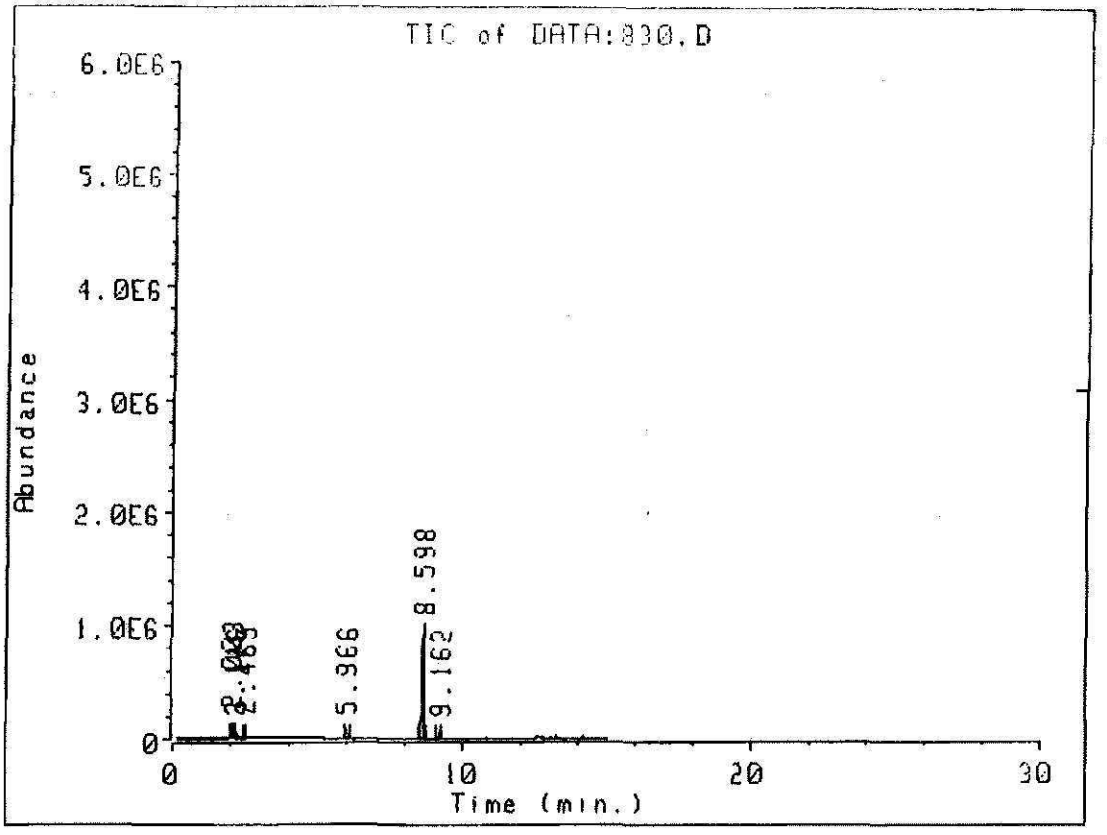
Retention Time (min.)	Compound	% Prob.	CAS
2.084	Acetaldehyde	70	75-07-0
6.001	1-Propanol	89	71-23-8
6.892	1-Butanol, 3-methyl-, acetate	70	123-92-2
7.061	1-Propanol, 2-methyl-	86	78-83-1
9.671	1-Butanol, 3 methyl	87	123-51-3
9.991	Ethyl orthoformate	60	122-51-0
10.423	3(2H) Furanone, dihydro-2-methyl-	83	3188-00-9
10.989	Propane, 1,1-diethoxy	52	4744-08-5
11.326	2-Heptanol	52	543-49-7
11.494	Propanoic acid, 3 ethoxy ethyl ester	70	763-69-9
11.896	Formamide	60	75-12-7
13.043	Octanoic acid, ethyl ester	79	106-32-1
13.858	Acetic acid	88	64-19-7
14.098	Acetic acid, diethoxy-, ethyl ester	60	6065-82-3
14.724	Benzaldehyde	96	100-57-7
15.052	1-Octanol	76	111-87-5
15.366	Propanedioic acid, diethyl ester	58	105-53-3
16.089	Decanoic acid, ethyl ester	79	110-38-3
16.651	Butanedioic acid, diethyl ester	78	123-25-1
17.376	Undecanoic acid, ethyl ester	79	627-90-7
18.955	Benzeneethanol	76	60-12-8
22.408	Butanedioic acid, hydroxy diethyl ester	86	626-11-9



TIC of DATA:R-818-3.D
CONC. R-818, EXT. CH_2CL_2

Peak #	Ret Time	Type	Width	Area	Start Time	End Time
1	4.129	BV	0.747	164291729	2.224	4.343
2	9.390	BV	0.077	51657685	9.164	9.527
3	14.086	PV	0.163	223070757	14.020	14.721
4	14.990	VB	0.032	5997250	14.949	15.137
5	15.954	PV	0.035	1993047	15.862	15.981
6	16.596	VV	0.041	6002249	15.524	16.679

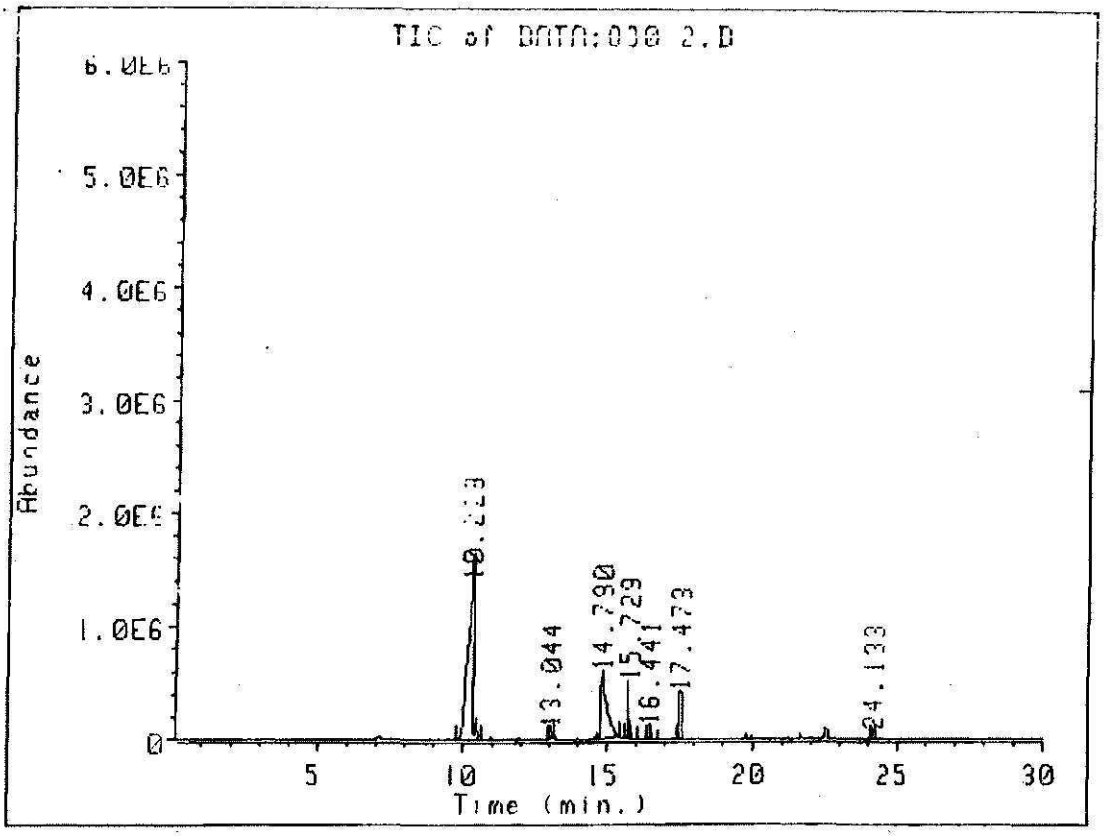
FIG. 9.— Capillary column GC-MS total ion chromatogram of R-818 reduced dichloromethane extract.



TIC of DATA:830.D

Peak #	Ret Time	Type	Width	Area	Start Time	End Time
1	2.003	VV	0.038	2548306	1.949	2.039
2	2.116	PV	0.028	1228714	2.079	2.152
3	2.465	BB	0.024	1071885	2.431	2.529
4	5.966	BV	0.063	3380387	5.897	6.083
5	8.598	BV	0.048	32343795	8.457	8.657
6	9.162	BB	0.031	1160433	9.004	9.236

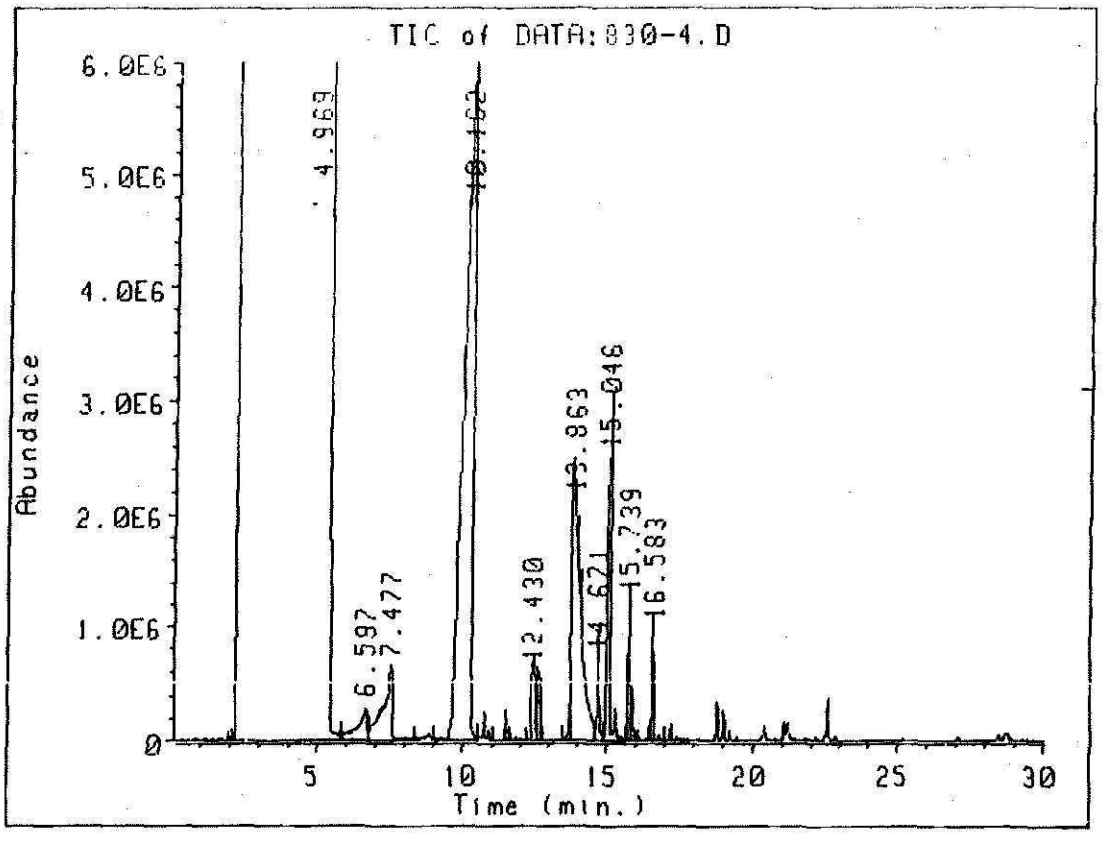
FIG. 10.—Capillary column GC-MS total ion chromatogram of neat R-830.



TIC of DATA:830-2.D

Peak #	Ret Time	Type	Width	Area	Start Time	End Time
1	10.229	BH	0.267	220237489	9.717	10.609
2	13.004	BV	0.082	7497653	12.953	13.170
3	14.790	BV	0.176	83683842	14.759	15.382
4	15.729	BB	0.044	15760626	15.601	15.821
5	16.441	BB	0.044	3386783	16.366	16.508
6	17.473	BB	0.047	15231604	17.393	\$7.551
7	24.133	BB	0.059	4264161	24.049	24.211

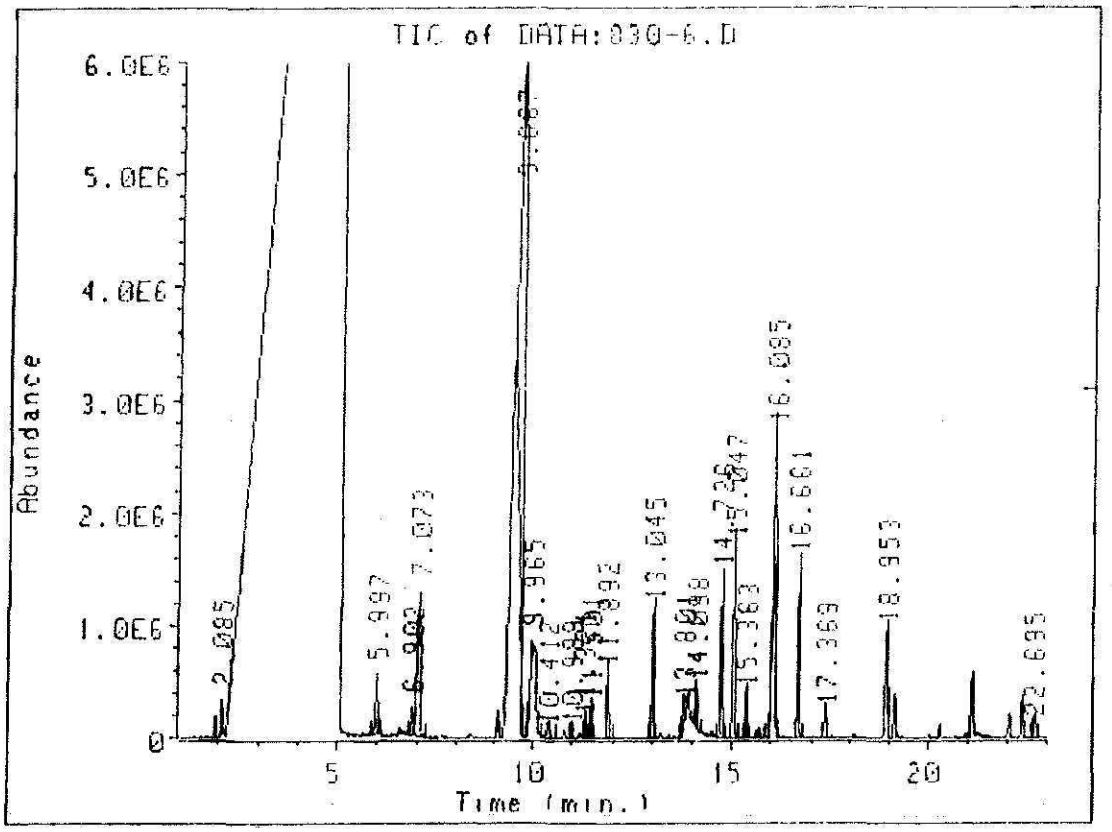
FIG. 11.—Capillary column GC-MS total ion chromatogram of R-830 reduced dichloromethane extract.



TIC of DATA:830-4.D

Peak #	Ret Time	Type	Width	Area	Start Time	End Time
1	4.969	BV	1.328	12246059030	2.103	5.800
2	6.597	VV	0.375	62588035	5.800	6.765
3	7.477	VV	0.379	143937025	6.765	8.336
4	10.162	VV	0.341	1417052241	8.986	10.517
5	12.430	PB	0.217	85335911	11.04	12.778
6	13.863	BV	0.350	511804393	13.497	14.600
7	14.671	VV	0.074	35721285	14.600	14.904
8	15.046	VV	0.149	162348448	14.904	15.621
9	15.739	VV	0.167	7393385	15,621	16.476
10	16.538	VV	0.133	33866552	15.476	16.973

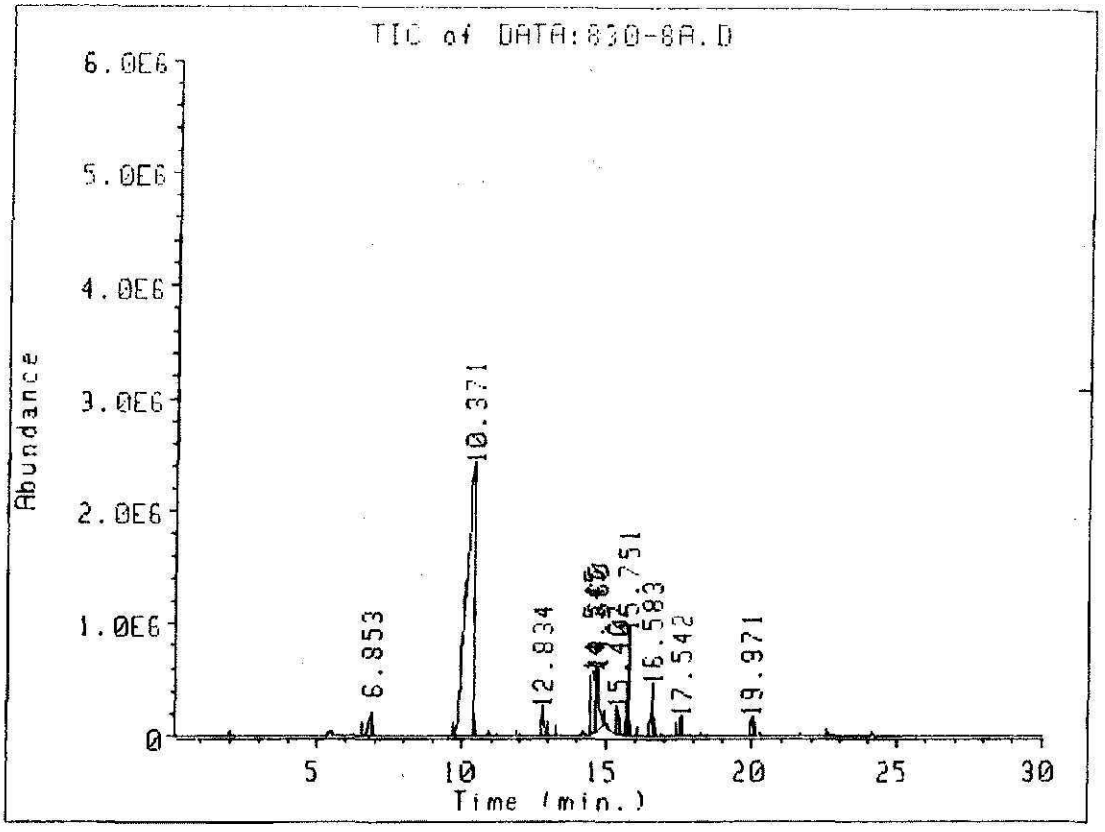
FIG. 12.—Capillary column GC-MS total ion chromatogram of R-830 reduced dichloromethane extract.



TIC of DATA:830-6.D

Peak #	Ret Time	Type	Width	Area	Start Time	End Time
1	2.085	VV	0.022	4001012	2.060	2.127
2	5.997	BV	0.060	20654212	5.839	6.079
3	6.902	BV	0.043	7259927	6.801	6.934
4	7.073	VB	0.073	65682477	6.934	7.228
5	9.667	BV	0.102	42658654	9.456	9.731
6	9.965	BBA	0.035	4938828	9.834	10.006
7	10.412	PB +	0.000	6107000	10.210	10.577
8	10.989	BH	0.031	2353177	10.928	11.017
9	11.321	PB	0.033	4997149	11.270	11.354
10	11.501	BH	0.035	8175542	11.436	11.540
11	11.892	BB	0.042	18803204	11.842	12.000
12	13.045	BH	0.050	39566821	13.943	13.114
13	13.801	PBA	0.077	11345822	13.740	13.867
14	13.098	BBA	0.027	6822127	14.057	14.235
15	14.726	PV	0.039	38421003	14.620	14.817
16	15.047	BV	0.051	58039990	14.951	15.156
17	13.363	BB	0.032	9276282	15.301	15.411
18	16085	BBA	0.061	44207966	15.941	16.099
19	16.661	BH	0.047	48325510	16.580	16.748
20	17.369	BH	0.046	9495155	17.268	17.419
21	18.953	BB	0.018	4576586	18.932	19.015
22	22.695	BB	0.056	8107223	22.639	22.783

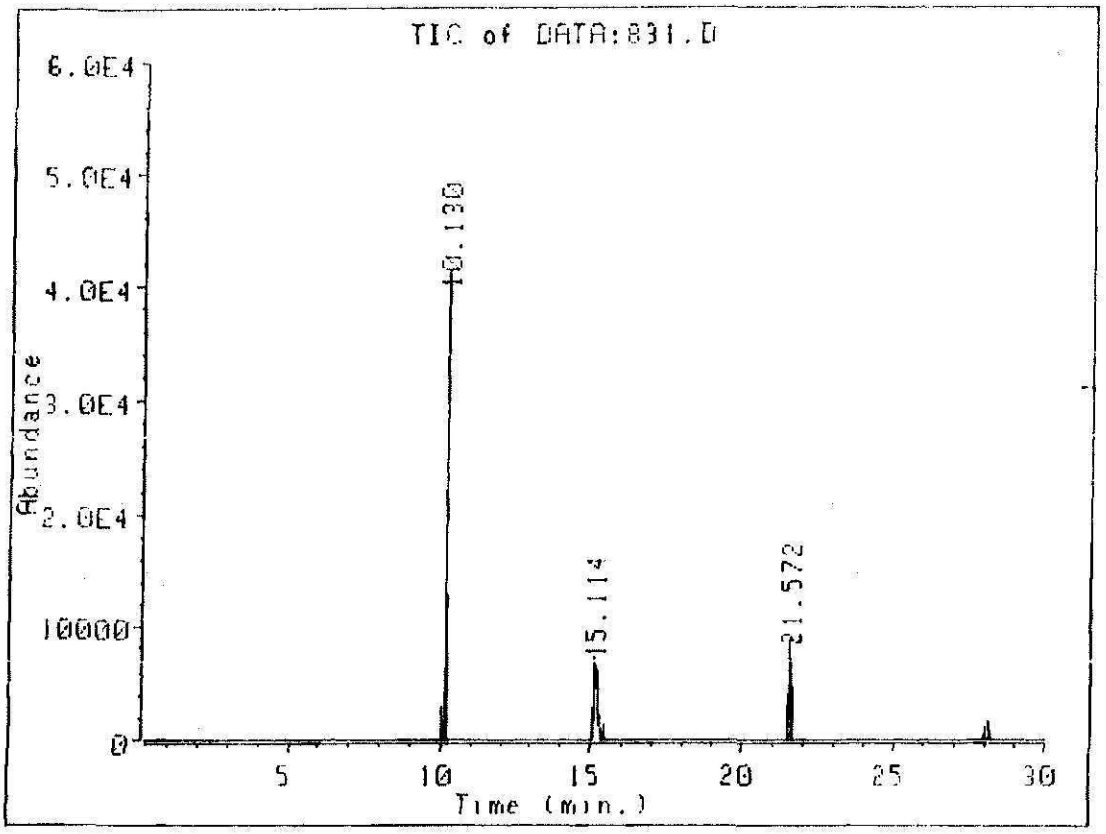
FIG. 13.—Capillary column GC-MS total ion chromatogram of R-830 reduced dichloromethane extract.



TIC of DATA:830-8A.D

Peak #	Ret Time	Type	Width	Area	Start Time	End Time
1	6.853	BH	0.107	16179082	6.518	6.923
2	10.371	BH	0.239	4757811291	9.694	10.447
3	12.834	BH	0.071	13586039	12.778	13.019
4	14.545	BV	0.082	35240132	14.483	14.626
5	14.660	VV	0.095	40371454	14.626	14.931
6	15.407	BV	0.037	6367665	15.350	15.481
7	15.751	BB	0.058	40111437	15.627	15.833
8	16.583	BH	0.058	20063677	16.466	16.624
9	19.971	BB	0.059	7037315	19.891	20.068
10	19.971	BB	0.059	7037315	19.891	20.068

FIG. 14.—Capillary column GC-MS total ion chromatogram of R-830 reduced dichloromethane extract.

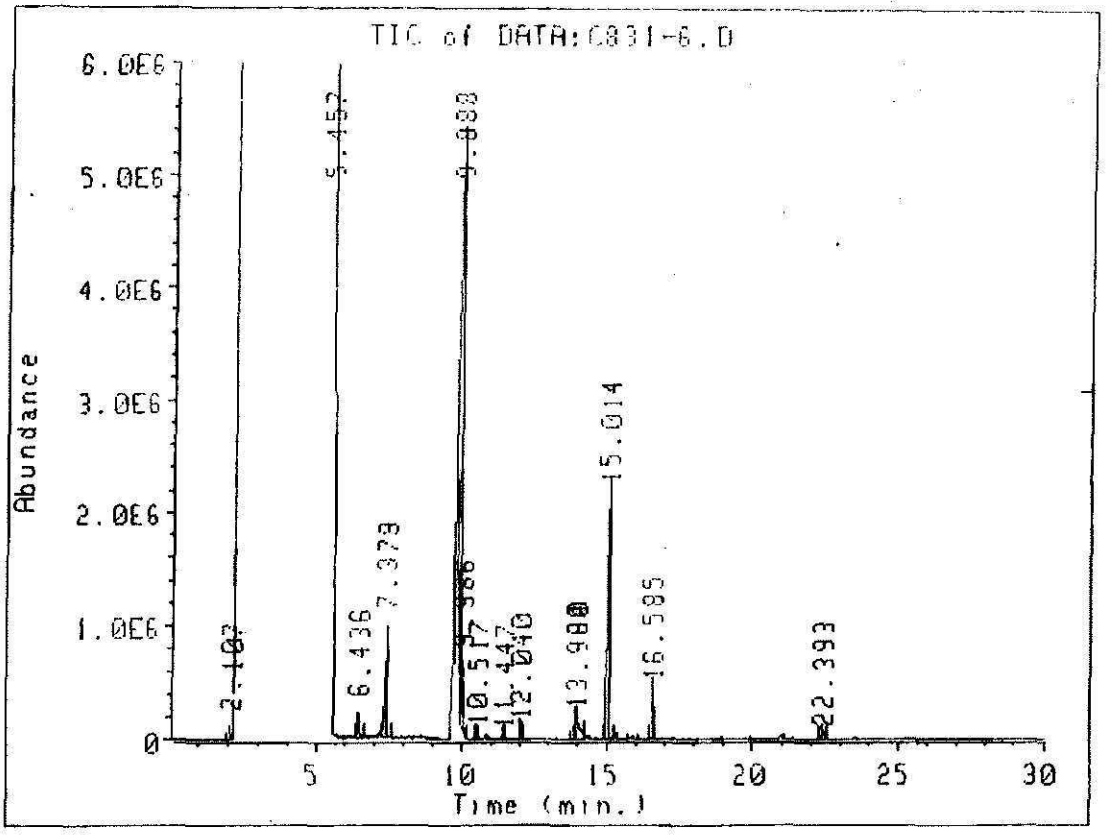


Peak #	Ret Time	Type	Width	Area	Start Time	End Time
1	10.130	BH	0.032	756035	10.068	10.159
2	15.114	BB	0.066	202575	15.056	15.190
3	21.572	VH	0.053	289863	21.524	21.600

FIG. 15.—Capillary column GC-MS total ion chromatogram of R-831 reduced dichloromethane extract.

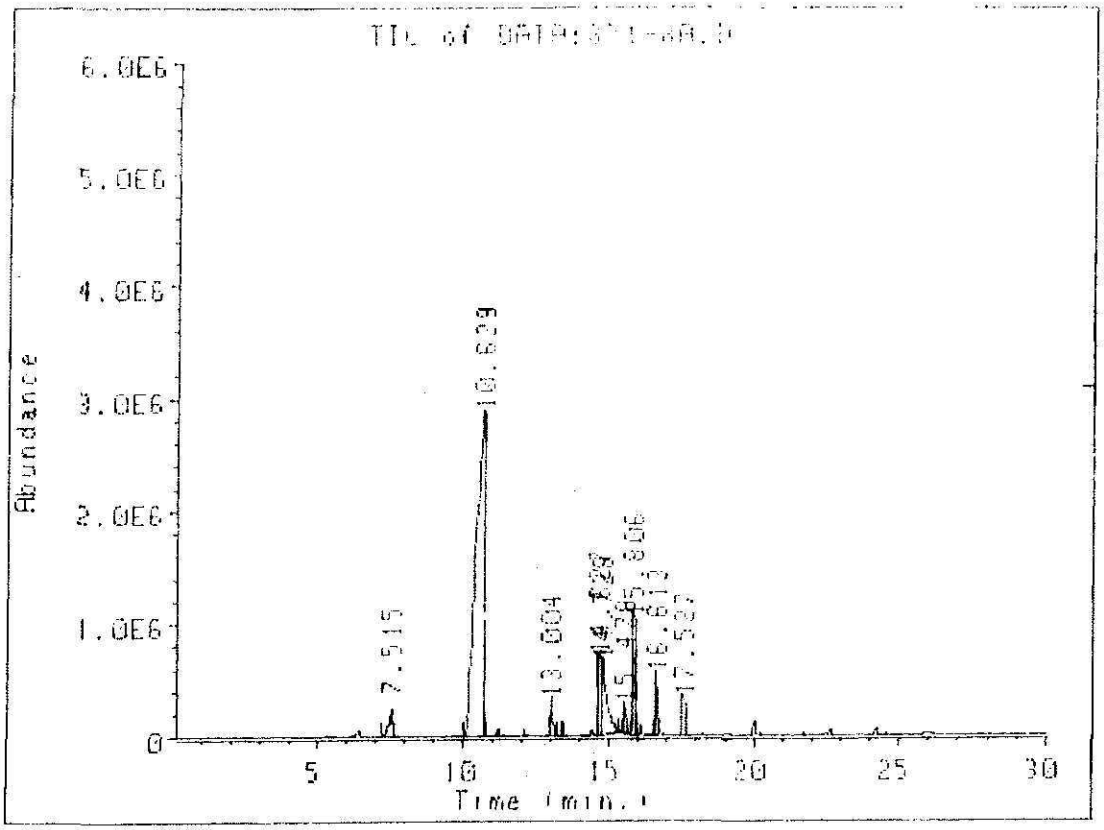
to improve the correlation value, the concentration of the component in the sample must be increased to reach a greater difference between the signal noise and the corresponding component signal intensity. The present procedure resulted in about a 250 to 500 fold increase in the concentration of components, as compared with that of the neat sample, and the vast majority of components resolved with this technique are at concentration of 20 ppb or less.

Although the technique was evaluated successfully and numerous components were separated in the different chromatograms, the extensive data are still being evaluated for future publications. Standard compounds must be acquired to make more positive GC-MS identification, especially of those compounds with probability values below 90%. Moreover, compounds separated in the packed column system must be properly matched with appropriate standard to be able to validate the quantitative procedure.



Peak #	Ret Time	Type	Width	Area	Start Time	End Time
1	2.107	BB	0.028	1806987	2.079	2.193
2	5.457	VV	0.034	46204741	5.435	5.472
3	6.536	VB	0.056	8402553	6.323	6.623
4	7.379	VB	0.054	37907309	7.223	7.576
5	9.888	BV	0.059	231494953	9.748	9.917
6	9.986	PV	0.061	23388401	9.917	10.157
7	10.517	VB	0.061	4308740	10.421	10.558
8	11.447	PH	0.029	2059494	11.415	11.489
9	12.040	BB	0.036	4447070	11.998	12.156
10	13.960	BH	0.055	9554926	13.883	13.976
11	13.994	VH	0.107	18657165	13.976	14.223
12	15.014	BV	0.047	66536359	14.886	15.238
13	16.585	BB	0.035	12315706	16.470	16.665
14	22.393	BB	0.066	6332514	22.261	22.541

FIG. 16.—Capillary column GC-MS total ion chromatogram of R-831 reduced dichloromethane extract.



Peak #	Ret Time	Type	Width	Area	Start Time	End Time
1	7.515	BB	0.121	22935261	7.178	7.598
2	10.629	BH	0.244	571634724	9.994	10.724
3	13.004	BH	0.068	16722005	12.939	13.209
4	14.627	BV	0.070	3947491	14.589	14.700
5	14.728	VV	0.147	79296150	14.700	15.307
6	15.478	BB	0.039	7335849	15.407	15.577
7	15.806	BB	0.063	51084775	15.698	15.904
8	16.613	BV	0.052	21905187	16.488	16.665
9	17.527	BB	0.047	11820033	17.448	17.633

FIG. 17.—Capillary column GC-MS total ion chromatogram of R-831 reduced dichloromethane extract.

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