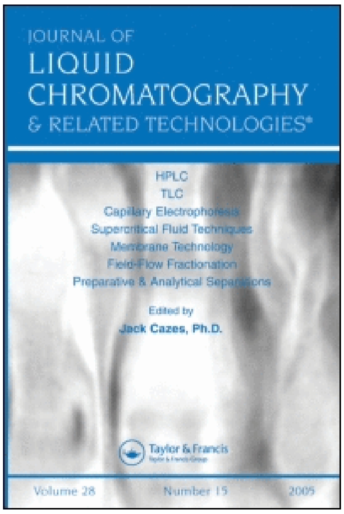

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SIMPLE TLC-GC METHOD FOR SEMI-QUANTITATIVE SEPARATION OF LIPID CLASSES IN COASTAL SEDIMENTS

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ABSTRACT.

A simple method combining quantitative TLC and GC methods was used to isolate, quantify and identify the lipidic constituents in the sandy sediments of the northern Rio de la Plata coast.

INTRODUCTION.

The analysis of lipidic constituents is important in several fields, including biological studies, chemical taxonomy, contamination control, soil analysis and geochemical studies (1-12,19-20). A correct determination not only can give an idea of the lipidic content, but also of its likely origins and previous history (6,9-12,16,19).

Several methods have been described, usually combining column (2,3,5,8), thin layer (3,7,8,18), gas (2,3,5,20) and/or high performance liquid chromatographic methods (5). It became apparent to us that given the usual constituents, a combined TLC-GC method developed for studying vegetable epicuticular waxes could be applied with advantages to these problems (13,14,17).

MATERIALS.

Precoated chromatoplates were used for preparative TLC (Merck 5554- DC-Alufolien, 20x20 cm x 0.20 mm thickness, Kiesegel 60G F_{254}). Solvents are redistilled in glass.

Gas chromatography was performed with a Pye-Unicam 104 and a Shimadzu GC-6AM with dual FID. Columns were OV-17 5% on Chromosorb 80-100 mesh, 1.5 m lenght, 3 mm ID and HP-1 (25m x 530um). GC-MS was performed on an HP-5970B mass detector, using an SE-30 fused-silica capillary column (15m x 0.25mm).

METHODS.

Samples were collected at Pocitos (POC), Jaureguiberry (JAU), La Pedrera (PED) and Punta del Diablo (PDD).

Samples were prepared by collecting the top (2 cm) of sand from a 0.25 m^2 surface, and mixed rigorously.

1 Kg fractions were extracted in a Soxhlet apparatus for 24 hs with methanol-toluene (70:30, 1.5 L). The extracts were evaporated under vacuum and redissolved in chloroform:methanol (1:1, 200 uL) for application to the plates. The plates were developed 8 cm with petrol ether:toluene (80:20, v/v), air-dried and redeveloped to 18 cm with chloroform:acetone (95:5, v/v). The bands were revealed with UV light (254 n.m.), scraped, and extracted twice with chloroform:methanol (1:1, 2 mL). The combined extracts were dried under a nitrogen stream, and redissolved in chloroform, prior to the injection in the GC. Compounds were identified by co-chromatography against authentic samples.

RESULTS.

Samples from the northern coast of the Rio de la Plata (Uruguay) were extracted with methanol-toluene (70:30). The extracts were applied to preparative TLC plates and developed using an unidirectional bidimensional technique (13,17). The bands were eluted and quantitated by GC.

The results are shown in Table I (crude residue % w/w), Table II (percentage of different lipidic

Table I: Crude Residues in ppm (mg/Kg of sand)

Pocitos (POC)	80
Jaureguiberry (JAU)	10
La Pedrera (PED)	10
Punta del Diablo (PDD)	10

Table II: Percentage of Different Lipidic Fractions (w/w).

	POC	JAU	PED	PDD
hydrocarbons	29.2	16.9	8.7	56.6
aromatic hydrocarbons	38.5			
alcohols	9.0	62.1	8.0	9.3
acids	23.2	21.0	83.3	34.1

fractions w/w) and Tables III-VI (composition of the fractions).

DISCUSSION.

This simple and economic method allows for a rapid and efficient way of separating the lipidic constituents in the extracts. It can be adapted to other extraction techniques (5) and the isolation of other specific fractions can be improved by changing the conditions of the TLC separation (3,13,17). It avoids the saponification step before the separation of the fractions, thus keeping relevant information

Table III: Aromatic Hydrocarbons Fraction Composition (% w/w)

12,13-Dihidro-Naphto[2,3-B:2',3-D]Thiphene	12.6
Anthracene	6.7
4,5-Diphenyl-3H-1,2-Dithiole-3-Thione	9.4
1-Phenyl-Naphthalene	9.8
Fluoranthene	18.1
Benzo[c]Phenentrene	15.1
Naphtacene	18.9
Chrysene	9.4

Table IV: Hydrocarbon Fractions Composition (% w/w).

	POC	JAU	PED	PDD
15		7.1	7.1	
16	0.9	tr	8.3	
17	8.6	2.8	5.0	
18	4.7	1.9	4.7	
19	3.8	4.8	19.2	
20	3.6	1.9	3.0	
21	2.8	3.8	10.1	3.5
22	8.7	7.6	5.0	2.7
23	2.4	12.3	5.9	tr
24	3.2	15.2	9.7	tr
25	5.2	8.6	4.6	24.0
26	4.3	6.7	3.8	3.9
27	5.7	27.6	3.9	9.8
28	8.6	6.7		8.3
29	6.7	tr		19.3
30	6.8	tr		2.0
31	7.4	tr		4.7
32	3.5	tr		1.2
33	3.1			
34	1.0			
U.I.	8.8		9.9	

related to the extract composition of the original constituents (5,7,8). The unidirectional bidimentional TLC manages to separate apolar and polar constituents in one operation, thus making unnecessary two chromatographic separations (2,7,8).

The patterns of lipidic components observed are interesting to consider. Sample (Pocitos) is the only one to show PAs in detectable amounts, compounds which were probably the rain-washoff from nearby road-works.

The figures for saturated hydrocarbons (Table II and IV) also show that sample (Pocitos) is heavily contaminated by hydrocarbons of mineral origin (the presence of equivalent percentages of odd and even C homologues), whereas the distribution patterns for samples (Jaureguiberry, La Pedrera y Punta del Diablo) with even C homologue predominance are consistent with a natural biological origin (1,13,15-17). This same can be said about the fatty acids (Table V), where although the great abundance of C16 and C18 homologues could be ascribed to human activity, their natural biological origin cannot be discarded (1).

The method described is rapid, simple and effective. Its low cost is most adequate for repetitive sampling when "worry zones" of high pollution have to be identified or when contamination control strategies are being tried out.

Table V: Alcohol Fractions Composition (% w/w).

	POC	JAU	PED	PDD
14			2.9	
16	38.8	26.9	45.9	
18		22.4		
20	25.9		19.6	
22	20.5			
24	2.6		0.7	
26				31.0
28	1.9	50.6	1.0	42.0
30			7.8	5.4
32	10.3		2.0	17.2
34	tr	1.1		
36		0.9		
U.I.			18.6	4.3

Table VI: Fatty Acids Fractions Composition (% w/w)

	POC	JAU	PED	PDD
12		21.6		
13				
14		7.2		1.3
15				1.0
16	10.8	56.2	13.8	50.8
17				1.2
18	22.5	15.0	26.6	13.7
19	1.0			0.3
20	3.5		4.9	0.8
21	1.0		7.8	
22	6.1		4.7	3.4
23	2.3			
24	5.3		5.7	0.4
25	1.9			1.5
26	3.9		3.7	7.5
27	1.0			0.4
28	1.1		0.6	0.9
29	0.4			
30	33.9			1.7
31	5.3			
U.I.			31.3	14.1

In our case, three samples from the Eastern Rio de la Plata-Atlantic Ocean coast show no apparent worrying compositions. but those from (Pocitos), within Montevideo's limits, give reason for the improvement of contamination-control measures.

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