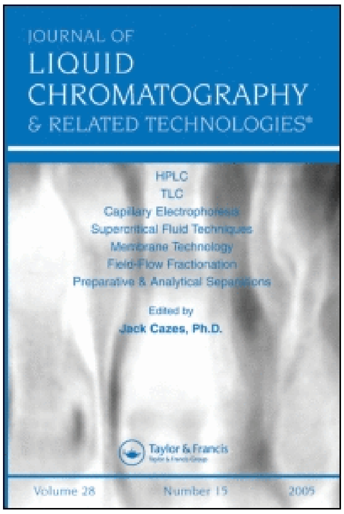

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TLC-Scanning for Direct Quantitation of Natural Waxes

Ruben Martínez[a]; Patrick Moyna[a]
[a] Farmacognosia y Praductos Naturales Facultad de Química, Montevideo, Uruguay

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TLC-SCANNING FOR DIRECT QUANTITATION OF NATURAL WAXES

RUBEN MARTÍNEZ AND PATRICK MOYNA
Farmacognosia y Productos Naturales
Facultad de Química
Av. Gral. Flores 2124
Montevideo, Uruguay

ABSTRACT

H_2SO_4/charring is used as visualization method for quantitative TLC scanning of waxes. The appropriate technical details are described.

INTRODUCTION

The range of components usually present in waxes (1) makes their direct quantitation difficult. This has been achieved by quantitative elution in Column Chromatography (CC) (2) or by combined Thin Layer Chromatography-Gas Liquid Chromatography (TLC-GLC) (3). For a limited group of components direct GLC of the wax has been tried (4). TLC scanning techniques seem particularly appropriate for this problem (5). Four stages of the TLC technique have to be controlled for adequate quantitation (5): plates with even surfaces;proper application of samples; adequate solvent mixtures; good visualization methods. For working on waxes even laboratory-prepared plates give adequate results. As sample spotting is simultaneous, it is simple to control the application stage (1,3,6). Slight variations of the solvent mixtures used in wax analysis will give good separations for most fractions present. This leaves visualization as the main difficulty: most wax constituents lack chromophores and do not fluoresce. With the exception of the more general visualization reagents, few cover well the wide range of wax components.

Given the available information on H_2SO_4/charring (6,7), the method was selected as worth assaying as a visualization procedure with quantitative capability.

MATERIALS AND METHODS

Plates were prepared with Silica-gel G (E. Merck, Nr. 7730).

All solvents were analytical grade (Mallinckrodt, USA).

Pure acids (C_{16},C_{18}, C_{26}, C_{30}) and hydrocarbons (C_{24},C_{26}) were from Aldrich (USA).

Pure alcohols (C_{16}, C_{18}, C_{20}) were from Fluka (Switzerland).

Triterpenes and esters were prepared from natural samples by CC (3,8,9).

Wax samples were prepared from <u>Colletia paradoxa</u> by the methods described (8).

A TLC Scanner (Shimadzu CS-9000) was used with the following parameters:

Photo mode: ABS REF
Zero set mode: STORED
Scan mode: ZIG-ZAG
Swing width: 10.0 mm
Delta "Y": 0.20 mm
PKF filter: 50
Drift line: 10.00
Minimum width: 5.0
Minimum area: 1000

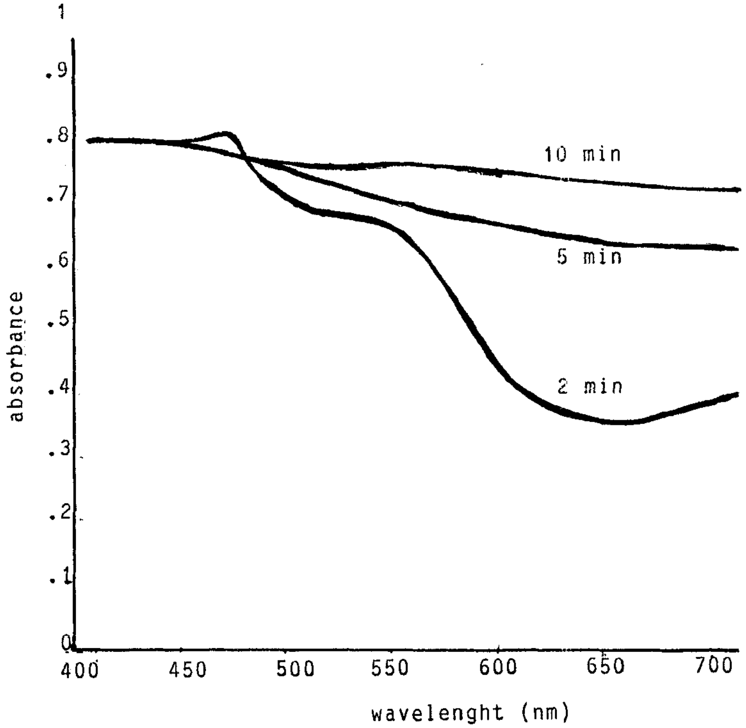

FIGURE 1 - INFLUENCE OF OVEN TIME

RESULTS AND DISCUSSION

The usual charring conditions (H_2SO_4:H_2O (1:1); 200°C oven temperature) (6) were used to establish the basic operation parameters. The influence of the amount of charring agent and oven time were determined and are shown in Figures 1 and 2. It could be established that on these conditions a maximum response could be obtained after 2 minutes, independently of the amount of charring agent applied when measuring below 450 nm. Over 450 nm the maximum could only be approached with increased time.

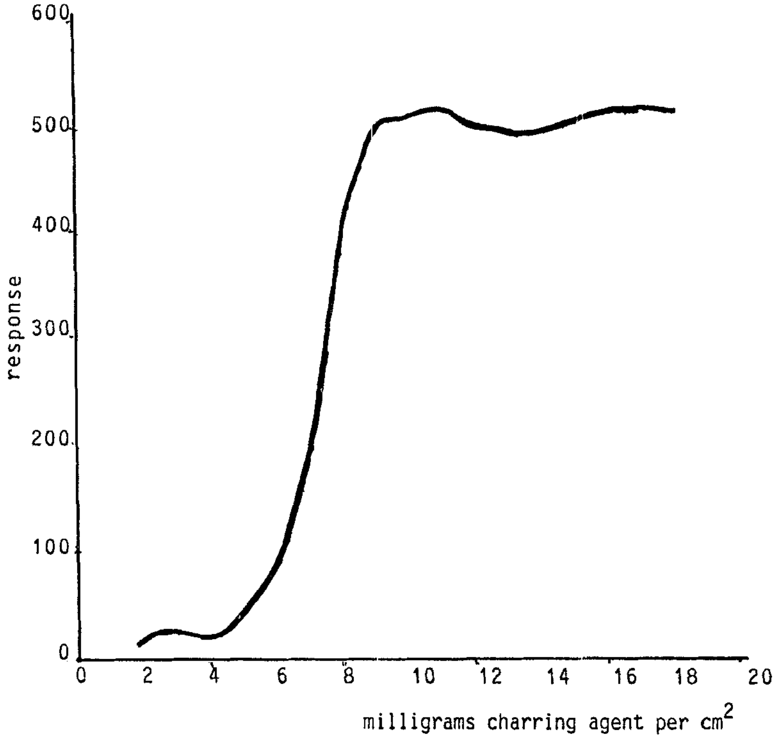

FIGURE 2 - INFLUENCE OF AMOUNT OF CHARRING AGENT

(*) Response is expressed in "Area" values of the TLC-scanner for a sample of 30 mg of acids.

The operating conditions thus established (10 min; 200°C oven temperature; 4 g charring agent per 20 x 20 cm plate) were used to scan the **absorbance-450 nm/concentration** ratios for pure wax components. The results are shown in Figure 3. It can be observed that there is a concentration over which the results are non-linear. These "saturation" thresholds are different for the different fractions and the slope under

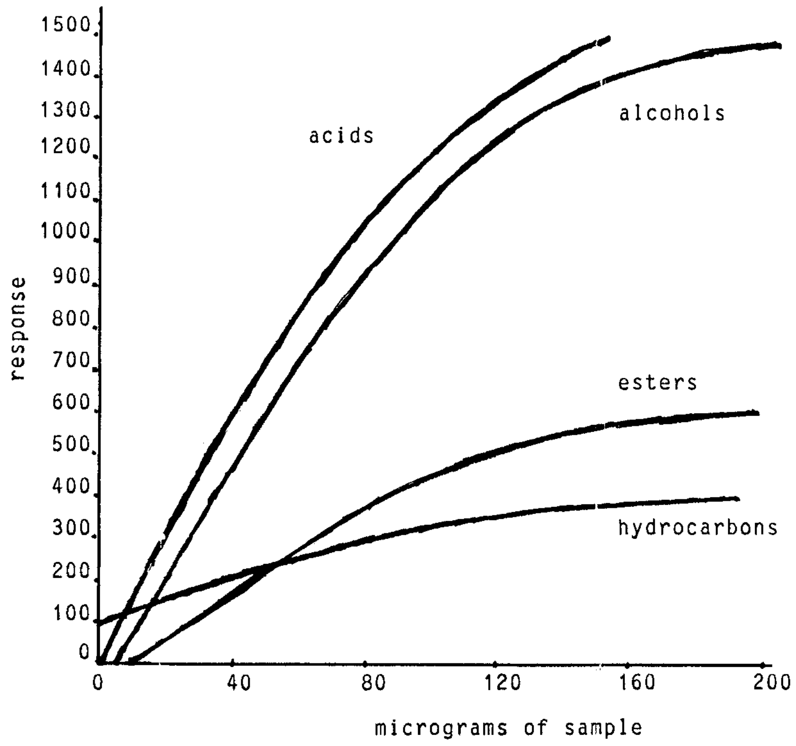

FIGURE 3 - RESPONSE FOR DIFFERENT COMPONENTS

(*) Response is expressed in "Area" values of the TLC-scanner.

Table 1 - Linear Range and Correlation Data for Wax Components

Component	Linear range (micrograms per plate)	Ratio (Acids = 1)
Linear Acids	20 - 100	1.00
Linear Alcohols	20 - 100	0.89
Esters	40 - 120	0.18
Hydrocarbons	40 - 120	0.10
Triterpenes		
Taraxerol	1 - 5	4.66
Oleanoic Ac.	2 - 10	2.84
Taraxerone	10 - 50	1.74

Table 2 - Composition of Colletia paradoxa

	by TLC-GC (8)(*)	by TLC-scanning
Hydrocarbons	22 %	22 %
Ketones	18 %	16 %
Free Acids	14 %	15 %
Free Alcohols (**)	13 %	17 %
Esters	10 %	8 %

(*) The TLC-GC values are corrected for the polar compounds present in the original sample (8).

(**) "Free Alcohols" is expressed directly in taraxerol and needs further refinement. It does not take into account that it is a mixture of 3 % alkanols and 97 % triterpenic alcohols (lupeol, germanicol, taraxerol, 2:1:1).

this limit is specific for each component. The correlation data are shown in Table 1.

The method was tried on the wax of <u>Colletia paradoxa</u> and the results were compared against those obtained by other quantitation approaches (8). This wax was selected as previously analysed samples were available and also for its broad range of components. The results are shown in Table 2.

The TLC scanning results are directly related to the original wax composition by the correlation data, while other methods tend to discard the more polar fractions that cannot be eluted from CC or be observed by GLC.

The correlation is good, that for the "Free alcohols" fraction being the lowest. In this wax, this fraction is 97 % terpenic alcohols and only 3 % alkanols so its quantitation needs further refinement.

ACKNOWLEDGEMENTS

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