

## Rapid Paper Chromatography of Carboxylic Acids

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$C_1$  to  $C_{14}$  straight chain fatty acids have been separated by chromatography on dimethyl sulphoxide impregnated paper. The acids migrated as almost round spots and a complete separation was obtained within less than 2 hours. The method was also found to be useful with alicyclic and higher dicarboxylic acids.

In the course of an investigation on the paper chromatography of sugar acetates, using dimethyl sulphoxide (DMS) as the polar, stationary phase<sup>1</sup>, it was found that the normal fatty acids in the range formic to myristic acid could be separated in a similar way. The present investigation includes the separation of normal fatty acids and some alicyclic and dicarboxylic acids (Table 1). When compared to other solvent systems used for the paper chromatography of these acids, those containing DMS seem to give a good resolution in quite a short time.

The papers were usually first impregnated with DMS, the samples were then added as solutions of the free acids and the chromatograms developed with light petroleum, isopropyl ether or a solution of DMS in ethyl ether. The spots were detected by spraying with an indicator solution. The total time required for the separation of fatty acids was about 45 min to 2 h.

As is to be expected, the  $R_f$  values increase with the number of carbon atoms in an homologous series, with the polarity of the eluant and with the moisture content of the paper. Of monocarboxylic acids having the same number of carbon atoms, those with a straight chain carbon skeleton seem to move faster than those having a cyclic structure. There is a significant difference between normal and isobutyric acid (Table 1), but not between the isomeric valeric acids.

To obtain predictable results it is essential to control the moisture content of the papers (see experimental part); it is usually most convenient to run the chromatograms under dry conditions, but slightly moist conditions will sometimes improve the separation (e.g. of (+)-camphonanic and hinokiic acid, Table 1). The spots are generally almost circular or only very slightly tailing. A conceivable explanation is that the acids in DMS solution are not appreciably dissociated or dimerised. If tailing occurs, this may be caused by too high a

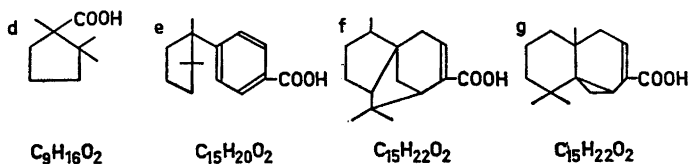
Table 1.  $R_F$  values of carboxylic acids on DMS impregnated paper.

Solvents	Light petroleum		Isopropyl ether	Ethyl ether — — DMS 24:1 v/v <sup>c</sup>
	Dry <sup>a,c</sup>	Moist <sup>b</sup>		
<i>Acids:</i>				
Formic			0.06	0.18
Acetic			0.12	0.27
Propionic			0.21	0.38
<i>n</i> -Butyric	0.03	0.06	0.29	0.48
<i>iso</i> -Butyric	0.04	0.07		
<i>n</i> -Valeric	0.06	0.10	0.38	0.55
Hexanoic	0.09	0.18	0.47	0.61
Heptanoic	0.14	0.29	0.57	
Octanoic	0.20	0.42	0.64	
Nonanoic	0.28	0.55	0.71	
Decanoic	0.35	0.67	0.77	
Hendecanoic	0.42	0.75	0.83	
Lauric	0.49	0.82		
Myristic	0.59			
Palmitic	0.66			
Stearic	0.68			
(+)Camphonic <sup>d</sup>	0.21	0.45		
Cuparenic <sup>e</sup>	0.16	0.42		
Cedrenic <sup>f</sup>	0.31	0.66		
Hinokiic <sup>g,4</sup>	0.21	0.50		
Glutaric				0.04
Adipic				0.05
$\alpha,\alpha$ -Dimethyladipic				0.15
Sebacic				0.21

<sup>a</sup> Conditioning with silica gel impregnated with DMS

<sup>b</sup> Conditioning with silica gel impregnated with DMS containing 10 % v/v of water.

<sup>c</sup> Diffuse solvent front



water content in the solvents, by insufficient impregnation with DMS, by the application of too large amounts of samples or by crystallisation of the samples on the paper. Evaporation of volatile acids from the chromatograms becomes apparent only on prolonged development. Thus formic, acetic, butyric and valeric acids (*ca* 20  $\mu$ g of each) could still be detected on chromatograms after 5 h development with light petroleum.

Preparative separations of sesquiterpenic acids have been made on thick filter paper or silica gel columns impregnated with DMS <sup>2</sup>. With silica gel — DMS, adsorption effects may occur as has been indicated by current work at this institute on the resolution of a tropolone—phenol mixture from plant material.

## EXPERIMENTAL

*Paper.* Whatman No. 1 filter paper was cut into strips 23 or 46 cm long, with the starting line 5 or 7 cm, respectively, from the upper end.

*Solvents.* DMS was of a technical quality\* (99.5 % purity) with less than 0.3 % of water. Other solvents were of reagent grade.

*Impregnation of papers.* The papers were passed at an even rate through a 25 % v/v solution of DMS in toluene, uniformly blotted between layers of filter paper and dried at 60° for 1 min to remove the toluene. This process was repeated once and the papers were then immediately placed between glass plates leaving only the starting line and the paper above it uncovered. (After application of the samples, the papers were transferred to the chromatogram tanks in such a manner that absorption of moisture was avoided.)

When the reproducibility is unimportant, the impregnation may be simplified, e. g. by blowing off the toluene with hot air.

*Application of samples.* Usually 1–2  $\mu$ l of 2 % solutions of the acids in ethyl ether were applied on the paper, placing the spots at least 1.5 cm from the paper edges and 1 cm from each other on short papers; on long papers the corresponding distances were 2 and 1.5 cm, respectively. 5–100  $\mu$ l samples of acetic acid have been run on short papers using the ethyl ether – DMS eluant. Normal chromatograms of acetic acid have also been obtained by applying 1–2  $\mu$ l of aqueous sodium acetate to the starting line before the impregnation with DMS. After impregnation acetic acid was liberated by adding enough of a saturated solution of oxalic acid in acetone-ethyl ether (1:1) to cover the acetate spot.

*Development.* All chromatograms were developed by the descending technique. The following solvents were used:

Light petroleum, b. p. 60–71° (Skellysolve B), not purified.

Isopropyl ether, sodium dried.

Ethyl ether (sodium dried) containing 4 % v/v of DMS.

When light petroleum was used as the eluant, the papers were 46 cm long and the chromatogram tanks were equipped with wire gauze bags containing silica gel impregnated with DMS or DMS-water to obtained standardised moisture conditions. With silica gel impregnated with DMS-water (9:1) there was only a little tailing, but this became very pronounced with DMS-water (8:2).

For safety reasons all experiments with ethyl or isopropyl ether were done in small chromatogram tanks using papers 23 cm long. The tanks contained no drying agent but were frequently cleaned and filled with fresh solvent.

*Detection of spots.* The spots were detected by spraying with the mixed indicator of Duncan and Porteous\*: Methyl red (200 mg) and bromothymol blue (200 mg) dissolved in formalin (100 ml) and ethanol (400 ml). The pH is adjusted as required below by adding dilute sodium hydroxide solution. Without drying, the chromatograms were briefly exposed to ammonia vapours, then evenly sprayed with indicator solution, treated with ammonia as before and finally sprayed with more indicator till red or orange spots were obtained against a stable green background. The spots were usually stable for more than an hour. If the indicator is too acid, the background colour will not be stable, and if it is too alkaline trace amounts of acids will not be detected. (Duncan and Porteous recommend pH 5.2 and several ammonia treatments after spraying only once with indicator.) It is advisable to mark faint spots as soon as they appear. Strong, blurred spots become sharper if more indicator is applied or if the indicator is more alkaline.

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\* Purchased from Nitroglycerin AB, Gyttop, Sweden.