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Note

Thin-layer chromatographic method for the identification of organic acids

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The interest in the analysis of organic acids has increased in recent years, particularly in biochemistry where one wishes to follow the metabolism of organic acids in biological systems. There is also a need for an accurate analytical method in other fields, for example in the food industry, especially those industries producing fruit juices and other refreshing drinks. Although paper, liquid and gas chromatography have found wide application in the detection of organic acids, thin-layer chromatography (TLC) is the method of first choice for a quick and reliable analysis of several samples. Therefore, many articles have been written describing TLC methods for organic acids[1–6].

The method presented here is a further development of existing methods, but has been substantially improved by using pre-coated TLC plates and a new solvent system.

EXPERIMENTAL

In order to achieve the best reproducibility, pre-coated silica gel plates (Sil G 25, 20 × 20 cm; Macherey, Nagel & Co., Düren, G.F.R.) were used. The plates were dried at 105° for 1 h before use. A suitable concentration of the acids in the samples and standards was 0.2–0.5%. In order to ensure that all of the acids were converted into the acid form, 10% concentrated formic acid was added to all of the samples and standards; 1 μl of this solution was then applied to the TLC plates. When the spots had dried, the plates were placed in a developing tank containing one of the following solvent systems: Solvent system 1: *n*-pentyl formate–chloroform–formic acid (70:15:15), and solvent system 2: *n*-pentyl formate–chloroform–formic acid (20:70:10). The freshly prepared solvent was poured into the tank 1 h prior to use in order to ensure that the chromatography took place in an atmosphere saturated with solvent vapours.

After 1.5 h the solvent front had migrated *ca.* 15 cm. The plate was then removed, and dried in a current of air at room temperature for 24 h in order to remove the last traces of formic acid. In order to locate the spots of the acids, the plate was dipped for a short time in a reagent consisting of 0.1 g of bromocresol green, 500 ml of ethanol and 5 ml of 0.1 *M* NaOH. The acids appeared as yellow spots on a blue background.

DISCUSSION

This TLC method for the separation of organic acids is used routinely for the identification of acids in fermentation broths and enzymatic reaction mixtures. The method is an improvement on previously described methods in that it is quick and gives very reliable determinations. The accuracy is obtained by using pre-coated TLC glass plates together with *n*-pentyl formate in the solvent system. Since both *n*-pentyl formate and chloroform are volatile, chromatography must take place in a saturated atmosphere if one is to expect reproducible R_F values for the individual acids. Application of the detection reagent is critical, and the solvent must not be present. Formic acid is the last solvent to evaporate, and in order to ensure that no trace of it is left the plate is kept overnight in a stream of air in a fume cupboard.

The use of two solvent systems makes identification based on R_F values more accurate, since many of the acids which have similar R_F values in one system may be separated in the other, as is illustrated in Table I and Figs. 1 and 2.

TABLE I

R_F VALUES OF ORGANIC ACIDS

Acid	*Solvent system 1*	*Solvent system 2*
Picric acid	0.76	0.74
Hexanoic acid	0.75	0.82
Salicylic acid	0.72	0.78
n-Valeric acid	0.71	0.77
Benzoic acid	0.69	0.79
Phenylacetic acid	0.68	0.74
Phenoxyacetic acid	0.64	0.63
Pyruvic acid	0.60	0.46
Mandelic acid	0.57	0.44
Phthalic acid	0.55	0.41
Glutaric acid	0.54	0.41
Maleic acid	0.50	0.30
α-Hydroxybutyric acid	0.50	0.38
Fumaric acid	0.49	0.37
Adipic acid	0.48	0.36
α-Oxoglutaric acid	0.45	0.23
Succinic acid	0.43	0.28
Glyoxylic acid	0.43	0.21
Malonic acid	0.40	0.20
Lactic acid	0.36	0.24
Glycolic acid	0.31	0.16
Malic acid	0.26	0.08
Glyceric acid	0.24	0.08
Citric acid	0.22	0.04
Tartaric acid	0.19	0.03
Ascorbic acid	0.15	0.03
Boric acid	0.07	0.05
Nicotinic acid	0.05	0.02
Oxalic acid	0 0.30 (diffuse)	0-0.17 (diffuse)

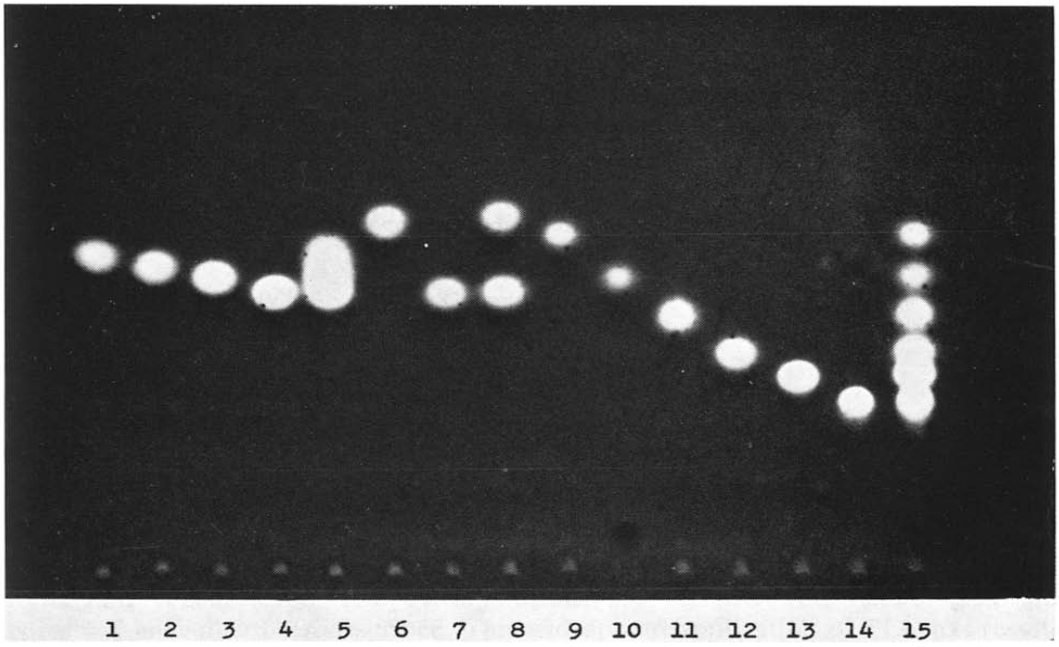

Fig. 1. Chromatogram of organic acids on a pre-coated silica gel plate (Sil G, 20 × 20 cm). Solvent system, *n*-pentyl formate–chloroform–formic acid (70:15:15). Detection reagent, bromocresol green. Samples (1 μl): 1 = adipic acid; 2 = glutaric acid; 3 = succinic acid; 4 = malonic acid; 5 = samples 1–4; 6 = fumaric acid; 7 = maleic acid; 8 = samples 6 and 7; 9 = mandelic acid; 10 = α-hydroxybutyric acid; 11 = lactic acid; 12 = glycolic acid; 13 = malic acid; 14 = citric acid; 15 = samples 9–14.

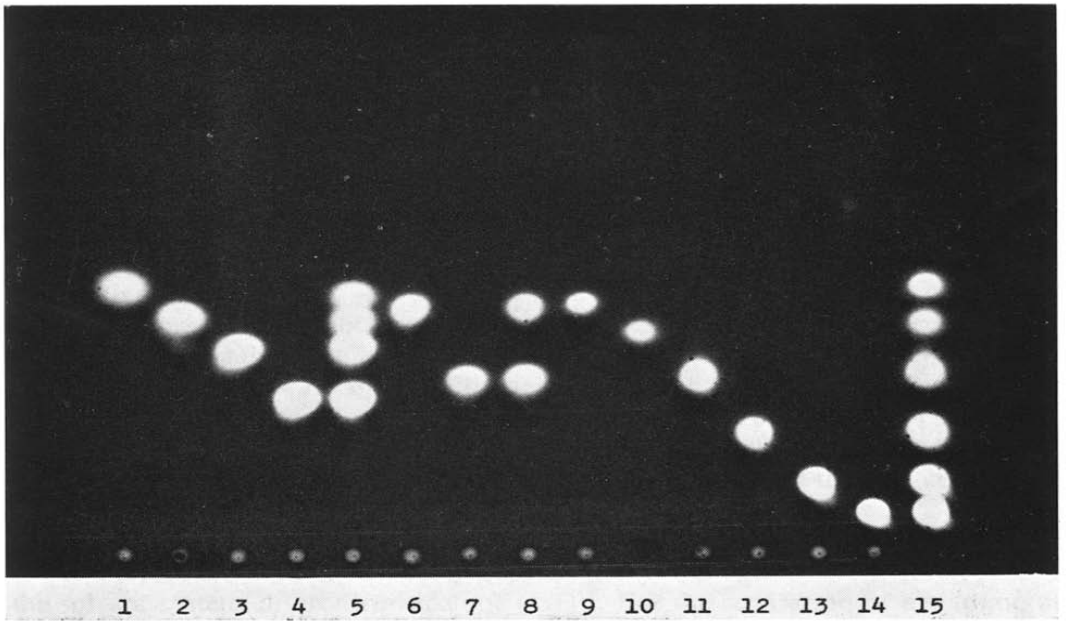

Fig. 2. Chromatogram of organic acids on a pre-coated silica gel plate (Sil G, 20 × 20 cm). Solvent system, *n*-pentyl formate–chloroform–formic acid (20:70:10). Other details as in Fig. 1.

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