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Note

Normal-phase thin-layer chromatography on silica gel with simultaneous paraffin impregnation for subsequent reversed-phase thin-layer chromatography in a second dimension

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Two-dimensional chromatography was first described by Consden *et al.*[1] for the separation of amino acids by paper chromatography. The technique is especially useful for the separation of complex mixtures which are unresolved by chromatography in a single solvent system. Two-dimensional chromatography has therefore been extensively applied to difficult separations, and many of these have recently been reviewed[2]. Normally in two-dimensional thin-layer chromatography (TLC), solvent systems with different selectivities are employed to achieve resolution but the underlying separation mechanism remains unaltered. There are examples where different separation mechanisms have been employed for each dimention, using either mixed stationary phases[3], or modification of the stationary phase by impregnation following chromatography in the first dimension[4]. In spite of their advantages these techniques have not been widely adopted. The resurgence of interest in reversed-phase TLC on bonded phases has resulted in the rediscovery of two-dimensional TLC with different separation mechanisms, using a combination of normal-phase (NP-TLC, adsorption) and reversed-phase chromatography (RP-TLC, partition). This may be achieved on either commercially prepared TLC plates such as the Whatman Multi K, which have a 3-cm strip of silanised silica (C_{18}) adjacent to a 17 × 20 cm strip of silica gel, or by silanising a section of a silica TLC plate in the laboratory[5]. Our experience with RP-TLC on paraffin coated silica gel TLC plates[6] prompted us to examine two-dimensional TLC with normal and reversed-phase chromatography on coated rather than bonded plates. The method which we have developed is based upon the incorporation of 7.5% paraffin in the normal-phase solvent system used to obtain the initial separation. During chromatography in the first dimension there is a concomitant coating of the silica gel with paraffin which, following the evaporation of the solvent, results in a TLC plate which may then be used for subsequent reversed-phase TLC in the second dimension. This approach and a number of variations have been investigated using a range of model compounds including ecdysteroids, aminophenols, antipyrine and several non-steroidal anti-inflammatory drugs.

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EXPERIMENTAL

Materials

Precoated plastic-backed silica gel TLC plates 20 × 20 cm with fluorescent indicator, manufactured by Macherey, Nagel & Co., were purchased from Camlab, Cambridge, U.K. Spectrosol paraffin used for coating the plates was purchased from BDH, Poole, U.K., and was included in the solvent systems used for coating the TLC plates at 7.5% (v/v) as described in Wilson *et al.*[6]. For post-chromatographic impregnation a hexane–paraffin (92.5:7.5) solution was used. Solvents used for chromatography were of technical grade. Ascending chromatography was performed in glass TLC tanks (20 × 20 × 5 cm), saturated with the solvent. Samples were applied using a 1-μl glass capillary as solutions in methanol.

TLC solvent systems

Normal-phase solvent systems were as follows: ecdysteroids, chloroform–ethanol (4:1); aminophenols, chloroform–acetone (1:1); non-steroidal anti-inflammatory drugs, ether–chloroform–acetic acid (2:1:0.05); antipyrine, chloroform–acetone (3:7).

Reversed-phase solvent systems were as follows: ecdysteroids, methanol–water (1:1); aminophenols, water; non-steroidal anti-inflammatory drugs, ethanol–water (1:1); antipyrine, ethanol–water (1:1).

RESULTS AND DISCUSSION

Initially the R_F values for each of the model compounds were determined using the normal-phase solvent system on silica gel. This process was repeated using the reversed-phase solvent systems on silica gel TLC plates precoated with paraffin. Paraffin (7.5%) was then added to each of the normal-phase TLC solvent systems and the test compounds re-chromatographed. A comparison of the R_F values and spot characteristics obtained in the presence and absence of paraffin in the mobile phase demonstrated that inclusion of paraffin had little effect. TLC plates coated with paraffin during normal-phase chromatography were then re-chromatographed in the second dimension using the appropriate reversed-phase solvent system. The R_F values obtained on reversed-phase TLC in this way were comparable to those obtained on RP-TLC in a single dimension. Therefore, for the range of compounds studied it would appear that inclusion of 7.5% paraffin in the normal-phase solvent system has no adverse effects on chromatography, and that there are no practical problems with subsequent RP-TLC. Representative TLC plates are illustrated in Figs. 1–4. It is likely that under some circumstances the inclusion of paraffin in the solvent might either be difficult (due to solubility–miscibility problems), or may adversely affect chromatography. In such cases it might be possible to coat the TLC plates after the normal-phase separation. This was achieved for ecdysteroids by developing the plate in a weak non-eluting solvent, (hexane), containing 7.5% paraffin. Following drying, the plate was developed in the reversed phase in the usual way. The results obtained using this post-chromatographic paraffin coating were similar to those obtained with the inclusion of paraffin in the normal-phase solvent system. This approach is similar to that adopted by Craske and Edwards[4] for the resolution of dinitrophenyl hydrazones, where, following the normal-phase separation, the plate was coated with Carbowax 400 prior to RP-TLC in the second dimension.

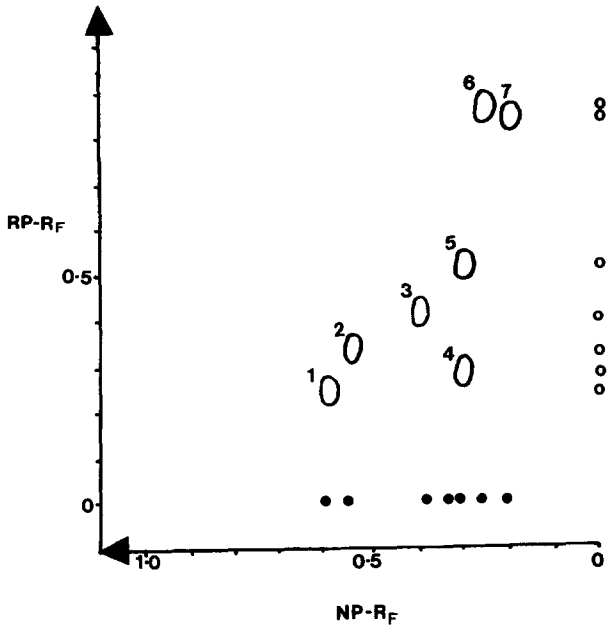

Fig. 1. Non-steroidal anti-inflammatory drugs. (●) R_F on NP-TLC, (○) R_F on RP-TLC. 1 = ibuprofen; 2 = ibufenac; 3 = methyl analogue of isoxepac; 4 = indomethecin; 5 = isoxepac; 6 = salicylic acid; 7 = 5-methyoxysalicylic acid.

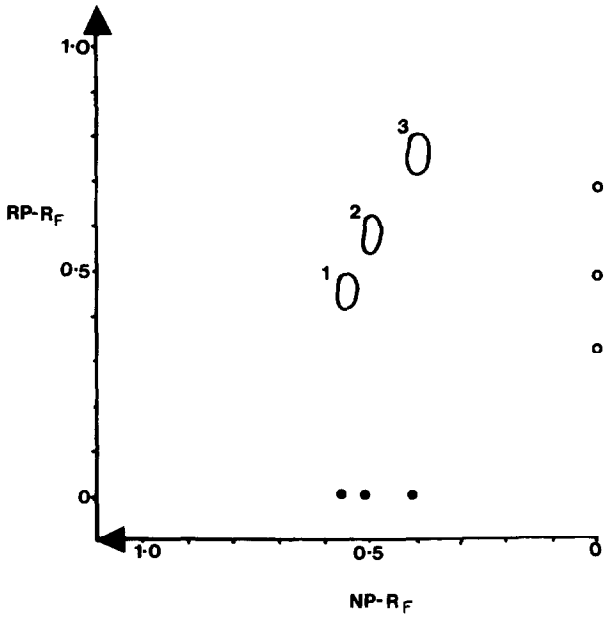

Fig. 2. Aminophenols. (●) R_F on NP-TLC, (○) R_F on RP-TLC. 1 = *p*-aminophenol; 2 = *o*-aminophenol; 3 = *m*-aminophenol.

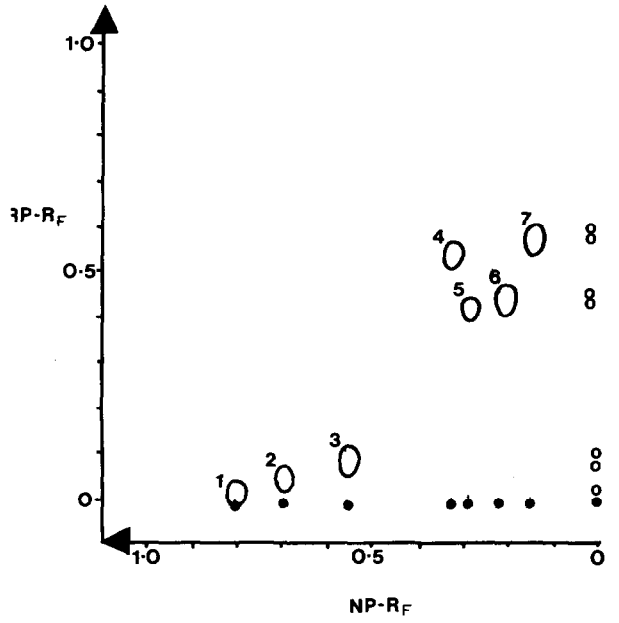

Fig. 3. Ecdysteroids. (●) R_F on NP-TLC, (○) R_F on RP-TLC. 1 = carpesterol; 2 = acetyl-pinnasterol; 3 = pinnasterol; 4 = poststerone; 5 = 2-deoxy-20-hydroxyecdysone; 6 = ecdysone; 7 = 20-hydroxyecdysone.

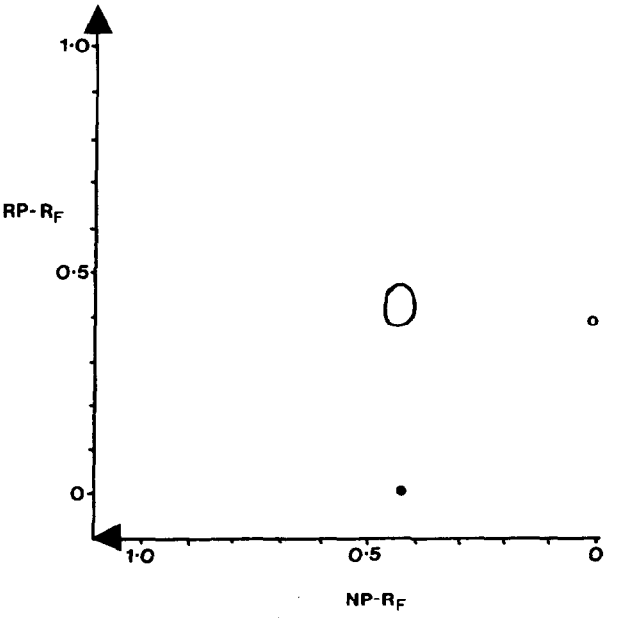

Fig. 4. Antipyrine. (●) R_F on NP-TLC, (○) R_F on RP-TLC.

Lastly, we investigated reversed-phase chromatography followed by normal-phase chromatography using plates partially coated with paraffin (obtained by dipping the silica gel TLC plate in hexane containing 7.5% paraffin to a depth of 3 cm). Non-steroidal anti-inflammatory drugs were used as a model. Following chromatography, the paraffin was removed by a second development in hexane (in the direction of development used for the second dimension), prior to the normal-phase separation. Failure to remove the paraffin before commencing the normal-phase development results in the migration of a zone of paraffin close to the solvent front. This can interfere with the chromatography and detection of compounds which have high R_F values. Development in hexane should extend at least 5 cm beyond the intended development distance for the normal-phase separation. The R_F values obtained for the non-steroidal anti-inflammatory compounds following this procedure were close to the expected values.

CONCLUSION

We have experienced no difficulty in performing two-dimensional chromatography using a reversed-phase separation on paraffin-coated plates for one dimension and normal-phase chromatography for the other. Of the systems we have examined the simplest and least time consuming is normal-phase chromatography with paraffin added to the mobile phase, followed by RP-TLC for the second development. This eliminates both pre or post-chromatographic paraffin coating, and the washing step required by the alternative methods. Compared to the preparation of mixed stationary phases or partially silanised silica-gel TLC plates in the laboratory, paraffin coating is simple and rapid. Whilst the silanising reagents used to prepare RP-TLC plates are extremely reactive and great care must be exercised in their use, no such difficulty exists with paraffin coating. In addition, many silanised plates are intensely hydrophobic[7,8], which restricts the amount of water which can be incorporated in the mobile phase and means that direct application of urine, plasma or bile is not possible. No such restrictions apply to paraffin-coated plates. Paraffin-coated plates are also much cheaper than partially silanised plates (either commercial or homemade). Providing that normal-phase chromatography with concommitant paraffin impregnation is used this method need not be any less convenient or time consuming that the use of partially silanised plates. Another advantage to paraffin coating is that a stock of partially silanised TLC plates does not have to be kept as plates can be coated when required. Paraffin-coated plates therefore represent a useful alternative to mixed stationary phases or partially silanised plates for two-dimensional TLC using both normal and reversed-phase separations.

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