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Note

Conditions for the use of Merck silica gel 60 F_{254} plates in the standardized thin-layer chromatographic technique for lichen products

CHICITA F. CULBERSON
Duke University, Durham, N.C. 27706 (U.S.A.)
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Thin-layer chromatography is an important tool for the identification of secondary products in lichens. A standardized method using three solvent systems has been developed[1,2] to facilitate the recognition of these compounds, and R_F data, obtained by using this method, have been reported for well over 200 natural products and their derivatives.

The standardized method involves running the samples in three solvent systems, determining R_F classes for each spot from the positions of control compounds on each plate, and sorting a deck of punched cards for the three R_F classes found for each unknown. An essential feature of this system is that the R_F classes are determined on every plate by the positions of two substances (atranorin and norstictic acid), so that moderate changes in absolute R_F values do not interfere with the identification. Although the method works even if R_F values change, it fails if relative R_F values change.

Recently, the Merck Company reduced the concentration of binder in the silica gel F_{254} plates (catalog number 5765) that were used to determine R_F values reported for the standardized method of identifying lichen products. Although the reduced concentration of binder in the new plates, now called silica gel 60 F_{254} (catalog number unchanged), improves the quality of the chromatograms, it also drastically changes some relative R_F values. Since the standardized method depends upon relative R_F values in three solvent systems, the new plates cannot be used under the conditions originally described. Compounds chromatographed on the new plates show changes in R_F values that depend upon the solvent system and the chemical nature of the substance. These effects are most serious for compounds that run with R_F values greater than 0.5 and when comparing substances of differing acidity. This note describes methods for improving the results obtained by using the new plates.

Of the three solvent systems used for the standardized method only solvent A (benzene–dioxane–acetic acid, 180:45:5) shows R_F values and R_F classes reasonably close to those previously reported. Chromatograms on the new plates can be run in this solvent without any change in procedure.

When chromatograms are prepared on the new plates using solvent B (*n*-hexane–ethyl ether–formic acid, 130:80:20), R_F values and classes change for compounds that normally run between atranorin and norstictic acid. The values for compounds with a free carboxyl group are depressed as compared with those for similar compounds that lack this functional group. In solvent C (toluene–acetic acid, 200:30), the chromatograms now show a pronounced secondary solvent front between the positions of the control substances atranorin and norstictic acid. Many compounds previously well separated in this solvent system now seem to be identical because they run with the secondary front. Most of the compounds so affected are carboxylic acids that originally gave R_F classes of 5 or 6. Compounds with R_F classes from 1 to 4 are generally not changed on the new plates.

The silica gel 60 F_{254} plates give better results in solvents B and C if they are equilibrated for 5 min in an acidic atmosphere just before being placed in the developing tanks. For chromatograms to be run in solvent B, the spotted plate is equilibrated in an atmosphere of 60% aqueous formic acid. The pre-treatment should be checked by comparing perlatolic acid with atranorin: these two compounds should have nearly identical R_F values in solvent B if the original R_F classes of most of the other lichen products are to be maintained. Without the pre-treatment, perlatolic acid runs below atranorin, and pre-treatment for 5 min in the atmosphere over undiluted formic acid (99%) raises the spot for perlatolic acid above that of atranorin. Dilution of the formic acid to approximately 60% lowers perlatolic acid to the same R_F value as atranorin. To equilibrate the spotted plate, it is placed in a tank over the formic acid solution, but the plate should not touch the solution at any time: the plate is removed after 5 min and run immediately in the usual way. If the R_F values of the atranorin and norstictic acid spots are consistently low in solvent B, they can be raised by increasing the proportion of ethyl ether and decreasing that of hexane, so that the solvent has the composition *n*-hexane–ethyl ether–formic acid, 120:90:20, for example.

For chromatograms to be run in solvent C, the spotted plate should be equilibrated for 5 min in an atmosphere over glacial acetic acid: this treatment eliminates the secondary solvent front. In both solvents B and C, the pre-treatments described above will restore most of the R_F classes observed for lichen products to those reported in the literature, but spots in R_F classes 5 and 6, especially those near the boundary between these two classes, should be searched for in both classes.

The reduction in the concentration of binder used in the commercial silica gel plates effectively changes the activity of these plates for certain compounds chromatographed in certain solvent systems. Although it is unfortunate to encounter a change in these plates once a standardized method had been adopted for screening a large number of compounds, the uniformity of the coating on these plates makes them superior for this work to plates that are prepared in the laboratory.

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REFERENCES

1 C. F. Culberson, *J. Chromatogr.*, 72 (1972) 113.
2 C. F. Culberson and H. Kristinsson, *J. Chromatogr.*, 46 (1970) 85.