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## Polyamide–kieselguhr layer chromatography of antipyretics

The separation of antipyretics on thin layers of silica gel[1–3], alumina[4] and polyamide[5] has been reported. Recently, polyamide mixed layers have been successfully used for the identification of several types of compounds, *e.g.* polyamide–silica gel for red food dyes[6], preservatives[7], water-soluble vitamins[8] and polyamide–kieselguhr for yellow food dyes[9]. These mixed layers have the advantages of good resolution and easy handling. In this note, the separation of eight antipyretics on polyamide–kieselguhr is described. For comparison, thin-layer chromatography on only polyamide and on only kieselguhr is also described.

*Experimental*

*Materials.* The polyamide chip was Nylon 6, type 1022B, of UBE Industrial Ltd. (Osaka, Japan). The solvents were reagent grade of Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

*Preparation of polyamide–kieselguhr layer.* Ten grams of polyamide chip were dissolved in 80 ml of 90% formic acid; then 20 ml of distilled water were added. After gentle warming (below 40°) and stirring, a homogeneous solution was obtained that was then cooled to room temperature. 50 g of Kieselguhr G (Merck) were added. Of the previous solution 250 ml were poured into a dish (15 × 20 × 3 cm) into which a glass plate (12 × 16 × 0.1 cm) was dipped. Both sides of glass were covered homogeneously. The glass was hung for 2 min over the dish to let the excess solution drain off. It was then dried in air for 3 h and heated at 100° for 30 min.

*Preparation of polyamide and of kieselguhr layers.* Before proceeding as described in the previous method, 20 g instead of 10 g of polyamide were dissolved, but without adding Kieselguhr G. Dilute slurries of Kieselguhr G (45 g in 100 ml of water) were sprayed at 1.5 kg/cm$^3$ pressure from a distance of 20 cm onto 8 horizontal glass plates (12 × 16 cm) which were then dried at 100° for 30 min. The thickness of the layers was about 250 $\mu$.

*Chromatographic procedure.* A 0.5% alcoholic solution of samples was applied to the start line 1.5 cm from the bottom of the layer, and the plate was developed by the ascending technique. The chambers had been equilibrated with the respective solvent system for 30 min before use.

*Visualization.* The layers were sprayed with a 0.07% alcoholic solution of Rhodamine B, and the spots could be observed under UV light at 254 m$\mu$.

*Results and discussion*

The $R_F$ values of polyamide–kieselguhr mixed layers, kieselguhr layers and polyamide layers with two solvent systems are given in Table I. It has been found that the results obtained using the mixed layers show better separation, lower $R_F$ values and sharp spots. Also a 10-cm ascent from the origin is more rapid using the mixed layers than when polyamide layers are employed. The addition of a small amount of acetic acid in the solvent mixture is essential to obtain small and sharp spots. After visualization, the mixed layers can be easily removed from the glass plate after a brief immersion in water followed by careful peeling of the layer using

TABLE I

CHROMATOGRAPHIC DATA

Solvent I: chloroform–cyclohexane–glacial acetic acid–dioxane (40:60:1:10). Solvent II: chloroform–cyclohexane–glacial acetic acid (40:60:1). P–K, $R_F$ value obtained on polyamide–kieselguhr layer; K, kieselguhr layer; P, polyamide layer.

| *Substance* | *Solvent I* | | | *Solvent II* | | |
|---|---|---|---|---|---|---|
| | *P–K* | *K* | *P* | *P–K* | *K* | *P* |
| Sulpyrin | 0.00 | 0.70t[b] | 0.00 | 0.00 | 0.75t | 0.00 |
| Salicylic acid | 0.14 | 0.88 | 0.24 | 0.12 | 0.80 | 0.44 |
| Salicylamide | 0.31 | 0.91 | 0.30 | 0.20 | 0.90t | 0.58 |
| Acetylsalicylic acid | 0.48 | 0.89 | 0.58 | 0.37 | 0.94 | 0.80 |
| Quinine HCl | 0.58 | 0.85t | 0.74 | 0.53 | 0.84t | 0.77 |
| Phenacetin | 0.68 | 0.97 | 0.70 | 0.59 | 0.95 | 0.42 |
| Antipyrine | 0.84 | 0.99 | 0.85 | 0.85 | 0.97 | 0.92 |
| Aminopyrine | 0.94 | 0.98 | 0.91 | 0.93 | 0.97 | 0.96 |
| Time (min)[a] | 60 | 20 | 400 | 60 | 20 | 600 |

[a] Time required to ascend 10 cm from origin.
[b] t = tailing.

a spatula. The separated layers can be filed in a notebook for record purposes. The contamination of free salicylic acid in aspirin (acetylsalicylic acid) or its commercial preparations can be easily detected by this method.

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