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APPLICATIONS OF VAPOR-PROGRAMMED THIN LAYER CHROMATOGRAPHY
TO SEPARATIONS OF CLOSELY RELATED COMPONENTS

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Among the chromatographic techniques, thin layer chromatography (TLC) and, to a lesser extent, paper chromatography stand out because of their relative simplicity and low cost. Yet, these flat-bed techniques are also characterized by an additional factor which is not always fully recognized: beside the mobile and stationary phase, there is a substantial vapor phase, its size being determined by the geometry of the developing chamber. Ideally, this vapor phase should be in equilibrium with the mobile and stationary phases. To this end, tank saturation procedures were introduced, consisting of lining the walls with filter paper and introducing the developing solvent into such a tank at least one hour prior to the introduction of the chromatogram sheet and to the actual development of the chromatogram. These procedures became standard practice in PC and when TLC started its rapid development in the late fifties and early sixties, the same saturation procedures were adopted. In the late sixties, however, various research groups became more interested in the role that the vapor phase played in TLC. Vanhaelen¹ studied the various saturation phenomena in TLC and came to the following conclusions:

1. Complete saturation of the developing chamber takes very long, especially with multicomponent solvents. Even with the aid of filter paper saturation times may be in the order of several hours.
2. When a TLC plate is introduced into a completely saturated chamber, significant amounts of vapor will be absorbed by the dry sorbent layer (SiO_2 , Al_2O_3), thus disturbing the vapor equilibria.
3. The above disturbance can be avoided by equilibrating the dry plate during the saturation period, e.g. with the tank in tilted position as to avoid the solvent to reach the plate. However, this leads to considerable diffusion of the substances at the starting points, thus resulting in rather poor separations.
4. Sandwich chambers, especially developed to minimize the influence of the vapor phase give suitable saturation during, but not prior to, development, and the resolution often compares unfavorably with normal chambers.

It thus became apparent that the commonly used saturation procedures do not give saturation at all and that the actual development process takes place under non-equilibrium conditions. Other workers^{2,3}, demonstrated that the vapor phase played an important role in the separation efficiency and that by manipulating the composition of the vapor phase one could exert marked effects on the migration rates of the components under investigation.

As a result of the critical evaluation of the vapor processes in TLC a new trend was advocated in those years, namely the use of unsaturated chambers, in which development of the plate is started immediately after introduction of the solvent⁴. Under these circumstances, the chamber atmosphere is almost devoid of solvent vapor at the beginning, but becomes gradually more and more saturated during the development process. As indicated above, it was realized that the "saturation" procedures in TLC could only be considered as an attempt to standardize the vapor conditions but that such standardization would not automatically provide optimum separation con-

ditions. Indeed, it was found that increased resolving power could be obtained in unsaturated chambers, in particular with multicomponent solvent systems. It was suggested that the better separations were due to vapor adsorption on the dry sorbent, which, in the case of multicomponent solvent systems will result in a gradient of the more polar vapor component(s), showing increased polarity from bottom to top⁵. Accordingly, the faster running spots will gradually meet areas where more of the polar vapor component(s) has been adsorbed, which will result in an accelerated migration of these spots. The lower spots will meet areas with a smaller amount of polar vapor and will not be accelerated to the same extent. This will be extremely suitable for closely related substances which will be pulled apart by the gradient of adsorbed vapor.

Although the influence of the above gradient of adsorbed vapor on the improved resolution has been questioned^{6,7}, the use of unsaturated chambers has increased substantially since 1968 with many authors reporting improved separation. It is also interesting to note that in an interlaboratory investigation, primarily carried out to evaluate reproducibility of R_f -values, a significantly improved separation was obtained with a single-component solvent in unsaturated chambers⁸.

Though the use of unsaturated chambers can provide improved separation, there are several restrictions that should be carefully distinguished. First, one cannot expect that optimum vapor conditions are always obtained simply by using an unsaturated chamber. Vapor formation and adsorption are dependent on the properties of the individual solvent components used, and will thus vary from solvent to solvent. In addition, a certain vapor gradient may be excellent for one group of substances but may be mediocre or totally unsatisfactory for another group. Second, it should be realised, as was pointed out above, that the "saturated" chamber in most cases becomes unsaturated when the plate is introduced so that at least part of the development will take place under unsaturated conditions. The latter effect will be more pronounced with the more

polar solvent systems which are being adsorbed to a high extent and, accordingly, switching to even less unsaturated conditions in the unsaturated chamber may not lead to further improvements⁹. Finally, it will be clear that the vapor conditions in a TLC system are dependent on factors such as the properties of the sorbent (significant differences may be observed even from batch to batch), geometry of the developing chamber, temperature and relative humidity.

Therefore, in order to obtain full benefit of the influence of solvent vapor in TLC it seemed necessary to search for developing techniques which would provide an efficient control of the vapor processes before and during development and which could be adapted to the needs of any specific separation problem. This has resulted in the design of the Vapor-Programming chamber or VP-chamber which is shown in Fig. 1. The chamber is commercially obtainable from C. Desaga, Heidelberg, German Federal Republic. The underlying principle is that the plate is being developed in a horizontal position and with the sorbent layer facing a number of troughs which are filled with liquids (single- or multicomponent) of different compositions. Thus, the use of the VP-chamber permits vapor adsorption from the underlying troughs and by filling the latter with appropriate liquid mixtures, the vapor conditions can be programmed and optimized over the entire plate. A detailed description of the VP-chamber has appeared elsewhere^{10, 19}.

The VP-chamber was especially designed for use in separations of closely related substances. When such substances are exposed to classical TLC techniques, it is often seen that they migrate closely together, with little or no difference between their respective R_f -values. Since we had observed enhanced separations in unsaturated chambers which we attributed to a vapor gradient across the plate showing increased polarity from bottom to top, it was felt that the VP-chamber would be ideally suited for establishing such gradients. The following example will indicate how a suitable VP-development system is set up.

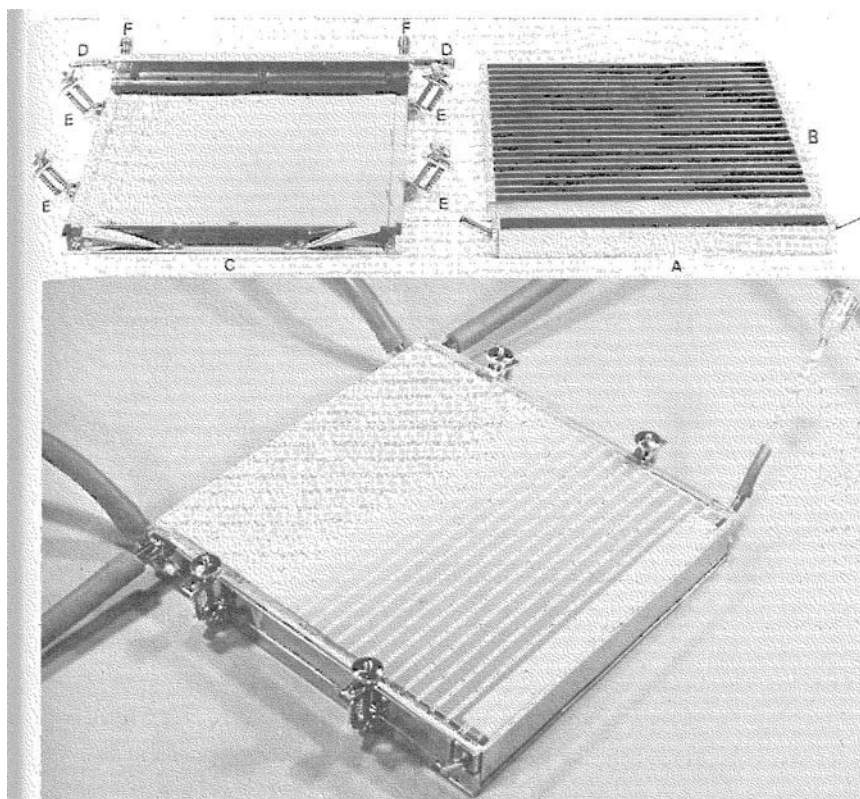


FIGURE 1

The Vapor Programming Chamber.

Solvent reservoir (A), Trough chamber (B), and Base plate (C). The latter is fitted with a tube for passing warm water (D), assembly clamps (E) and an internal tube system for water circulation, the inlet and outlet (F) to be connected to a thermostat. The bottom photograph shows the VP-chamber at work. The developing solvent makes the sorbent transparent, with the underlying troughs and the filter paper strip that supplies the solvent from the reservoir becoming visible. The solvent reservoir is to be filled with about 30 ml of solvent; each trough should be filled with about 5 ml of liquid.

The sulfonamides represent an important class of chemotherapeutics but their separation is rather difficult to perform because of their close similarities in structure. With ether-methanol (90+10) as developing solvent, some separation can be achieved by classical TLC as can be seen in Fig. 2^A, but the spots remain on the lower part of the plate. If a slightly more polar developing solvent is used such as ether-methanol (80+20) the spots move to higher positions on the plate, but the overall separation becomes worse (Fig. 2^B). In VP-TLC one should use a developing solvent of rather low polarity, giving the substances under investigation a low migration rate. In the case of the sulfonamides, ether-methanol (90+10) proved to be quite suitable. The troughs underneath the starting points (e.g. the first two troughs) are filled with liquids of the same composition as the developing solvent. In the next troughs we then start to use solvent mixtures that show increased polarity e.g. by introducing larger percentages of methanol. When the fastest moving spot enters the area in which more methanol is present, its migration rate will be accelerated and it will move ahead of the rest of the mixture; then the second fastest spot comes under the influence of increased amounts of methanol, will move faster, etc. This process is repeated whenever a spot enters an area of increased polarity. However, one difficulty arises in that solute spots are of finite size and hence, migration of the upper part of a solute spot will be accelerated over that of the lower part each time the spots enter an area of higher polarity. This may result in severe tailing if this process is repeated trough after trough. Fortunately, however, tailing can be adequately suppressed by interspersing troughs of low polarity between the troughs containing the polar mixtures. The combination of accelerating troughs followed by one, two or three decelerating troughs produces compact spots without affecting the improved separation efficiency. The resulting separation is shown in Fig. 2^C. Additional operating conditions were: temperature 22°C, cooling temperature VP-chamber 19°C, spacer thickness 0.5 mm, relative humidity 30%, saturation time 10 min., development 80 min.

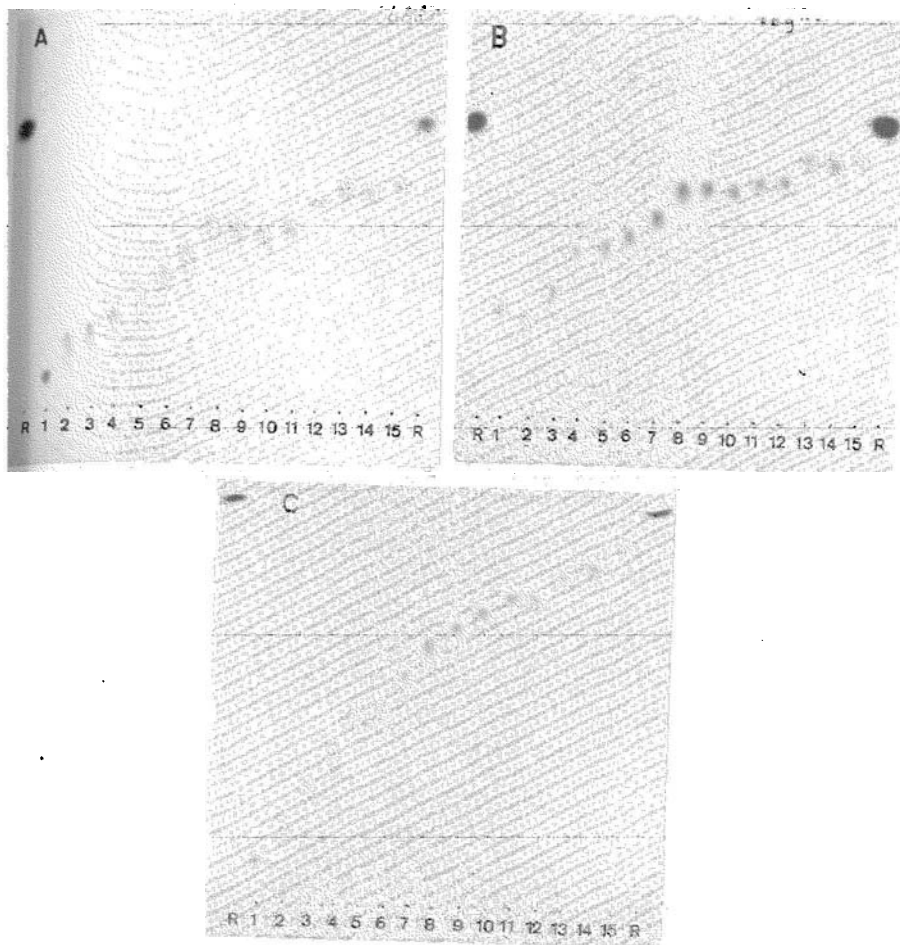


FIGURE 2

Separation of Sulfonamides under Neutral Conditions.

- (A) Normal development with ether-methanol (90+10)
- (B) Normal development with ether-methanol (80+20)
- (C) VP-development with ether-methanol (90+10) as developing solvent and the following vapor program: troughs 1, 2 and 3 = ether; trough 4 = ether-methanol (80+20); trough 7 = ether-methanol (50+50); trough 10 = ether-methanol (20+80); troughs 13, 16, and 19 = methanol; all other troughs contained benzene-ether (50+50) as decelerating liquid.

Sorbent: Silica gel GF 254 (Merck); 1 = sulfaguanidine, 2 = sulfamethizole, 3 = sulfisomidine, 4 = sulfathiazole, 5 = sulfadiazine, 6 = sulfacetamide, 7 = sulfamerazine, 8 = sulfadimidine, 9 = sulfapyridine, 10 = sulfamethoxypyrimidine, 11 = sulfamethoxypyridazine, 12 = sulfisoxazole, 13 = sulfadimethoxine, 14 = sulfanilamide, 15 = sulfaphenazole, R = reference compound 4-nitroaniline. Visualization: UV light of 254 nm.

Because of their amphoteric character, a separation of sulfonamides under alkaline conditions can also be envisaged, but, using classical methods, the results are rather poor as can be seen in Figs. 3^A and 3^B. Fig. 3^C represents the separation obtained in the VP-chamber using the bottom layer of chloroform-methanol-25% ammonia (70+20+10) as solvent in combination with a chloroform-acetone-methanol-ammonia vapor gradient. Chloroform, saturated with ammonia was used as decelerating liquid.

In the following section some additional examples of VP-TLC separations are shown. The details given with regard to vapor programs, temperatures, saturation times, etc. should be considered as guide lines rather than rigid conditions that should be maintained at all times.

Sterols. Normal thin layer chromatography procedures are usually not capable to separate sterols containing one double bond from the corresponding saturated compound. Special techniques are necessary such as silver nitrate impregnation, continuous development or reversed phase partition systems, which all have their disadvantages. By means of VP-TLC on silica gel we were able to obtain adequate separations between cholesterol and cholestanol and between β -sitosterol and stigmastanol¹¹, which is depicted in Fig. 4.

Aflatoxins. During recent years the analysis of aflatoxins has had widespread attention because of their extremely toxic properties to animals and presumably also to humans. Adequate separation of the 4 common aflatoxins, B₁, B₂, G₁ and G₂ has proved rather difficult. The use of an unsaturated chamber gave improved resolution^{12,13}, but although the separation may look complete visually, quantitative fluorodensitometry of such chromatograms still caused difficulties due to overlapping of the recorded peaks. VP-TLC was found to give distinct enhanced resolution so that fluorodensitometric evaluation of the chromatogram showed completely separated peaks with the recorded curve always coming back to the baseline¹⁴. The results are demonstrated in Figs. 5 and 6.

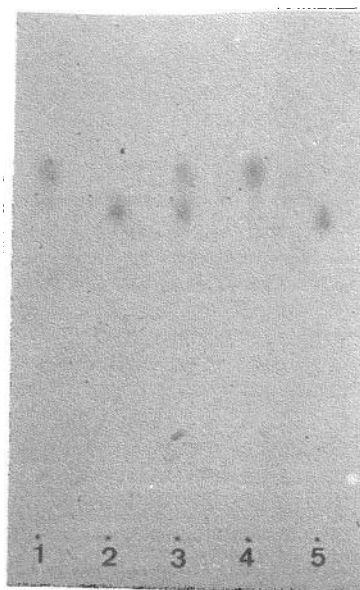


FIGURE 4

Separation of Sterols

VP-development with benzene-ether (90+10) as solvent and the following vapor program (B = benzene, E = ether, Ca = carbon tetrachloride): trough 2 = Ca-E (80+20), trough 5 = Ca-E (60+40), trough 8 = Ca-E (60+40), trough 11 = Ca-E (40+60), troughs 12-21 = Ca, all other troughs contained B-E (90+10) as decelerating liquid. Temp. 20°C, rel. humidity 40%, saturation 10 min, development 180 min, spacers 0.5 mm, cooling 19°C, sorbent Silica gel GF 254 (Merck). 1 = β -sitosterol, 2 = stigmastanol, 3 = mixture of 1 and 2, 4 = cholesterol, 5 = cholestanol. Visualization: conc. H_2SO_4 spray followed by charring.

Carbohydrates. TLC of this class of compounds has been cumbersome due to the highly polar character and the structural similarity of the sugars. Lato and co-workers¹⁵ extensively studied the TLC separation possibilities of carbohydrates. They reported excellent separations on buffer-impregnated plates, but the development times were in the order 6-8 hours. With the VP-chamber however, this can be reduced to about 3 hours using acetone-methanol-water vapor gradients. Compact spots are obtained and the system provides adequate



FIGURE 5

Separation of Aflatoxins

VP-development with chloroform-acetone (96+4) as solvent and the following vapor program (B = benzene, C = chloroform, Ac = acetone, M = methanol): trough 3 = C-Ac-M (85+13+2), trough 6 = C-Ac-M (75+22+3), trough 9 = C-Ac-M (65+31+4), trough 12 = C-Ac-M (55+40+5), trough 15 = C-Ac-M (55+40+5), trough 18 = C-Ac-M (55+40+5), all other troughs contained B-C (60+40) as decelerating liquid. Temp. 27°C, rel. humidity 60%, saturation 10 min, development 120 min, spacers 1 mm, cooling 24°C, sorbent Silica gel G-HR (Machery and Nagel). Spots from top to bottom: aflatoxins B₁, B₂, G₁ and G₂, respectively. Visualization: UV light of 254 nm.

resolving power for use in routine analysis of simple carbohydrate mixtures¹⁶. A separation of some mono- and disaccharides is depicted in Fig. 7.

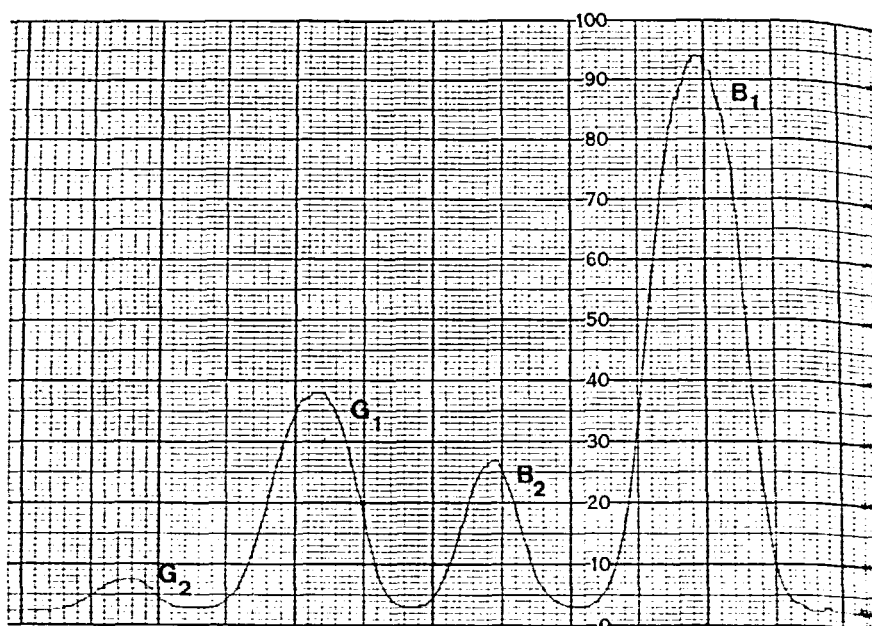


FIGURE 6

Fluorodensitometry of Aflatoxins

Fluorodensitometric record of a natural mixture of aflatoxins B₁, B₂, G₁ and G₂ (right to left) after separation by means of VP-TLC. Separation conditions as in Fig. 5.

PREPARATIVE APPLICATIONS. When using preparative thin layer chromatographic techniques one often notices that substances which can be handled adequately by analytical TLC methods cannot be separated on a preparative scale due to the higher loadings and the broader initial band widths that result in the application of the substances onto the plate. Also, if an impurity or a decomposition product is to be separated and isolated from a parent compound, rather high sample loadings are necessary. Accordingly, the band of the latter may become so broad that it overlaps the much smaller band of the former. In such cases the increased resolving power of VP-TLC can be of great help to achieve complete separation. Two

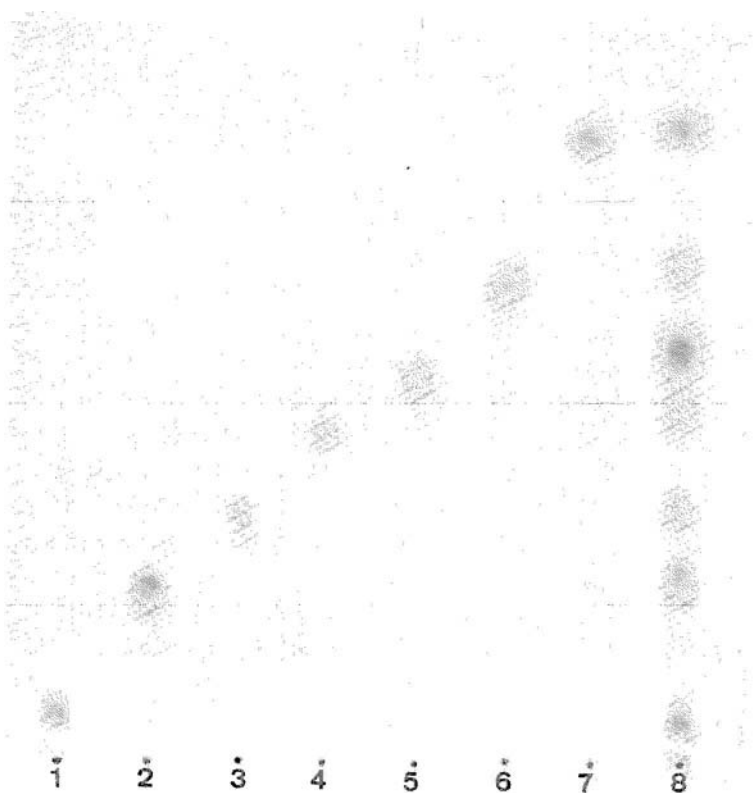


FIGURE 7

Separation of Carbohydrates

VP-development of some mono- and disaccharides with acetone-methanol-water (80+15+5) as solvent on Silica gel G (Merck) as sorbent, impregnated with 0.2M sodium dihydrogen phosphate solution and the following vapor program (Ac = acetone, M = methanol, W = water): trough 3 = Ac-M-W (70+20+10), trough 6 = Ac-M-W (60+20+20), trough 8 = Ac-M-W (40+30+30), trough 10 = Ac-M-W (20+40+40), troughs 12, 14, 16 and 18 = Ac-M-W (20+25+55), all other troughs contained acetone as decelerating liquid. Temp. 25°C, rel. humidity 60%, saturation 10 min, development 180 min, spacers 1 mm, cooling 24°C, 1 = lactose, 2 = sucrose, 3 = galactose, 4 = glucose, 5 = mannose, 6 = arabinose, 7 = xylose, 8 = mixture of 1-7 plus fructose, which coincides with mannose. Visualization: heating for 30 min at 100°C in an oven, then sprayed when still hot with a freshly prepared solution of 20 mg naphtoresorcinol in 10 ml of ethanol and 0.2 ml concentrated sulphuric acid and reheating for 5 min at 100°C to intensify the spot colors.

examples are given here, namely the preparative separation of three sulfonamides and the separation of a barbiturate impurity from the parent component. These separations were carried out on thick layers of about 1 mm on 20 x 40 cm plates but the same principles can be applied to layers of other thicknesses, provided the spacers that regulate the distance from the sorbent layer to the trough walls are properly adapted.

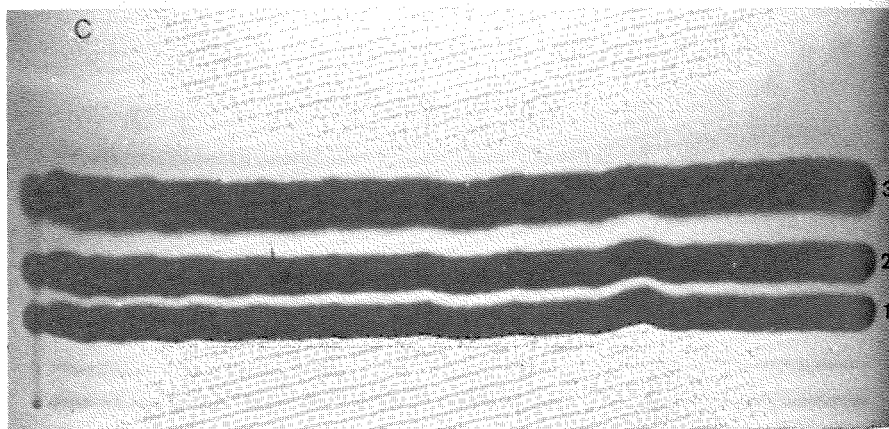
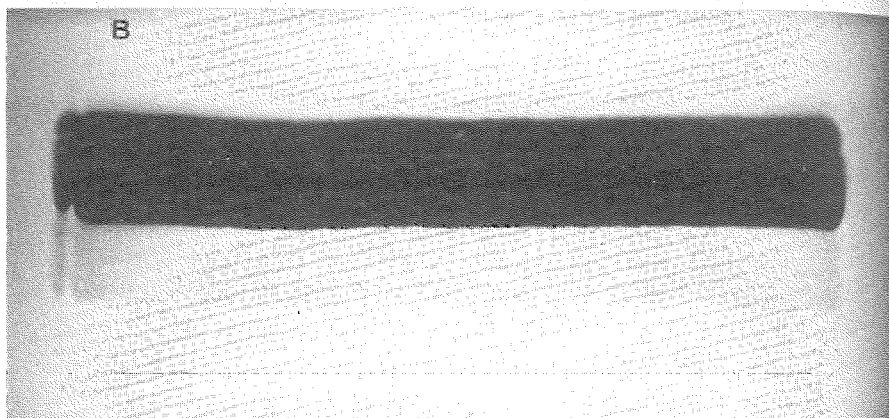
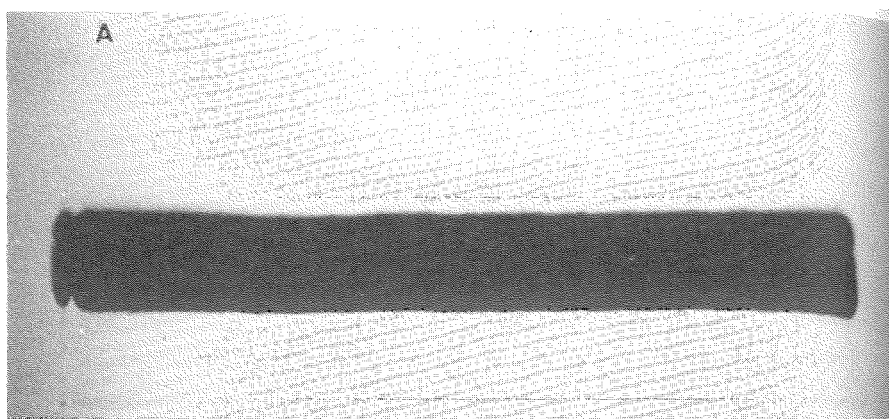
Sulfonamides. The Trisulfapyrimidine mixture according to the United States Pharmacopeia XVII (sulfadiazine, sulfamerazine and sulfadimidine) can be separated reasonably well in analytical TLC using classical development. This can be seen in Figs. 2^A and 2^B which were obtained with neutral solvent systems as well as in Figs. 3^A and 3^B, obtained with alkaline systems (spots 5, 7 and 8). Yet, if a separation needs to be done on a preparative scale, the bands overlap because of the quantities of material. This is demonstrated in Fig. 8^A, in which 10 mg of each substance was applied on 40 cm wide, 1 mm thick layers and developed with chloroform-methanol-20% ammonia (50+40+10). Repeated development, which has been suggested as a useful alternative for preparative separations, was unsuccessful in this case as can be concluded from Fig. 8^B. With the VP-chamber, all three substances could be completely separated using a chloroform-acetone-methanol-ammonia vapor gradient in conjunction with a chloroform-methanol-25% ammonia solvent (75+20+5)¹⁷. The result is depicted in Fig. 8^C. Rechromatography of the thus separated and isolated substances showed them to be chromatographically pure for at least 99%.

Butobarbital impurity. Butobarbital tablets (5-*n*-butyl-5-ethylbarbituric acid) were found to contain an unknown impurity by means of classical TLC, as shown in Fig. 9. Although the major and minor component separate fairly well, it can be observed that the separation efficiency decreases with increasing sample loads. When preparative TLC was applied in an attempt to isolate larger quantities of the unknown, the butobarbital band overlapped the one of the unknown to such an extent that identification of the latter could

not be achieved (Fig. 10^A). Preparative VP-TLC, however, was much more successful and provided complete separation of the two bands, using a chloroform-ether-methanol vapor gradient (Fig. 10^B). The thus separated and isolated impurity could rapidly be identified as being dibutylbarbituric acid (5,5-di-n-butylbarbituric acid).

The above examples are indicative of the potentials of VP-TLC. Its increased resolving power will be particularly useful when dealing with closely related substances which cannot be separated adequately with classical TLC procedures. An additional advantage of VP-TLC is that it can be used to guide already separated spots to particular areas of the chromatoplate, which are not occupied by other substances. An example is given in Fig. 11. In Fig. 11^A, a VP-TLC separation of 5 dyestuffs is shown using a carbon tetrachloride-methanol vapor gradient. The vapor program has been adjusted so as to give optimum separation of the spots and a full spread over the entire plate. This has the advantage that other dyestuffs with intermediate polarity can be guided to the open areas between spots B, A, C, A, and A₂. Yet, if the vapor gradient is reversed, *i.e.* if it shows decreasing polarity from bottom to top, the five dyestuffs, though still separated, can be kept within a rather narrow band, thus leaving plenty of "room" to accommodate other substances with higher polarities than A₂ or lower polarities than B. A third advantage of VP-TLC is that any liquid component can be utilized and its effects be applied through the vapor phase so that one no longer has to worry about solvent demixing problems.

Finally, a few guide lines may be given as to how a suitable vapor program can be obtained. Basically, the same principles that are used in the selection of a developing solvent for classical TLC can be applied: The first step is to examine how the spots migrate with single-component solvents in normal chambers, and to see what their separation efficiency is. Select those that show good separation efficiency and then try to work out a suitable vapor program by combining components with different polarities. If a vapor



gradient with increasing polarity from bottom to top is desired, select as the developing solvent a liquid (single- or multicomponent) with which the solutes show only limited migration; then, in the troughs, increase the polarity of the solvent mixtures therein by using increased amounts of more polar solvent components and intersperse the accelerating troughs with troughs containing decelerating mixtures. In general, the polarity of the gradient should match the polarity of the component to be separated, *i.e.* low polarity gradients should be used for lipophilic solutes, whereas high polarity gradients are required for hydrophilic components. If the spots run too high on the plate, one should decrease the polarity of the gradient, but if the spots remain too close to the starting point, increase the polarity. If the spots tend to show tailing, the gradient becomes too steep and one should consider a less steep one or use stronger deceleration (3 instead of 2 decelerating troughs or liquids of lower polarity).

The thickness of the spacers also play an important role in the separation, in that they determine the distance between the sorbent layer and the trough walls, and, accordingly, the degree of vapor diffusion from one trough area to another. If the spacers are

FIGURE 8

Separation of Sulfonamides on a Preparative Scale.
Sulfadiazine (1), sulfamerazine (2), and sulfadimidine (3), 10 mg each on a 20x40 mm plate with Silicagel PF 254 (Merck) as sorbent, layer thickness 1 mm when spread.
(A) Normal development with chloroform-methanol-25% ammonia (50+40+10).
(B) As above, but developed twice in the same solvent.
(C) VP-development with chloroform-methanol-25% ammonia (75+20+5) as solvent and the following vapor program (C = chloroform, Ac = acetone, M = methanol, A = 25% ammonia, C-A = chloroform, saturated with 25% ammonia): trough 2 = C-M-A (50+40+10), trough 6 = C-M-A (20+70+10), trough 10 = Ac-M-A (20+70+10), trough 14 = Ac-M-A (20+70+10), all other troughs C-A as decelerating liquid. Temp. 20°C, rel. humidity 60%, saturation 15 min, development 200 min, spacers 1.4 mm, cooling 19°C. Visualization: UV light of 254 nm.

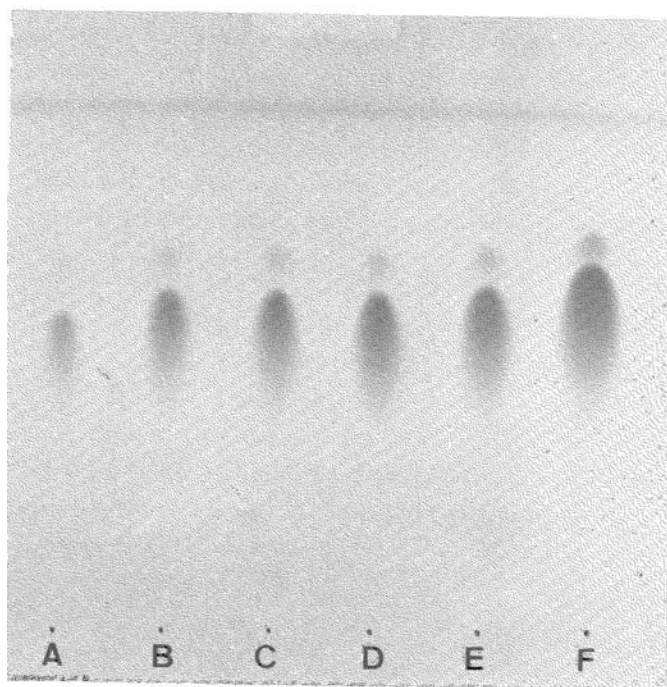


FIGURE 9

Analytical-scale Separation of Butobarbital and an Impurity. Thin layer chromatogram of a chloroform extract from butobarbital tablets. Upper spot: dibutylbarbituric acid; lower spot: butobarbital. Silica gel GF 254 (merck) developed with chloroform-ether (75+25). Sample: 1 tablet containing 100 mg of butobarbital, extracted with 5 ml of chloroform. Spotted quantity: A = 1 μ l, B = 2.5 μ l, C and D = 5 μ l, E = 10 μ l, F = 20 μ l. Percentage dibutylbarbituric acid calculated to be 2.7%. Visualization by UV light of 254 nm, after activation with ammonia vapor.

too thick, too much vapor diffusion will take place and the desired vapor gradient cannot be obtained, but, on the other hand, the wet sorbent layer should by no means be allowed to touch the trough walls. This will cause the developing solvent to disappear in the troughs and development comes to a halt. It should be observed, however, that most sorbents swell more or less when wetted by the

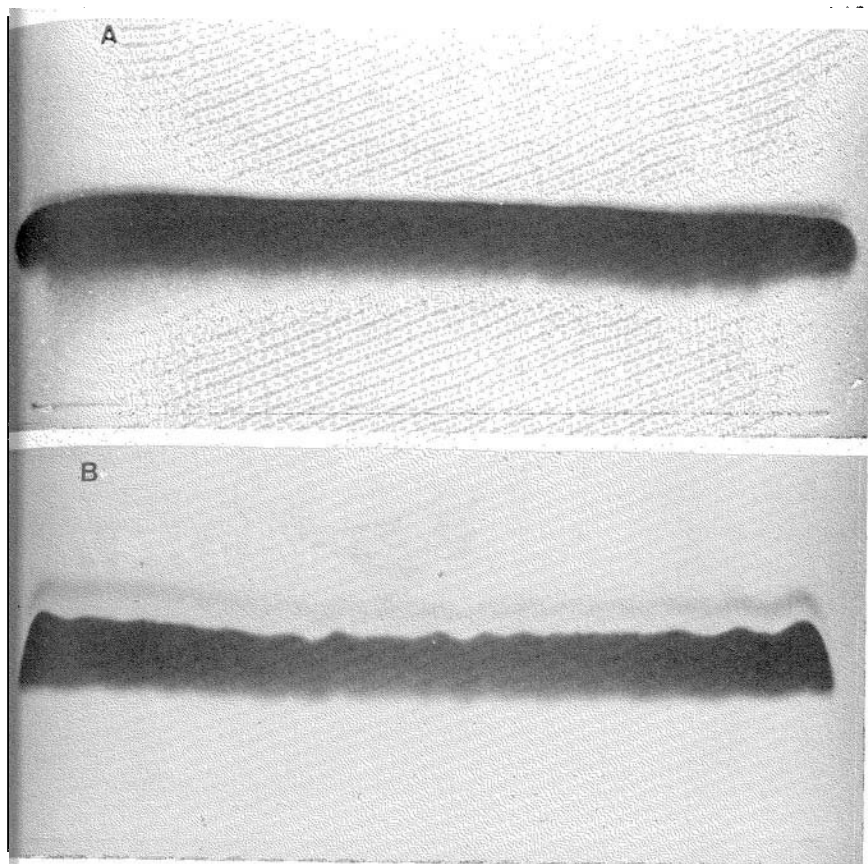
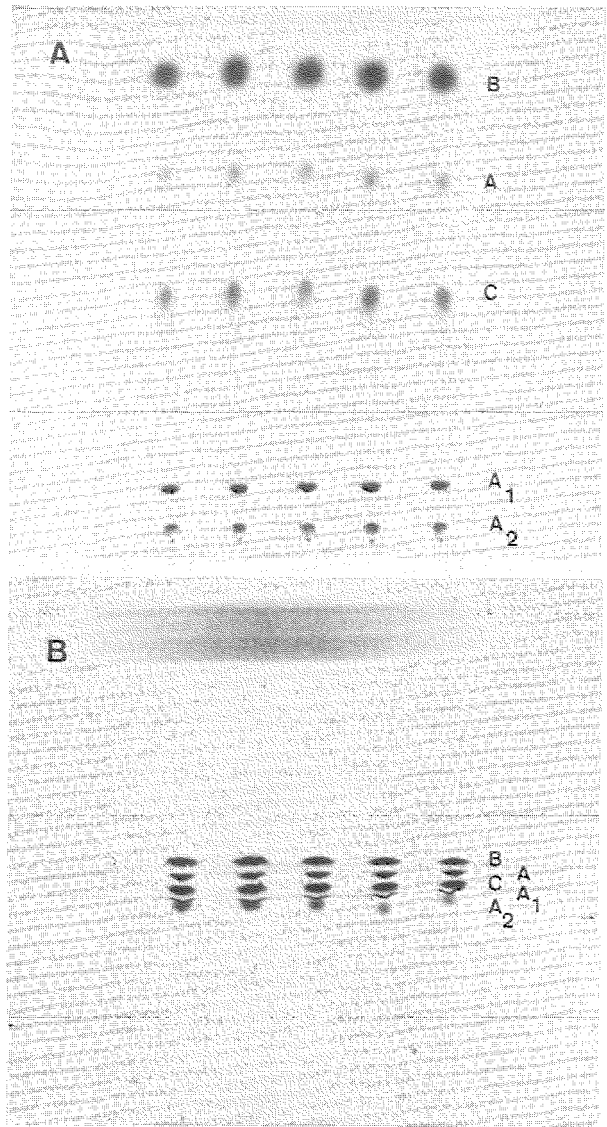


FIGURE 10

Preparative-scale Separation of Butobarbital and an Impurity.

- (A) Normal preparative TLC on 20x40 cm plates of Silica gel PF 254 (Merck), layer thickness 1 mm. Development with chloroform-ether (75+25).
- (B) VP-development with chloroform as developing solvent and the following vapor program (C = chloroform; E = ether, M = methanol): trough 2 = C-E (95 + 5), trough 4 = C-E (70+30), trough 7 = C-E (40+60), trough 10 = C-E (10+90), trough 13 = E-M (80+20), trough 16 = E-M (50+50), trough 19 = M, all other troughs contained chloroform as decelerating liquid. Temp. 22°C, rel. humidity 50%, saturation 10 min, development 90 min, spacers 1 mm, cooling 19°C. Silica gel PF 254 (Merck) layer thickness 1 mm when spread on 20x40 cm plates. Spotted quantity 100 mg, calculated as butobarbital. Visualization by UV light of 254 nm, after activation by ammonia vapor.



developingsolvent, the rate of swelling being proportional to the polarity of the solvent. The thickness of the spacers should be adapted to this phenomenon. With thin layers of 0.25 mm we found good results with spacers of 0.3-0.5 mm for low polarity solvents such as hexane, benzene, ether and chloroform; 0.5-0.8 mm for solvents containing acetone, ethylacetate, alcohols; and 1 mm strips were needed for solvents containing water, ammonia and acetic acid. For preparative layers the same principles apply.

In order to ensure that adequate and reproducible quantities of vapor are available for adsorption it is necessary to equilibrate the plate over the filled troughs for at least 10 minutes. After this saturation period, the solvent reservoir is filled and development is started.

It will be obvious that the various vapor processes are highly dependent on the temperature. Therefore, the VP-chamber must be thermostated and care should be taken that the chamber is not

FIGURE 11

Properties of the VP-Chamber with Different Programs.

- (A) Maximum spread within a class of components. Separation of 5 dyestuffs, using carbon tetrachloride as solvent and the following vapor program (Ca = carbon tetrachloride, M = methanol): trough 1 = Ca-M (95+5), trough 5 = Ca-M (80+20), trough 9 = Ca-M (60+40), trough 13 = Ca-M (60+40), trough 17 = Ca-M (20+80), all other troughs contained carbon tetrachloride as decelerating liquid. Temp. 21°C, rel. humidity 45%, saturation 10 min, development 85 min, spacers 0.5 mm, cooling 20°C.
- (B) Minimum spread within a class of components. Separation of the same 5 dyestuffs, using carbon tetrachloride-benzene (80+20) as solvent and a "reversed" vapor program *i.e.* the polarity of the program decreases from bottom to top (Ca = carbon tetrachloride, M = methanol): trough 1 = Ca-M (20+80), trough 2 = Ca-M (80+20), trough 5 = Ca-M (97.5+2.5), troughs 6-21 = carbontetrachloride. Further conditions as under A.
- Silica gel GF 254 (Merck), layer thickness 0.25 mm. A, A₁ and A₂ = the three anthraquinone isomers in Artisil Blue BSQ, B = Bütter Yellow, C = Cibacetred B₃.

placed in direct sunlight. Draught should also be kept to a minimum. In practice, it has proved to be useful to thermostat the VP-chamber at temperatures about 2-3°C below the ambient room temperature.

In VP-development, the top end of the plate (1 cm) is not accompanied by a trough underneath, but lies 0.5 cm over a tube through which warm water can be passed. Solvent reaching this area thus can evaporate and development can be continued until optimum migration of the spots has been achieved. To this end, colored reference solutes were found to be useful indicators through which migration can be monitored throughout development. The choice of the reference component should be in line with the polarity of the substances under investigation.

The reproducibility of the VP-chamber is of the same order as that in classical TLC, if factors such as ambient temperature, thermostated temperature, relative humidity, saturation, thickness of the spacers and of the sorbent layer, etc. are kept constant. Small variations in these parameters will have great influence on the vapor gradient and, subsequently on the reproducibility of the separation.

From the above, it will be clear that the VP-technique is more complicated than classical TLC and that it should certainly not be used in cases which can equally well be resolved by simpler methods. Yet, if classical TLC cannot provide adequate resolution, VP-TLC has a lot to offer. Its substantially improved resolving power is well worth the extra work needed to develop a suitable vapor program.

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