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Pharmaceutical applications of vapour-controlled* thin-layer chromatography

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In thin-layer chromatography, solvent vapour plays an important role. To obtain full benefit of the influence of vapour in the technique, a new chamber has been developed, providing full vapour control over the entire plate. This allows better separations of chemically related compounds. The properties of the vapour-control chamber are shown in separations of barbiturates and local anaesthetics.

RECENT work (de Zeeuw, 1968a) has shown that solvent vapour plays an important role in thin-layer chromatography, particularly with multicomponent solvents. Depending on the affinity of the vapour components for the adsorbent, varying amounts of solvent vapour may be adsorbed on the dry plate and separation of the components of any mixture is affected by this phenomenon. It has been shown (de Zeeuw, 1968b) that if the development is begun before the atmosphere in the tank is in equilibrium with the running solvent vapour (so-called unsaturated chambers), then separations with multicomponent solvents are more efficient than in the situation where equilibrium is established before commencing development (so-called saturated chambers). Because of ascending solvent the lower parts of the plate will adsorb less vapour than the upper parts, thus a concentration gradient of adsorbed vapour is formed from the bottom to the top of the plate. With multicomponent solvents this adsorbate will consist mainly of the more polar components. During development, the faster running spots will pass into plate areas enriched in the more polar components of the solvent, thus producing an acceleration in the rate of migration. At the same time the slower running spots pass through areas with a lower concentration of the polar components. The migration rate of these will thus be affected to a smaller extent and hence a better separation will result. In saturated chambers a concentration gradient is less easily formed, because when the plate is placed in a tank saturated with respect to the vapour of the running solvent the maximum amount of vapour will be adsorbed almost immediately. I therefore suggest that unsaturated chambers are preferable to saturated chambers when multicomponent solvents are used.

Little or no control can be exercised of course on the extent to which any gradient is developed on the thin-layer plate. This will depend mainly on the rate of evaporation of the solvent components and on their affinity for the adsorbent used. Thus, although the suggested technique with unsaturated chambers yields improved separations, the conditions are not necessarily optimum for every case.

The apparatus described below, however, does allow full vapour control over the entire plate, thus making it possible to affect the migration rate of each individual spot.

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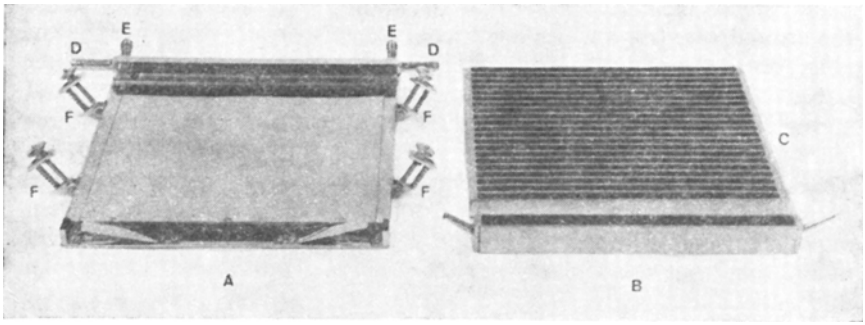
* Recently the name Vapor-Programmed TLC was introduced for this technique.

APPARATUS

The vapour-control chamber (de Zeeuw, 1968c) (VC-chamber) consists of three parts, a ground plate (A) 20 × 20 × 1 cm on which is placed a solvent reservoir (B) 20 × 1 × 2 cm, and a trough chamber (C) 20 × 17 × 1·5 cm, containing 21 troughs 19 × 0·6 × 1·3 cm, all of chromium-plated brass (Fig. 1a, b). In use the troughs are filled with mixtures of

a



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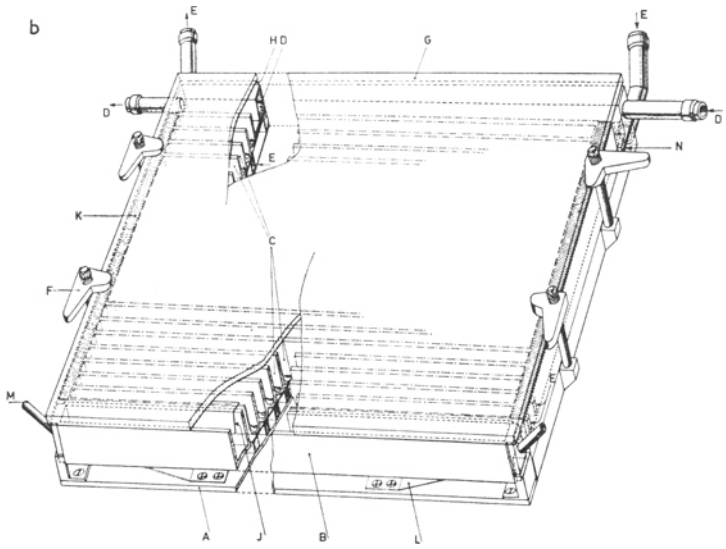

FIG. 1a. The components of the VC-chamber. A: ground plate, B: solvent reservoir, C: trough chamber with 21 troughs. The ground plate is equipped with a warm water tube (D), an internal tube system for water-thermostating, the inlet and outlet being visible at E, and fixation clamps (F).

b. Schematic view of the VC-chamber. A: groundplate, B: solvent reservoir, C: trough chamber with 21 troughs, D: warm water tube, E: water circulating system for cooling purposes (the arrows at D and E indicate the direction of water flow), F: fixation clamps, G: TLC plate, H: adsorbent, J: filter paper strip, K: spacer, L: metal springs, M: solvent inlet, N: asbestos layer for warm water tube insulation.

polar and non-polar solvents increasing in polarity from one to the other. The vapour from such solvent mixtures is used to equilibrate with the area of adsorbent directly over the particular trough concerned and thus to form the concentration gradient referred to above. Running solvent is placed in the reservoir (B), development of the plate, which takes place in a horizontal position with the adsorbent immediately facing the troughs, may be continued for an unlimited time, since excess solvent can evaporate from the end of the plate. For this purpose the end of the plate extends 0·5 cm over a warm water tube (D), which is attached to the groundplate (A) but insulated from it by asbestos. Running solvent is led on to the plate from the reservoir (B) by means of a strip of filter paper. The solvent reservoir is pressed gently to the plate by two metal springs. Adsorbent on the plates (20 × 20 cm) is removed from three sides to a width of 0·5 cm. During development the plate rests on two Teflon spacers of 0·5 mm placed on opposite edges. Thus, the space between the plate and the trough chamber is small enough to prevent vapour currents without the layer touching the troughs. Development of the thin-layer takes place with the ground plate thermostatted; inlet and outlet tubes of the water circulation system are shown at E. Four clamps (F) fix the plate to the chamber.

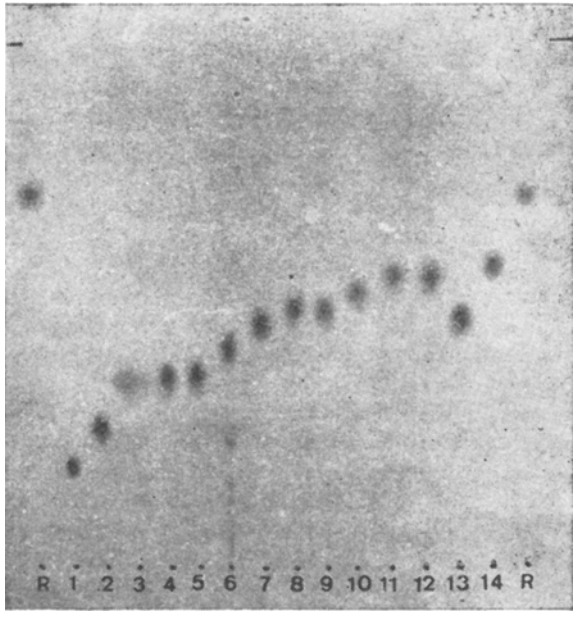

FIG. 2. Separation of 14 commonly used barbiturates with chloroform–isopropanol–25% ammonia (45:45:10) in saturated normal chambers on silica gel GF 254, Temperature 20·6°, relative humidity 39%, saturation 60 min, development 75 min., load 10 μg. 1 = heptobarbitone, 2 = phenobarbitone, 3 = brallobarbital, 4 = barbitone, 5 = allobarbitone, 6 = cyclobarbitone, 7 = aprobarbitone, 8 = butalbital, 9 = butobarbitone, 10 = amylobarbitone, 11 = pentobarbitone, 12 = quinalbarbitone, 13 = methylphenobarbitone, 14 = hexobarbitone, R = reference 4-nitroaniline.

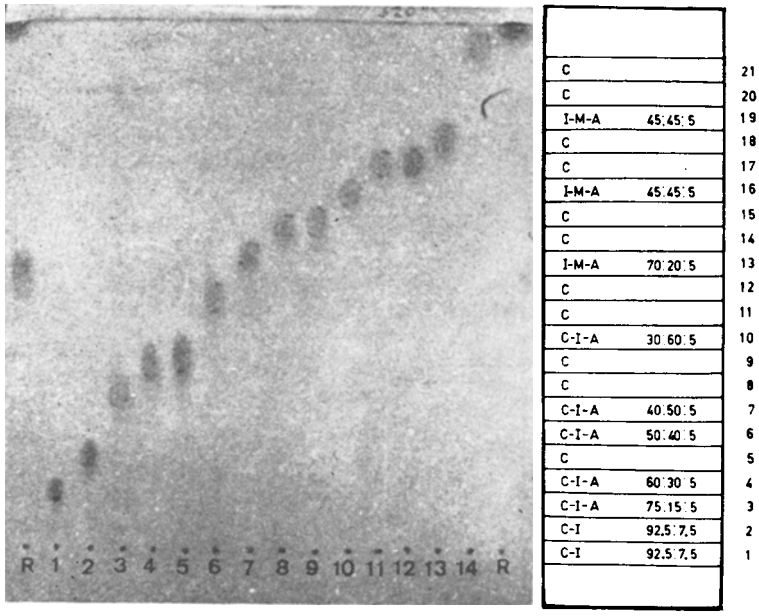

FIG. 3. The same substances as in Fig. 2, now separated in the VC-chamber with chloroform–isopropanol (92·5 : 7·5) (saturated with 25% ammonia) and a chloroform–isopropanol–methanol–ammonia vapour gradient. The position of the troughs and the liquid composition therein during development are shown at the right. Temperature 21·6°, relative humidity 40%, saturation 10 min, development 110 min, strips 1 mm, cooling ground plate 19°, Code: C = chloroform, saturated with 25% ammonia, I = isopropanol, M = methanol, A = 25% ammonia. Numbering as in Fig. 2. Note: the left-hand reference contains an impurity.

Because the solute spots are finite in size, migration of the upper parts of the spot will tend to be more rapid than the lower parts and tailing will consequently result. This may be prevented by interspersing troughs with solvents of low polarity between troughs containing the more polar mixtures. Troughs thus interspersed will exert a decelerating effect on the migration rate of the spots. When working with a chloroform–ether gradient for example, the following would suitably be used: troughs 1 and 2, chloroform; trough 3, chloroform–ether 90 : 10; trough 4, chloroform; trough 5, chloroform–ether 75 : 25; trough 6, chloroform; trough 7, chloroform–ether 60 : 40; and so on. In this way compact spots can be obtained.

Experimental

Solvents used were of reagent grade (Merck). All substances examined were 99·0% chromatographically pure, except cyclobarbitone which showed slight decomposition. Solvent compositions are given by volume.

Silica gel GF 254 (Merck) was used as adsorbent, in layers 0·25 mm on glass plates 20 × 20 cm. After spreading, the plates were air dried

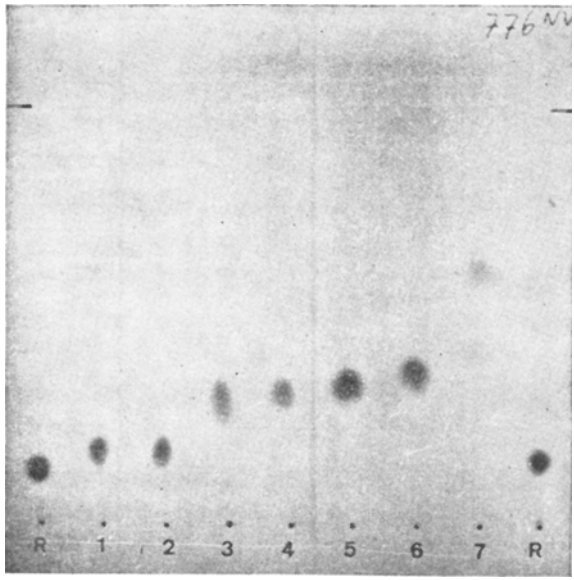

FIG. 4. Separation of chemically related local anaesthetics with hexane–chloroform–methanol (60 : 35 : 5) in saturated normal chambers on sodium hydroxide impregnated silica gel GF 254. Temperature 22·0°, relative humidity 40%, saturation 45 min, development 25 min, load 5 and 6: 10 μg, 1, 2 and 4: 15 μg, 3: 30 μg, 7: 50 μg. 1 = procaine, 2 = tutocaine, 3 = tetracaine, 4 = butacaine, 5 = ethylaminobenzoate, 6 = butylaminobenzoate, 7 = lignocaine.

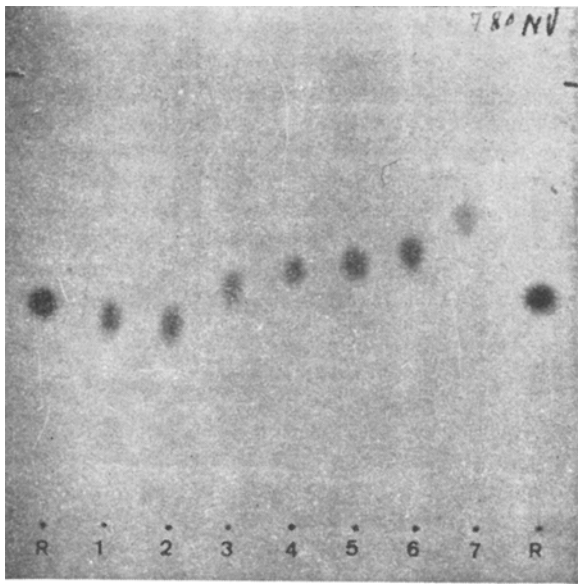

FIG. 5. The same substances and technique as in Fig. 4, with chloroform–methanol (95 : 5) as solvent. Temperature 22·0°, relative humidity 44%, saturation 45 min, development 30 min. Numbering as in Fig. 4.

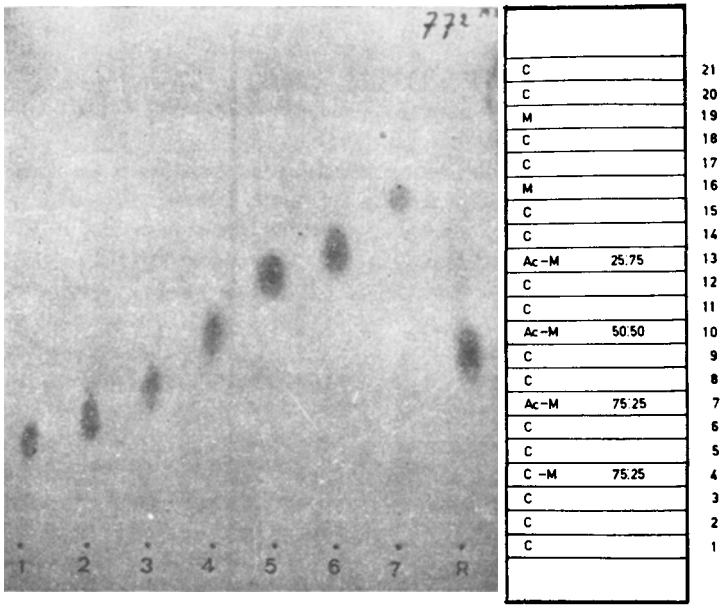

FIG. 6. The same substances as in Figs 4 and 5, now separated in the VC-chamber with hexane–chloroform–methanol (60 : 35 : 5) as solvent and a chloroform–acetone–methanol vapour gradient. The position of the troughs and the liquid composition therein during development are given at the right. Temperature 22°, relative humidity 45%, saturation 10 min, development 32 min, strips 0·5 mm, cooling ground plate 20°. Code: C = chloroform, M = methanol, Ac = acetone. Numbering as in Fig. 4.

(15 min), heated (30 min) at 110° in an oven with a fan, then cooled and stored in a desiccator.

Solutions (5 μl) in chloroform (barbiturates) or ethanol (anaesthetics), were applied with 10 μl micropipettes, 2·5 cm from the bottom edge of the plate, 1·5–2 cm apart.

Troughs were filled with about 5 ml each of the appropriate liquid mixtures, the plate fixed in position, and after allowing time for equilibration (10 min) the solvent reservoir was filled with 25 ml of running solvent.

All experiments were made at 20–22° and a relative humidity of 39–45%. Within these ranges reproducibility of the chromatogram runs was observed.

Normal chromatograph tanks, 21 × 21 × 9 cm, were used as controls. These contained 100 ml of solvent and were saturated with solvent vapour by lining the inner walls with filter paper. After 45–60 min the thin-layer plate was introduced and development started. Solvent was allowed to run 15–17 cm.

Spots were visualized under ultraviolet light of 254 mμ (Camag), chromatograms where photographed under two such light sources on Agfacolor CT 18 Diapositive film with an Asahi-Pentax camera, type SV,

with 49 mm ultraviolet "ghostless" filter; exposure time 15 sec, distance 70 cm, aperture 5·6.

On all plates, 4-nitroaniline reference (R), was run as a control substance.

Results and discussion

The separation of 14 commonly used barbiturates in the normal tank using chloroform–isopropanol–25% ammonia (45:45:10) is shown in Fig. 2. The spread of the spots is poor using about one-third of the plate only. Changing the ratio of solvent composition does not materially improve the separation or the spread. Fig. 3 shows the results obtained on the same compounds in a VC-chamber. The running solvent was chloroform–isopropanol (92·5:7·5), saturated with 25% ammonia used in conjunction with mixtures of chloroform–isopropanol–methanol–ammonia to form a vapour gradient. For the interspersed troughs of low polarity, solvent chloroform saturated with 25% ammonia was used. The spread of spots almost extends over the entire plate; the size of the spots is not significantly increased. Most of the barbiturates are now clearly separated, although there remain a few critical pairs. Such pairs can often be separated by application of slightly different gradients. The overall time required for this separation in the VC-chamber is almost the same as in the normal tanks. The time for development in the former is longer but this is compensated for by the fact that the saturation time can be reduced to 10 min due to the small space between the plate and the troughs.

A similar comparison of methods can be made with local anaesthetics. Seven chemically related substances chromatographed by the classical method are poorly separated using hexane–chloroform–methanol (60:35:5) and silica gel impregnated with sodium hydroxide as adsorbent* (Fig. 4). With a more polar solvent like chloroform–methanol (95:5) the spots run faster and separation is worse (Fig. 5). The results with the VC-chamber and the systems used in Fig. 4 are shown in Fig. 6. Chloroform–acetone–methanol was used for the vapour gradients. Separation is complete and can be used for identification purposes, with a spread of spots over the plate of about 50%. The time required for the separation of the anaesthetics in the VC-chamber is about 30% less than in the normal tank.

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* 0·1N sodium hydroxide is used to prevent retention of the basic substances at the starting point.

References

De Zeeuw, R. A. (1968a). *J. Chromat.*, **32**, 43–52.
De Zeeuw, R. A. (1968b). *Analyt. Chem.*, **40**, 915–918.
De Zeeuw, R. A. (1968c). *Ibid.*, in the press.