United States Patent [19]

Meyer

[11] **3,915,856**

[45] **Oct. 28, 1975**

[54] **METHOD OF CARRYING OUT PREPARATIVE THIN-LAYER CHROMATOGRAPHY AND APPARATUS FOR USE IN THE METHOD**

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[22] Filed: **Dec. 20, 1974**

[21] Appl. No.: **534,916**

[52] **U.S. Cl.** ..... **210/31 C**; 210/198 C

[51] **Int. Cl.**[2] **B01D 15/08**

[58] **Field of Search** ..... 210/31 C, 198 C

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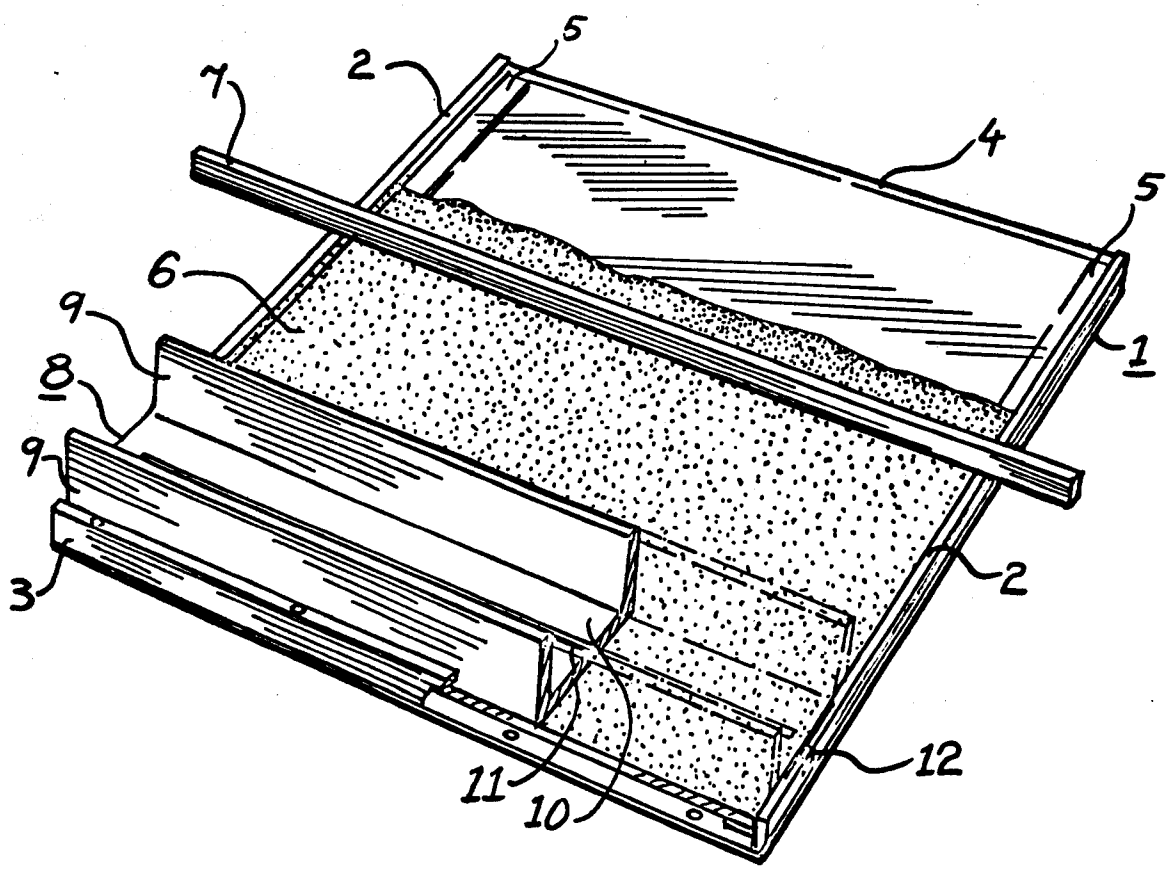
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Primary Examiner—John Adee
Attorney, Agent, or Firm—Fred Philpitt

[57] **ABSTRACT**

A method of carrying out preparative thin-layer chromatography comprising application of a layer of adsorbent material to a base plate, application of a sample in the form of a narrow strip in the adsorbent layer and immersion of the base plate in a solvent for the development of the chromatogram is disclosed wherein the sample is applied in solid form, the sample being dispensed in a groove in the adsorbent layer to form a strip which is then compressed or compacted. The invention also comprises apparatus for carrying out the method according to the invention which apparatus includes (a) a bridge member, (b) a holder for cutting blades, (c) an applicator and (d) a press of T-shaped cross-section.

12 Claims, 6 Drawing Figures



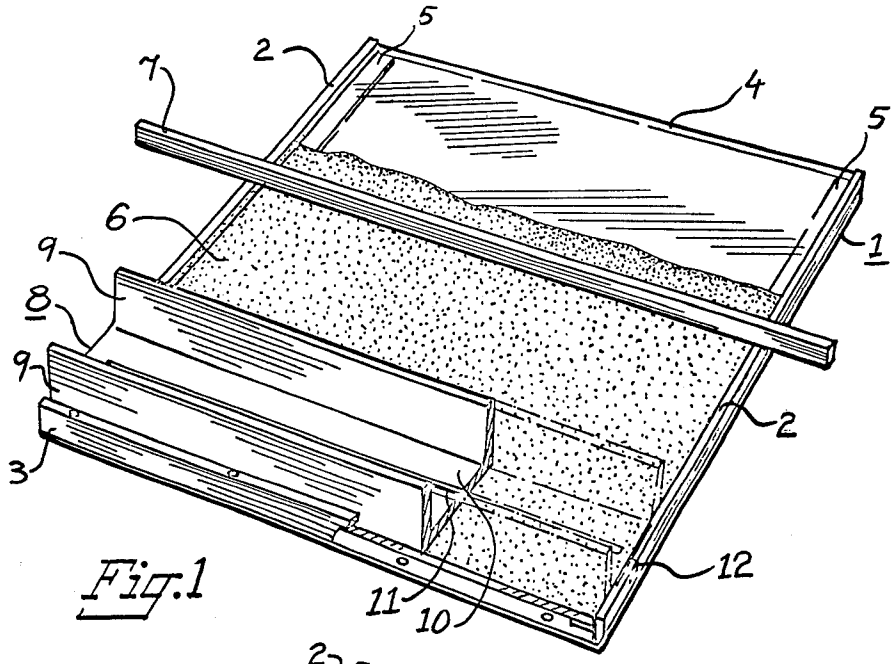


Fig. 1

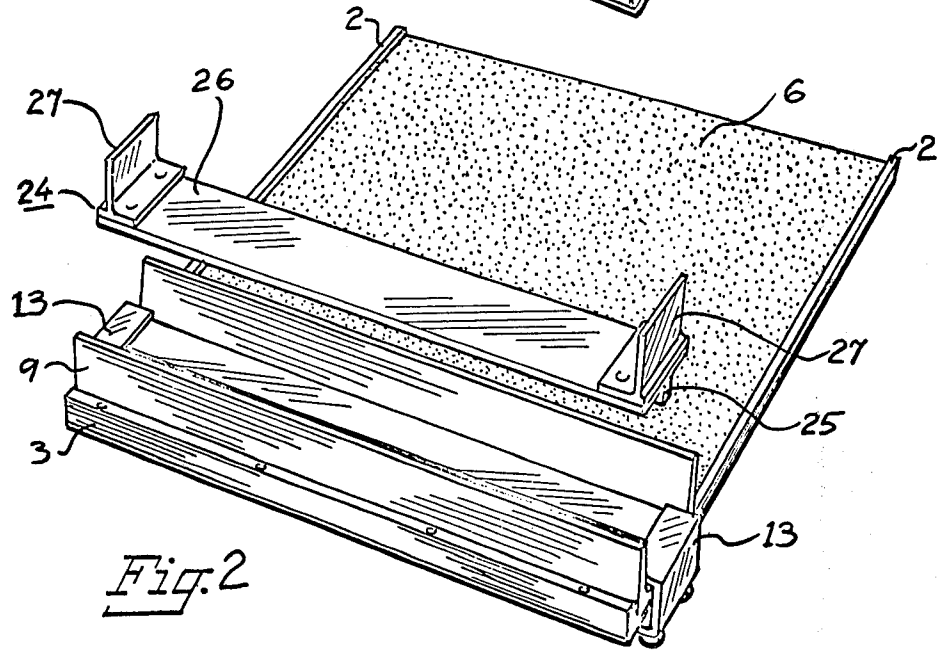


Fig. 2

Fig. 3

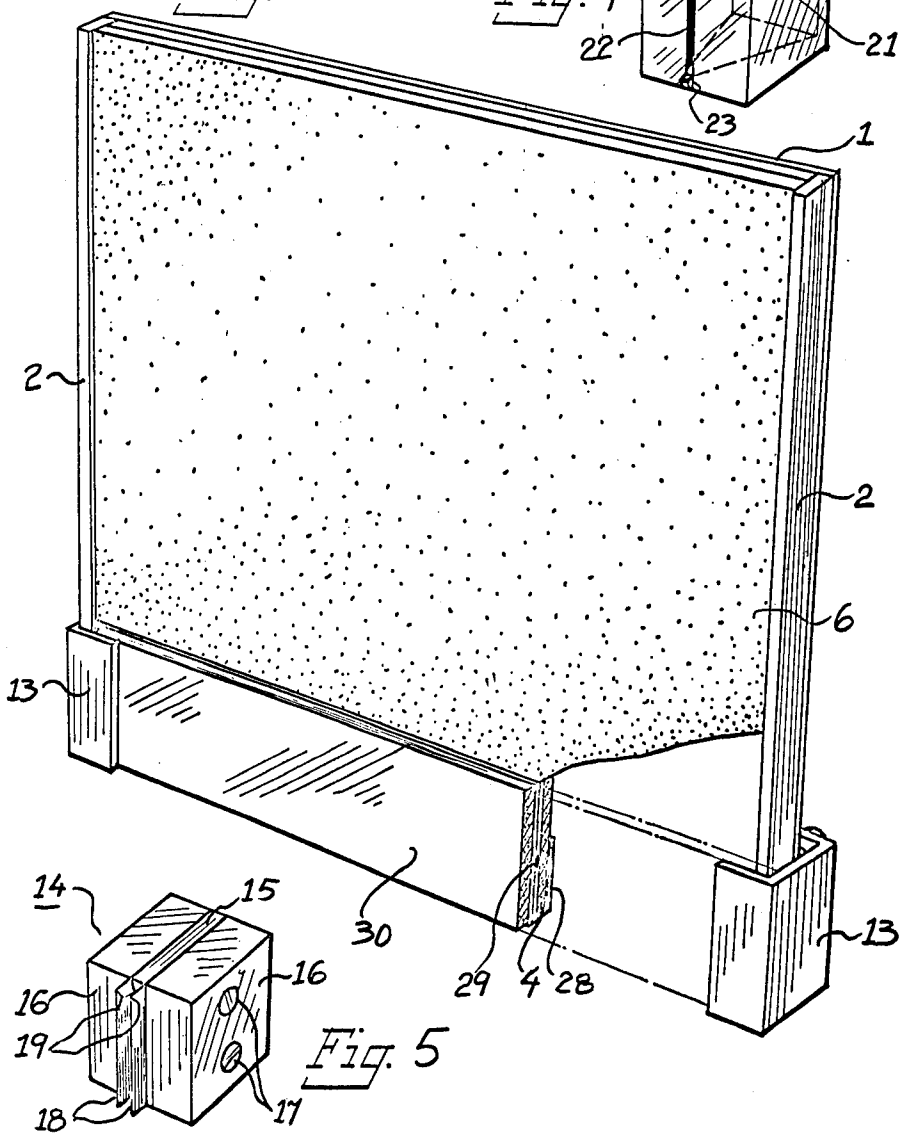


Fig. 4

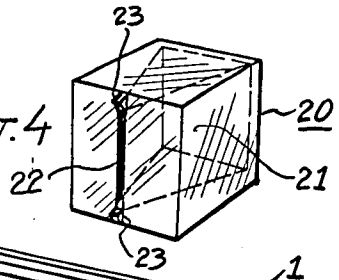
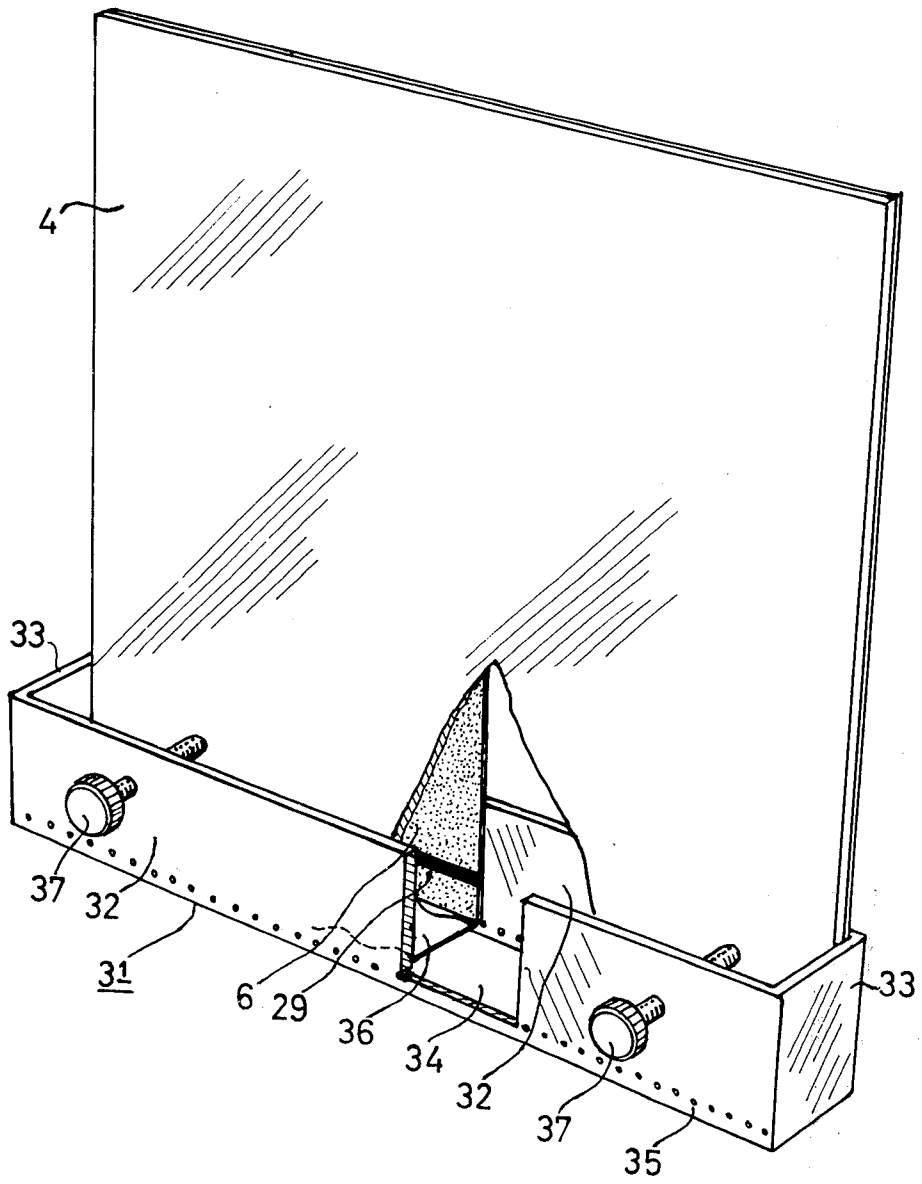


Fig. 5

Fig 6



METHOD OF CARRYING OUT PREPARATIVE THIN-LAYER CHROMATOGRAPHY AND APPARATUS FOR USE IN THE METHOD

The present invention relates to a method of carrying out preparative thin-layer chromatography comprising the application of a layer of adsorbent material to a base plate, application of a sample in a narrow strip in the adsorbent layer and immersion of the base plate in a solvent for the development of the chromatogram.

Hitherto, in preparative chromatography, the sample has been applied to the adsorbent layer in the form of a solution. When doing this, the sample has generally been applied in the form of a solution in water or in an organic solvent in a narrow strip on the adsorbent layer by means of an injection-syringe provided with a capillary. Among the disadvantages of this way of application the following may be mentioned:

1. The dissolved state of the sample renders it difficult to apply a defined dosage.

2. In view of the facts that the initial strip must be at most 2 to 3 mm in width and that the sample should be evenly distributed along the strip the application requires a comparatively long time, typically at least 20 minutes. Moreover, the solvent has to evaporate before a subsequent strip can be applied.

3. When applying a solution most of the sample is sucked up into the surface layer of the adsorbent and the concentration distribution of the initial strip is liabie to become non-uniform in length, width and depth.

It has now been found possible to avoid or alleviate these disadvantages by means of a method which is characterized in that the sample is applied in solid form, the sample being dispensed in a groove in the adsorbent layer to form a strip which is then compressed. The sample is applied either in pure form or in homogeneous mixture with adsorbent material in various weight ratios. The advantages gained by using this method are primarily that a carefully weighed amount of sample may be chromatographed and that the sample strip in the adsorbent layer may be made thoroughly homogeneous.

According to a preferred form of the method according to the invention, the sample strip and those parts of the adsorbent layer which, on immersion of the base plate in the solvent, are adjacent to or below the sample strip, are held against the base plate during the development of the chromatogram.

The present invention also provides apparatus for carrying out the method acoording to the invention, which apparatus includes

a. a bridge having longitudinal, high sides and a bottom which is provided with a narrow longitudinal slot,

b. a holder for cutting blades, which holder is designed for cutting out a groove in the adsorbent layer, following the slot of the bridge,

c. an applicator designed for dispensing the sample into the groove cut in the adsorbent layer and

d. a press of T-shaped cross-section, the stem of which fits into the slot of the bridge for compressing the sample in the groove.

Preferably, the cutting blade holder is provided with means for scraping the groove in the adsorbent layer to remove adsorbent material when said holder is moved to and fro in the bridge.

Preferably, the interior of the applicator has the shape of a funnel having an outlet at the base thereof,

said outlet being arranged to register with the slot of the bridge when the applicator is placed between the longitudinal high sides of the bridge. It is also of advantage to provide the applicator with two wedge-shaped levelling means on opposite sides of the outlet for levelling the sample in the groove.

The T-stem of the T-shaped press is preferably somewhat longer than the thickness of the bottom of the bridge. The press is also advantageously provided with finger grips.

Apparatus according to the present invention may also include:

e. a trough having vertical side and end walls and a bottom, at least one of the side walls being perforated near said bottom,

f. an insert member, which rests on the bottom of the trough and is shaped to provide a plurality of equally high elevations and

g. means for securing the base plate with the adsorbent layer applied thereon in close contact with one of the side walls of the trough.

The invention will now be illustrated, by way of example, with reference to the accompanying drawings, wherein

FIGS. 1 – 3 illustrate different steps of the method according to the invention,

FIG. 4 is a view in perspective of one form of applicator for apparatus according to the invention,

FIG. 5 is a view in perspective of one form of blade holder for apparatus according to the invention and

FIG. 6 illustrates an alternative to the step of FIG. 3.

In FIG. 1 there is shown a frame plate 1 comprising aluminium, stainless steel or any other material which will allow heating of a glass plate with adsorbent thereon lying on said frame plate to a suitable temperature, usually 120°C, for a sufficiently long time to activate the adsorbent, i.e., to remove completely water and the liquid in which the adsorbent was suspended when the thin layer plate was prepared. The frame plate 1 is provided with border members 2 along two parallel edges and a third somewhat higher and removable border member 3 at right angles to the border members 2. A base plate 4 is located within the borders, and may comprise glass or any other material which is resistant to prolonged heating to about 120°C and to the solvents in which the plate is immersed for the development of the chromatogram. The base plate 4 is placed on flat rods 5 of aluminium, stainless steel or a similar material laid out along the borders 2 in such a number that the upper surface of the plate 4 will be at such a vertical distance from the upper surface of the borders 2 as is required to accommodate the thickness of the adsorbent layer. An adsorbent layer 6 is applied to the base plate 4. This layer may, for instance, be produced by pouring onto the plate a suspension of silica gel or another adsorption agent in water or other suitable liquid, and levelling the suspension to form a uniformly thick layer by means of a rod 7 laid on the borders 2 at right angles thereto and drawn along said borders 2 to distribute the suspension into a layer of a well-defined uniform thickness on the plate 4. The layer is then allowed to dry, after which it is activated at about 120°C for 1 – 2 hours to remove the suspending medium. This also produces a small decrease in the thickness of the layer. Prepared plates with an adsorbent layer already applied thereon are commercially avail-

able and may also be used for the method according to the invention.

After the adsorbent layer 6 has been prepared a bridge 8 having two high longitudinal sides 9 and a bottom 10 with a longitudinal slot 11 is placed on the borders 2. The bridge is suitably provided with downwardly projecting pegs (not shown) which should engage into notches 12 in the borders 2. The bridge is suitably also chamfered at the ends thereof so that the unchamfered middle part of the bottom side is given the same length as the inside distance between the two borders 2 of the frame plate 1. In this way, the bottom side of the bridge 8 can lie close to the adsorbent layer. The slot 11 is situated about 10 mm from the edge of the layer.

From FIG. 2 it can be seen that the bridge 8 is secured to the frame plate 1 by fastening means comprising screw clamps 13. In this position a groove is cut in the adsorbent layer 6 by means of cutting blades mounted in a holder, for instance a holder of the type shown in FIG. 5.

The holder 14 shown in FIG. 5 comprises a central portion 15 and two side portions 16 held together with screws 17. Two cutting blades 18 are held by means of the screws between the central portion 15 and the side portions 16, one blade between each side portion 16 and the central portion 15, in such a position that the edges thereof will be at a distance from the bottom side of the central and side portions corresponding to the desired depth of the groove in the adsorbent layer. The distance between the outside surfaces of the cutting blades 18 corresponds to the width of the slot 11 of the bridge 8 and the overall width of the holder is equal to the internal distance between the longitudinal vertical sides 9 of the bridge. Tongues 19 are arranged between the cutting blades 18.

When cutting out a groove in the adsorbent layer the blade holder 14 is placed between the sides 9 of the bridge in such a way that the cutting blades 18 are inserted in the slot 11. Then the holder 14 is moved to and fro in the slot along the longitudinal axis of the bridge, and the blades 18 cut the outer edges of a groove in the adsorbent layer 6, which groove is scraped clean by the tongues 19.

Then the blade holder, and loose adsorbent material possibly remaining in the groove, are removed and the sample is introduced into the groove by means of an applicator which may, for example, be as shown in FIG. 4.

The interior of the applicator 20 shown in FIG. 4 forms a funnel 21 ending in a rectangular slot-shaped outlet 22 in the base of the applicator. Two wedges 23 of the same breadth as the slot 11 of the bridge 8 are arranged at the base of the applicator at the ends of the outlet 22. In operation, the applicator is placed between the sides 9 of the bridge 8 in such a way that the wedges 23 engage in the slot 11. A solid sample (which may be pure or mixed with adsorbent) is charged into the funnel 21 and the applicator is moved to and fro along the slot 11. During this operation the sample flows out through the outlet 22 and is distributed in the groove in the adsorbent layer where it is levelled by means of the wedges 23. The maximum thickness of the wedges 23 is adapted to the surface of the adsorbent layer in such a way that the surface of the levelled sample in the groove will be of about the same level as the surface of the adsorbent layer.

After the sample has been distributed into and levelled in the groove the applicator is removed and the sample is compressed by using a T-shaped press 24 (FIG. 2), the T-stem 25 of which fits into the slot 11 of the bridge. The upper part 26 of the press is of the same width as the internal distance between the longitudinal sides 9 of the bridge and is provided with two finger grips 27 for facilitating its insertion into the bridge and its removal therefrom. The stem 25 is somewhat longer than the thickness of the bottom 10 of the bridge.

After compression of the sample, the press 24 is removed and the bridge 8 and the plates 1 and 4 may if desired be immersed in a solvent for the development of the chromatogram. In order, however, to avoid flaking of that part of the adsorbent layer which on immersion of the plates into the solvent is situated beneath the strip of sample, one or other of the expedients illustrated in FIGS. 3 and 6, respectively, may suitably be adopted.

In FIG. 3 the removable border 3 (vide FIGS. 1 and 2) of the frame plate 1 has been removed and a sheet 28 of a porous material which does not contaminate the sample, the adsorbent layer or the solvent, for instance a filter paper, has been folded round the adsorbent layer 6 and the base plate 4 at the edge thereof which is nearest to the sample strip 29. The sheet 28 extends sufficiently far up the adsorbent layer that it covers the strip 29 of sample. A plate 30, made of glass or of any other material that is resistant to the solvent, is placed outside the sheet 28 between the borders 2, and is secured by fastening means, in this case the same screw clamps 13 as were used earlier for securing the bridge 8 to the frame plate 1 (FIG. 2).

Instead, as shown in FIG. 6, the base plate 4 may be completely removed from the frame plate and placed in a trough 31 having vertical side and end walls 32 and 33, respectively, and a bottom 34. The side walls 32 are perforated with apertures 35 situated near the bottom 34. The bottom 34 has an insert in the form of a slightly corrugated metal sheet 36 resting thereon, said sheet presenting a large number of points of support for the base plate 4 and the adsorbent layer 6 applied thereon. The base plate is placed in such a way that the adsorbent layer 6 is in close contact with one of the side walls 32 of the trough. The plate is secured in this position by fastening means comprising screws 37 passing through the opposite side wall. The height of the trough is such that the strip 29 of sample will lie below the top edge of the trough when the base plate 4 is resting on the sheet 36. The distance between the strip 29 of sample and the top edge of the trough suitably amounts to about 5 mm or possibly somewhat more. The apertures 35 may, for instance, have a diameter of 1 mm and be situated about 5 mm above the bottom 34 and about 1 cm apart. The trough is suitably made a millimeter or so longer than the width of the plate.

For the development of the chromatrogram, the base plate with the trough attached thereto is immersed in a solvent and the development may then be carried out in conventional manner.

It is easily recognized that the embodiment in detail of the components forming parts of the invention may be performed in many ways without departing from the idea of the invention. For that reason the invention is not limited to the embodiments of said components shown in the figures of the accompanying drawings.

What is claimed is:

1. A method of carrying out preparative thin-layer chromatography comprising application of an adsorbent layer to a base plate, application of a sample in the form of a narrow strip in the adsorbent layer and immersion of the base plate in a solvent for the development of the chromatogram, characterized in that the sample is applied in solid form, the sample being dispensed in a groove in the adsorbent layer to form a strip which is then compressed or compacted.

2. A method according to claim 1, wherein the sample is applied alone or in homogeneous admixture with adsorbent material.

3. A method according to claim 1, wherein the strip of sample and those parts of the adsorbent layer which, on immersion of the ground plate in the solvent, are adjacent to or below the sample strip are held against the ground plate during the development of the chromatogram.

4. A method according to claim 3, wherein a sheet of a porous material which does not contaminate the sample, the adsorbent layer or the solvent is folded round the adsorbent layer at the edge thereof that is nearest to the strip of sample, care being taken that the sheet covers the strip of sample, and the sheet is secured in this position during the developing of the chromatogram.

5. A method according to claim 3, wherein the base plate with the adsorbent layer applied thereon is caused to rest upon a plurality of points of support at a small distance from the bottom of a trough having vertical side and end walls and is secured in close contact with one of the vertical side walls with the adsorbent layer facing said one side wall, the height of said one side wall being adapted to the position of the strip of sample so that said strip will be situated somewhat below the top edge of said wall, said wall and the opposite side wall being perforated near the bottom of the trough.

6. Apparatus for carrying out the method according to claim 1, characterized in that it includes

a. a bridge having longitudinal sides and a bottom, said bottom being provided with a narrow longitudinal slot,

b. a holder for cutting blades, which holder is designed for cutting out a groove in the adsorbent layer by following the slot of the bridge

c. an applicator designed for dispensing the sample in the groove cut in the adsorbent layer, and

d. a press of T-shaped cross-section, the stem of which is arranged to fit into the slot of the bridge for compressing the sample in the groove.

7. Apparatus according to claim 6, wherein the blade holder is provided with means for scraping clean the groove in the adsorbent layer when said holder is moved to and fro along the slot in the bridge.

8. Apparatus according to claim 6, wherein the interior of the applicator has the shape of a funnel and has an outlet arranged in its base, said outlet being arranged to register with the slot of the bridge when the applicator is placed between the longitudinal sides of the bridge.

9. Apparatus according to claim 8, wherein two wedge-shaped levelling means are arranged on opposite sides of the outlet for levelling of the sample in the groove.

10. Apparatus according to claim 6, wherein the T-shaped press is provided with finger grips.

11. Apparatus according to claim 6, wherein the T-stem of the T-shaped press projects somewhat further than the thickness of the bottom of the bridge.

12. Apparatus according to claim 6, characterized in that it also includes

e. a trough having vertical side and end walls and a bottom, at least one of the side walls being perforated near said bottom (34),

f. an insert which rests on the bottom of the trough and is shaped to provide a plurality of equally high elevations, and

g. means for securing a base plate with an adsorbent layer applied thereon in close contact with one of the side walls of the trough.

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