

amine was eluted from the coating material by adding about 7 ml of the solvent used. Each flask was shaken well for 10 min, centrifuged and filtered into 10-ml volumetric flasks; solvent was added to the volume. A similar elution process was followed for a blank sample of the coating material alone.

Interferometric readings for each solution were taken using the blank as reference solution, and the values obtained were recorded next to the standard curve and compared.

Results

The results obtained for amphetamine sulfate in water solution are depicted in Fig. 1, where it appeared that a $94 \pm 2\%$ recovery of the compound used was achieved.

The technique described above can be considered simple, rapid and accurate for routine analysis.

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Apparatus and procedure for developing thin-layer plates under nitrogen

Thin-layer chromatography has proved to be a very useful technique for separating many types of compounds. However, some of the separations for which it is used involve easily oxidizable compounds such as the polyunsaturated fatty acids. When these spread out on thin layers of adsorbent they are especially prone to oxidation unless air is excluded during the spotting and developing procedures. BADINGS¹ separated a fatty acid ester mixture on thin-layer plates both in an atmosphere of nitrogen and in air. After extracting the esters from the adsorbent layers and determining their composition by gas chromatography, he found that the mixture separated in air contained 4% less methyl linoleate and 6% less methyl linolenate than that separated in nitrogen*.

An inherent difficulty in trying to develop chromatograms in an inert atmosphere with the usual TLC apparatus is that if the tank already contains the plates and

* Percentages were calculated from BADINGS' data.

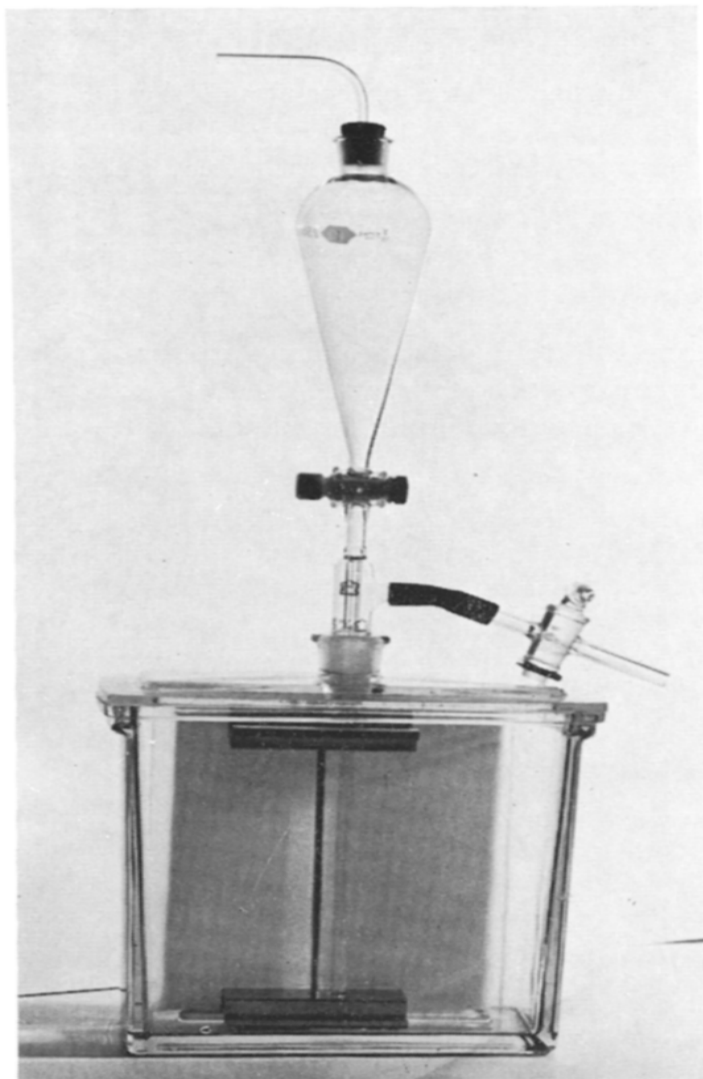


Fig. 1. Apparatus for developing thin-layer plates under nitrogen. Shown with optional steel clip for holding plates upright.

solvent when it is flushed with gas, the activity of the plates may be altered and the composition of the solvent may be changed, especially if it is a mixture of substances of different volatility. If plates or solvent are added after the flushing operation, air may get into the chamber. With the latter procedure carbon dioxide may be used for flushing since it is considerably heavier than air and not easily lost from the chamber. Apparently for this reason STAHL² recommends it in cases where nonbasic adsorbents are used. However, there may be other cases, such as in the chromatography of easily hydrolyzed substances, where the pH-lowering effect of the carbon dioxide upon adsorbed water or upon aqueous solvents used in reverse-phase systems may be undesirable.

Application boxes for spotting TLC plates in an inert atmosphere and chromatographic tank covers with gas inlet and outlet connections are now commercially available (Brinkmann Instruments, Inc.*), but the latter make no provision for

* Mention of trade names does not imply endorsement by the Department of Agriculture over other products which may be suitable.

adding the solvent after flushing. One apparatus for doing this has been devised by BADINGS.¹ The present paper describes an alternative apparatus (Fig. 1) which many may find easier to construct since it eliminates any need to drill through the tank or its cover.

The apparatus makes use of a Chromaflex tank cover (Kontes Glass Co. *) and an adapter which consists of a 24/40 standard taper joint with a tubulature at the top and a hose connection on the side. Preferably the adapter joint is cut to the length of that in the cover. A separatory funnel with Teflon stopcock is sealed to the tubulature end of the adapter. A long piece of wide-bore capillary tubing (2.5 to 3 mm I.D.) is passed through the adapter joint and sealed to the inner end of the tubulature so that the flushing gas, coming down through the separatory funnel, has to reach the bottom of the tank before it can pass out through the hose connection on the side of the adapter. The tubing must end near the bottom of the tank to prevent splashing and to increase flushing efficiency, but if a Chromaflex tank is used the tubing must clear the ridges on the bottom. The bores of stopcocks used with the apparatus should be of the same diameter as the capillary or larger.

The thin-layer plates are first placed in the tank, and the tank is flushed with nitrogen by means of tubing connected to the top of the separatory funnel. If the activity of the adsorbent is critical, the flushing can be carried out at a fixed time and rate with the aid of a flowmeter. The gas may be passed through water or through drying tubes as needed. The flowmeter should be placed at the head of the train to keep out adsorbent particles, etc. After the flushing procedure the developing solvent is added through the funnel. When an equilibration period is desired the tank may be lined with filter paper and tilted slightly before adding the solvent in order to keep the adsorbent dry. The Brinkmann stainless steel holder (shown in Fig. 1) may be used to keep the plates away from the liner, but is probably not essential. Holes are drilled in it to accommodate the capillary tubing.

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