Techniques to reduce errors in quantitative thin-layer chromatography using elution

In quantitative thin-layer chromatography (TLC) when it is necessary to identify one or more of the separated compounds of a mixture by I.R., U.V. or N.M.R. techniques, the usual method is to remove the adsorbent containing the spot of interest quantitatively from the plate, and to extract the powder by means of a suitable solvent. The solution containing the extract is however frequently polluted by organic impurities which are present in nearly all commercial adsorbents. Moreover, extremely fine adsorbent particles which cannot be eliminated, even by high speed centrifuging, contaminate the product to be examined. This contamination is of great inconvenience when quantitative measurements in the U.V. region are to be carried out, either because it has an influence upon the background absorption, or because it provokes spurious absorption bands. The presence of spurious bands is also an inconvenience when the I.R. spectra of the eluted products are to be taken for identification purposes.

Last year, MULDER AND VEENSTRA[1] published a method which overcame these difficulties. The basic principle of this method consists in concentrating the compound to be analyzed in a small area. This method yields good results but is rather time-consuming. In this communication, we propose two methods which have been used in our laboratory and which in our opinion are more adequate than the above-mentioned one.

Method 1

After the classical elution of the chromatogram in direction I in the usual manner, the plate is dried. A part of the adsorbent around the spots (visible under U.V. light if the adsorbent contains a fluorescent indicator) is scraped off in the way shown in Fig. 1A. Small pieces of ash-free filter paper (Whatman No. 1) cut into a point, are placed on the remaining strips of adsorbent (Fig. 1B). The whole is covered with

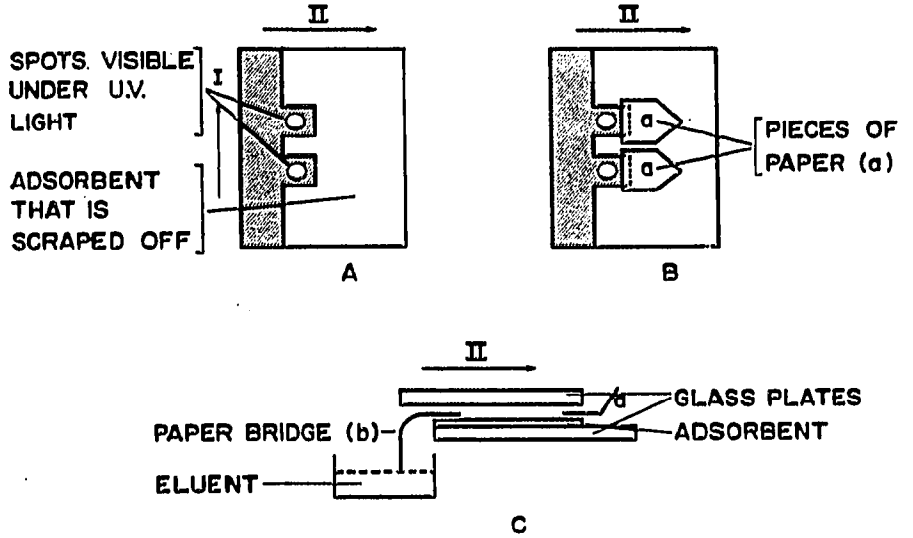

Fig. 1. Method 1. (A) After elution in direction I the adsorbent layer is partly scraped off. (B) On the remaining parts of adsorbent, small pieces of paper are placed. (C) The whole is eluted in direction II with a horizontal elution technique.

a second glass plate, leaving the top of the pieces of paper free. With a horizontal elution technique, using a paper bridge b of the same ash-free quality the plate is eluted for a second time with a fast-working eluent in direction II. In this way the products contained in the spots are pushed into the top of the pieces of paper (these tops are folded at an angle of 45°) (Fig. 1C).

Thereafter the top of the paper a is cut off and extracted quantitatively by the solvent in which the U.V., N.M.R. or I.R. analysis is performed.

Method 2

After separation in direction I, the layer is dried and the adsorbent is partly removed in the manner shown by Fig. 2. In an open thin-layer elution tank (in order to allow a good evaporation of the solvent), the plate is then eluted in direction II by means of a fast eluent. The products are concentrated in the point b of the remaining strips of adsorbent.

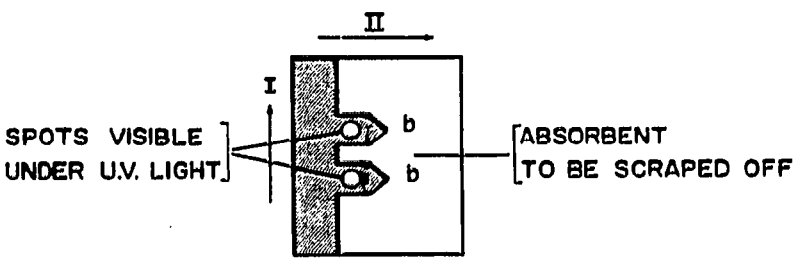

Fig. 2. Method 2. After elution in direction I the absorbent layer is partly scraped off and then eluted in direction II.

After this process the adsorbent on the top b is scraped off. This small quantity containing the product for examination, can then be extracted in the usual way without much fear of pollution.

The elution in the second direction can also be done by the horizontal elution technique as described in the first method.

In both methods a very low background absorbance in the U.V. region is obtained (background absorbance less than 0.003 in 5 ml of solvent in a 1 cm cell).

When I.R. spectra for identification purposes are to be taken, we allow the products to eluate out of the top of the adsorbent. When they show a good crystallinity, small crystals are produced on the glass plate in front of the point b. The extremely pure crystals are collected and their I.R. spectra recorded using the micro KBr-technique.

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