Quantitative thin layer chromatography of amines using an interference refractometer

Numerous techniques, such as direct measurement of the spot area[1,2], colorimetric U.V.[1,3], fluorescence[4], infrared[5], photometry[6] and microphotometry[7], have been reported for the quantitative estimation of amines and other compounds on thin-layer chromatograms.

In the present investigation the quantitative determination of a number of amines has been carried out using a Zeiss interference refractometer and a 80-mm cell.

Experimental

Thin-layer glass plates, 20 × 20 cm, coated with MN 300G cellulose powder (Macherey, Nagel & Co) according to STAHL[8], were used.

The solvent system employed was *n*-butanol–acetic acid–water (4:1:5, v/v).

Solutions of the amines were prepared by dissolving 500 mg of the pure compound in a solvent, in a 10-ml volumetric flask (stock solution). Using a micropipet, quantities up to 15 μg (in 2.5 μg intervals) were diluted in 10-ml volumetric flasks, and the measurement process followed directly after the determination of the zero point.

For this purpose the solvent to be used for the solution under measurement is placed in the twin-cell and the zero reading is taken. The reference substance is then removed from one half of the cell and the test sample is poured into it and allowed to stand until the temperature has become equalized (approximately 20 min), because at this stage only the lower interference band of the band system can be seen, the upper band having been displaced laterally due to the filling cell.

The actual measurement process consists in the determination of the amount of this displacement. The reading, less the zero point reading, is the required result for each solution. From the values obtained a standard curve was plotted.

For the elution of the spots, quantities up to 15 μg, as above, were applied to the thin-layer plates 1.5 cm from the bottom edge, and the chromatograms were run in the usual manner. After drying the plates, the spots were located using an U.V. lamp and were marked off. Each spot was then scraped off into small conical flasks and the

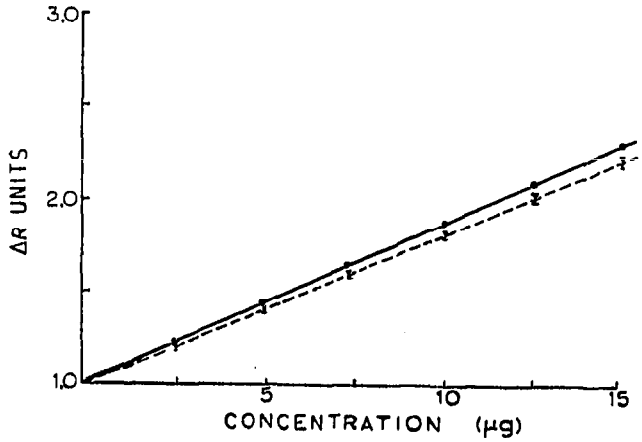

Fig. 1. Amphetamine sulfate recovery after elution of the spots from thin-layer chromatogram of cellulose. Key: straight line indicates the blank; broken line indicates recovery after elution; ΔR indicates differential refractive index units.

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 ± 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. BADINGS[1] 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.