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Improved preparative dry-column chromatography

Analysis of resolution by puncture sampling

LOEV *et al.*[1–4] introduced the use of nylon tubing and adsorbents with fluorescent indicators for preparative dry-column chromatography. Their procedure involved developing the column with homogeneous solvents and then locating the resolved products by scanning with short wavelength ultraviolet light. These modifications increased the utility of the system, but limited its applicability to ultraviolet light transparent solvents and to the resolution of compounds capable of "blanking out"[2] the fluorescence of the indicators.

We report an improved procedure which eliminates the use of adsorbents with fluorescent indicators, and is not limited to ultraviolet light transparent solvents. Our method increases the practical utility of the chromatographic system and can be extended to the resolution of radioactive compounds.

Experimental

Silica gel (Will Corp., grade 950, 60–250 mesh) was deactivated by addition of water (15 %, w/v). Nylon casings were purchased from Walter Coles and Co., Ltd., Plastic Works, London, S.E. 1, Great Britain. Packing of the column in the nylon tubing and deposition of the sample was carried out as described by LOEV and co-workers[1,2]. Following the development of the column with an appropriate solvent it was laid horizontally. The nylon column was then pierced at regular intervals of 1 or 2 cm (or otherwise) with disposable glass pipets ($5^3/_4$ in. × 7.0–7.4 mm O.D., heavy glass wall, VWR Scientific No. 14672-029) jamming some of the adsorbent into the end of the pipet. Usually the pipet was inserted to a depth sufficient to remove about 5 mm of adsorbent. The tip of the pipet was placed in a 10 × 70 mm test tube and five drops of methanol were added. The adsorbent was expressed from the pipet by gentle pressure and the product extracted with methanol. An aliquot of the methanolic extract from each tube was chromatographed on a 20 × 20 cm plate and the products visualized with a suitable reagent. The revealed distribution and qualitative boundaries of the resolved product were marked on the column. Satisfactory results were obtained by removing twenty samples from a 40-cm long column. The nylon column was sliced accordingly and the compounds eluted. Instead of monitoring by thin-layer chromatography the extracts could be scanned by gas–liquid chromatography.

Other adsorbents, alumina, Celite, etc., can be employed in a similar manner. If the compounds were radioactive the resolution of the products could also be assayed by the distribution of radioactivity in the extracts. Alternatively the adsorbent could be forced out of the pipette into counting vials containing scintillation fluid and counted directly.

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