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Note

Apparatus for the isolation of microgram amounts of compounds from thin layers by elution and direct Millipore filtration

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Thin-layer chromatography is commonly used for the isolation of a particular compound, the layer of sorbent containing that compound being scraped off the chromatogram and eluted with a suitable solvent. If, *e.g.*, mass spectrometry, is subsequently to be applied, it is necessary to have an eluate that is sufficiently concentrated. Therefore, when eluting microgram amounts, the total volume of the eluate should not exceed about 30 μl. When concentrating the eluate by evaporation, impurities in the solvent will also be concentrated and decomposition of the compound may also occur.

In order to avoid the need for evaporation after elution of microgram amounts, an apparatus has been developed that makes it possible to scrape off and collect the sorbent layer, elute the compound in a small volume and filter the resulting solution through a paper filter and a Millipore filter. In this way, microgram amounts of a compound can be isolated in a volume of 15–30 μl. In comparison with other techniques for the transfer of small amounts[1–6], this apparatus has the advantage of being specially constructed for filtration through a Millipore filter, instead of glass-wool[1–5] or cotton-wool[6]. With glass-wool or cotton-wool asfilters, the eluate is not free from particles from the thin layer. Filtration through the Millipore filter results in a particle-free eluate, which can be used directly for high-performance liquid chromatography and mass spectrometry.

APPARATUS

The device consists of three parts, the filtration section, the collector section and the conical tip (Fig. 1). The apparatus is made of PTFE and the diameter of the bore is 1.2 mm. The conical part of the tip fits into the collector section at side 1 (Fig. 1).

Part of the filtration section is milled, in order to simplify its connection with the collector section. The screw-thread used for connecting these two sections and the measure are defined by M8 $\times$ 1. The end of the filtration section is round, with a radius of 2.5 mm (R 2.5). As only a small part (1.2 mm^2) of the filters is used for filtration, it is necessary for the end of the collector section to be round, with a radius of 20 mm (R 20), in order to achieve a good connection. At side 1 the inner diameter

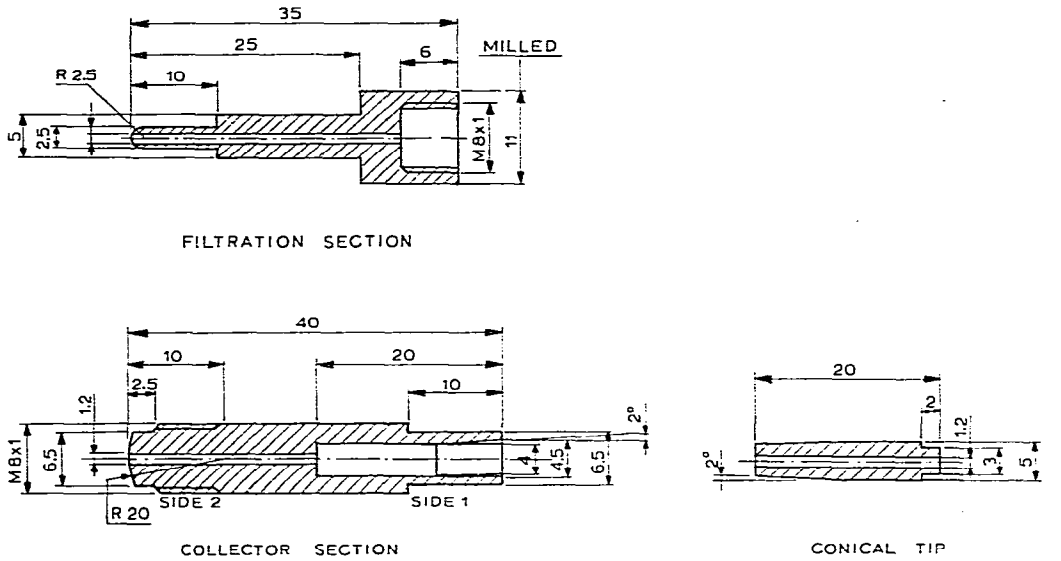

Fig. 1. Components of the apparatus. Measurements in millimeters.

is conical (2°) in order to obtain a sufficiently tight connection with the conical tip of the syringe, which is also conical. The diameter of the non-conical end of the conical tip is reduced from 5 mm to 3 mm over a distance of 2 mm in order to be able to scrape off small areas of sorbent.

Fig. 2 illustrates the collector sectionfitted with the conical tip, used as a scraper and sorbent collector.

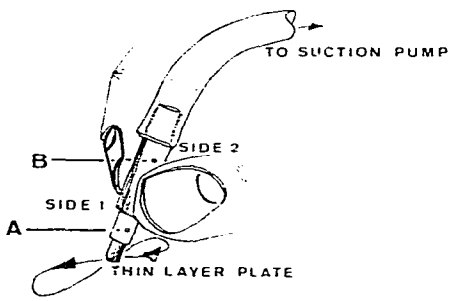

Fig. 2. Collection of sorbent from a thin-layer plate. A = Conical tip; B = collector section.

Fig. 3 illustrates the device for elution and filtration. The sorbent has been sucked into the collector section (B) (as illustrated in Fig. 2). The conical tip at side 1 has been disconnected, and also the tube at side 2, the side with the screw-thread leading to the suction pump.

Three kinds of filters are used: a Millipore filter, a paper filter and a collector filter. The Millipore filter (Sartorius-Membranfilter; Sartorius, Utrecht, The Netherlands; *e.g.*, S.M. 116 for organic solvents) (F, Fig. 2) and the paper filter (Munktell's

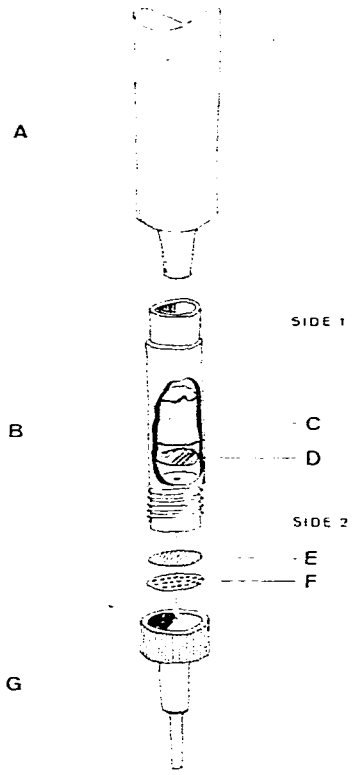

Fig. 3. Illustration of the apparatus set up for filtration after collection of the sorbent. A = Syringe; B = collector section; C = collected sorbent; D = collector filter; E = paper filter; F = Millipore filter; G = filtration section.

Swedish filter-paper; Grycksbo Pappersbruk, Grycksbo, Sweden; No. 2; A2-80-150) (E, Fig. 2), both with a diameter of 5.5 mm, are obtained by cutting the materials with a paper perforator. The collector filter (D, Fig. 2) stops the sorbent in the collector section; it has a diameter of 4 mm and is obtained by perforating a tissue of Kleenex Medical Wipes with a leather perforator.

OPERATION

After deciding which component on a thin layer has to be isolated, the operator brings a collector filter to side 1 of the collector section and pushes it to the bottom of the collector section with the aid of a small PTFE bar of diameter 3.8 mm and length 5 cm, then presses the narrow end of the conical tip into the collector section, also at side 1. Side 2 of the collector section is connected with a suction pump and the conical tip is moved over the thin-layer plate (Fig. 2). After the sorbent has been transferred into the collector section, the suction pump is disconnected and the conical tip is removed. The Millipore filter and the paper filter are placed in the filtration section and the collector section is connected with the filtration section (Fig. 3).

About 50–100 μl of a suitable eluting solvent are placed in the collector section

at side 1 (Fig. 3); finally, the fluid is pressed through the device with the help of a syringe (*e.g.*, Inaltera Supra, Henke-Sass Wolf, Tuttlingen, G.F.R., Luer Lok system; a 20-ml syringe is adequate) connected at side 1 (Fig. 3). The clear solution of the compound under investigation thus obtained can, without further purification and concentration steps, be injected into a high-pressure chromatograph or subjected to mass spectrometry.

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