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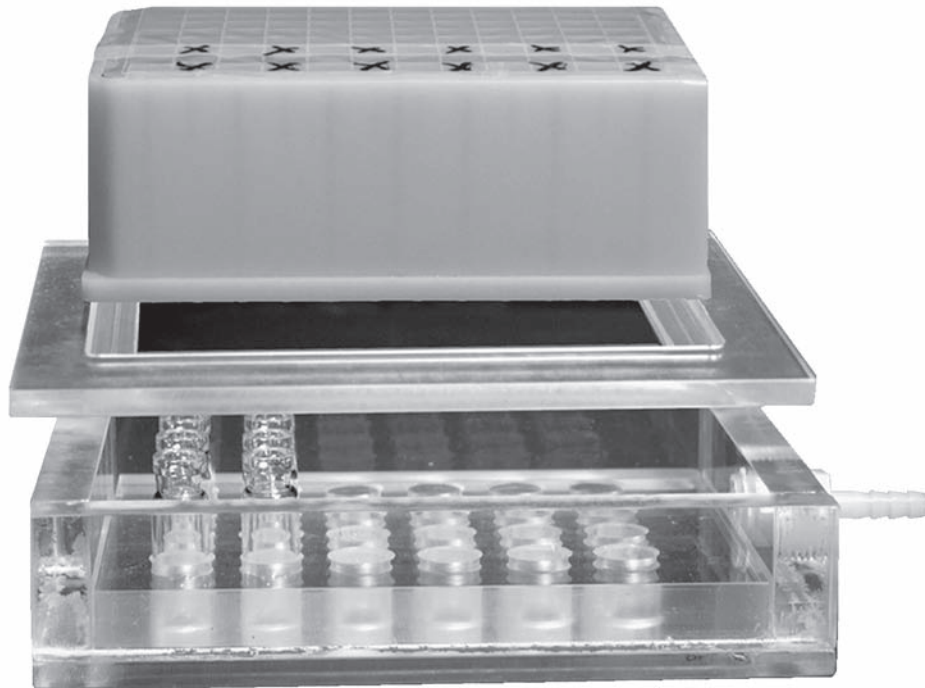
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Time- and Cost-Saving Apparatus for Analytical Sample Filtration

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Abstract

Simple and cost-effective protocols were developed for removing particulates from samples prior to analysis by high performance liquid chromatography and gas chromatography. A filter and vial holder were developed for use with a 96-well filtration plate. The device saves preparation time and costs.

Keywords: Analytical preparation, filtration.

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Time- and Cost-Saving Apparatus for Analytical Sample Filtration

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Many protocols for analysis by high performance liquid chromatography (HPLC) or gas chromatography (GC) require the centrifugation and filtration of samples prior to injection. Single-use filters are generally expensive because of manufacturing costs and quality control. Multiple-sample filtration devices (96-well filtration) are generally less costly per sample filtered, but they require the use of filtrate collection devices and the transfer of samples to autosampler vials. This is a time-consuming operation for laboratories that cannot afford programmable robotic positioning equipment.

In our laboratory, we use standard 11- by 32-mm (0.43- by 1.26-inch) (diameter by height) autosampler crimp seal vials. Filtration devices for 96-well microtiter dish samples are spaced 9 mm (0.35 inch) center to center. It is impossible to design a simple reusable device so that each drip tip is positioned directly over the mouth of each vial. The spacing of the drip tips below the filtration device accommodates 11-mm vials, but the vials must be spaced such that every other sample well is used in both the horizontal and vertical directions.

We designed a filter holder and autosampler vial positioning device that allows the filtration of samples, 24 at a time, directly into autosampler vials (Fig. 1). Samples can be filtered in four sets of 24 by moving a sliding lid and turning the filter. The base, vial holder, and lid are made from AcryliteGP acrylic sheet (Cryo Industries, Mt Arlington, New Jersey), although any clear bondable sheet will suffice. The base is glued together and the lid is attached to the base with a layer of silicone vacuum grease. This filtration device uses Whatman (Clifton, New Jersey) 2-mL UNIFILTER 0.45- μ m hydrophilic polyvinyl difluoride (PVDF) microplates.

Before use, plates are sealed with an adhesive film (Polyolefin film, Nunc-232701, Fisher Scientific, Pittsburgh, Pennsylvania) to reduce vacuum loss through unused filter chambers. A slice is made in the film above those wells to which samples will be added. Vacuum is connected to the apparatus, and the samples are placed in the wells. The filtration process can be monitored through the transparent sides of the device. If the vacuum applied is insufficient to hold the filter device to the lid, clear plastic tape is applied along all four sides of the filter to provide a better seal. The

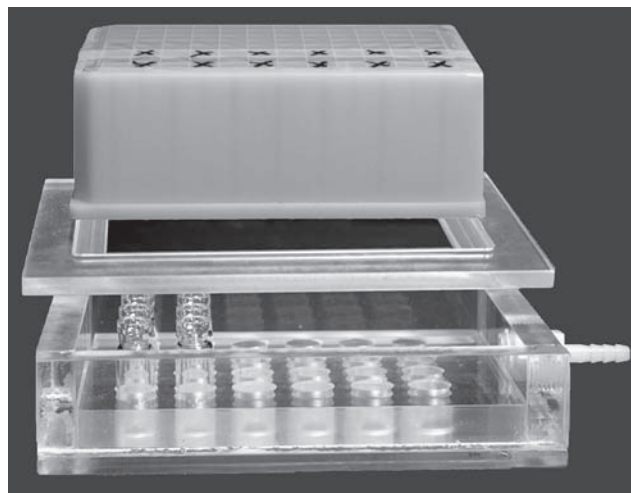


Figure 1—Apparatus for sample filtration: 96-well filter (sealing film in place), lid of positioning apparatus, and box with autosampler vial holder. Vacuum is applied through a side-port containing a hose barb.

top film is resealed after filtration by placing clear plastic tape across a row with open wells and marking the tape over the used wells (Fig. 1).

The sides of the filter holder and the autosampler vial holder are made from 12.7-mm (1/2-inch) stock; the bottom and top are made from 6.35-mm (1/4-inch) stock. The device is assembled as a bottom box with internal dimensions of 131.25 by 95.25 by 28.8 mm (5.17 by 3.75 by 1.13 inches) and external dimensions of 156.65 by 120.65 by 35.15 mm (6.17 by 4.75 by 1.38 inches). The depth of the box was chosen so that the filter drip tips could be positioned directly over the openings of the autosampler vials, ensuring proper alignment and no splash-over. The holes (12-mm, 0.47-inch) are drilled on center, as indicated on the autosampler vial holder. The vial holder can be glued to the bottom of the box, but leaving it free facilitates cleaning. The lid has an interior opening and routed interior edge (Fig. 1). The dimensions of the lid and vial holder are shown in Figure 2. A hole is drilled in the center of one end of the box and tapped to receive a hose barb fitting for attaching the vacuum line.

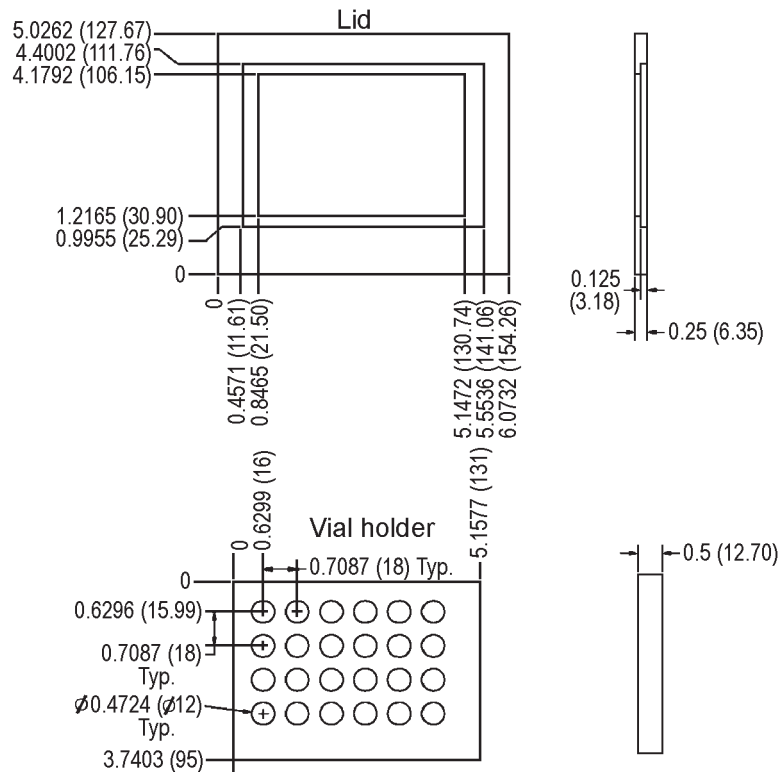


Figure 2—Dimensions of lid and vial holder. Measurements are in inches and millimeters (in parentheses).

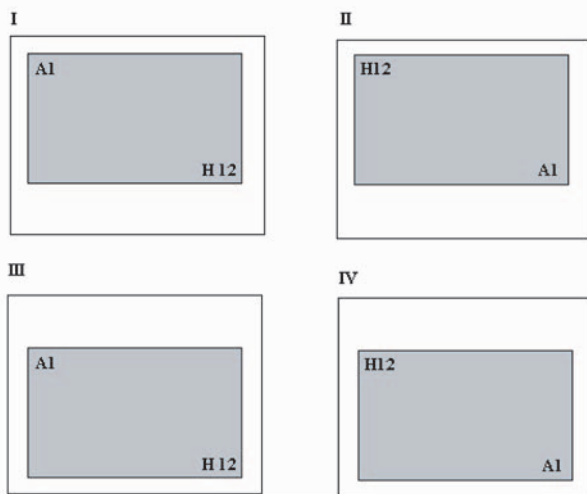


Figure 3—Configurations for positioning filtration device over autosampler vials. A1 and H12 refer to the first well in row A and last well in row H, respectively. In positions I and II, the sliding lid is set flush to top of apparatus; in positions III and IV, lid is set flush to bottom of apparatus. In position I, rows A, C, E, G, and odd-numbered columns are aligned with vials. In position II, filter has been rotated 180°; rows H, F, D, B, and even-numbered columns are aligned with vials. In position III, rows B, D, F, H, and odd-numbered columns are aligned with vials, and in position IV, rows G, E, C, A and even-numbered columns are aligned with vials.

Transparent material should be used in the construction of the bottom box to permit visual inspection of vial alignment and filtration system. The dimensions of the device allow for positioning the drip tips into the necks of the autosampler vials. If vials of different heights are used, the height of the vial holding apparatus must be adjusted so that the drip tips are properly positioned above the vials.

Figure 3 shows various configurations used to position the filtration device over the autosampler vials. Four different sets of 24 positions can be obtained by positioning the sliding lid and changing the orientation of the filter on the lid. The device can be used for sets of 24 or fewer samples.

Dispensing the samples and filtering them into the vials takes less time than using manual syringe filters. A set of 24 samples can be applied to the filter after centrifugation to remove debris. After the last sample is applied, filtration is completed in about 1 minute. If problems arise with a given sample, it can be removed from the filtration manifold and filtered with a syringe filter.

The cost savings depend on the relative cost of the multiple filtration manifold compared to the cost of syringe filters plus the syringe. In our procedures, this apparatus has saved well over \$0.60 per sample in direct costs in addition to reducing sample preparation time.