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A procedure for the micro-phase extraction of lipophilic drugs from biological fluids with low-density organic solvents

The use of small volumes of organic solvents for the extraction of lipophilic drugs from biological fluids in which the ratio (v/v), between the organic solvent and the aqueous phase is of the order of 1:50 to 1:100 ("micro-phase" technique) has important advantages over conventional extraction procedures in the case of many drugs (Ramsay & Campbell, 1971; Aggarwal, Bath & Sunshine, 1974). These include elimination of the time-consuming and destructive concentration of extracts by means of distillation techniques and the fact that "cleaner" extracts with less back-ground interference in chromatographic separations are obtained. Although the method has many other advantages in the extraction of a variety of drugs, it has not yet gained wide acceptance in drug analysis schemes. This is perhaps due to the inherent limitation of the method due to the necessity of using only solvents that are heavier than water, such as chloroform, carbon disulphide and carbon tetrachloride with relatively high polarities limiting their selectiveness in extraction.

To overcome this problem we have used successfully the simple and easy-to-construct apparatus depicted in Fig. 1 for the extraction of aqueous solutions with small volumes of low-density organic solvents. It may be constructed from an ordinary 10 ml glass-stoppered test tube by drawing out the bottom portion into a thick walled capillary tube (capacity approximately 100 μl). A thick walled side arm (3 mm internal diameter) is then attached as shown.

The capillary end is closed with a small plug (polythene tube) and the aqueous solution to be extracted (e.g. 5 ml of urine suitably buffered and saturated with respect to sodium chloride) is poured into the tube. The glass stopper is then replaced and the tube inverted as shown in Fig. 1. The required volume (usually 50–100 μl) of the organic phase is then injected through the open capillary end with the aid of a syringe and the unit shaken on a Vortex mixer for 3–5 min with the open capillary end pointing upwards. After centrifugation to separate the phases, a syringe filled with saturated aqueous sodium chloride solution is attached to the side

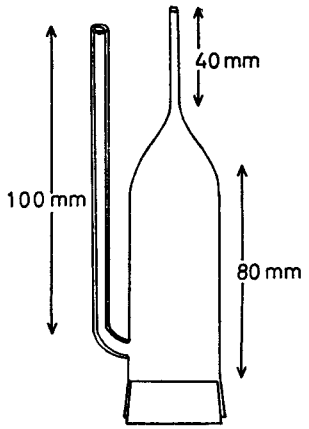

FIG. 1. Apparatus for the extraction of drugs with low-density organic solvents.

arm with the aid of a polythene tube and the sodium chloride solution slowly injected into the tube until the top of the fluid layer in the tube has risen into the capillary end. At this stage the organic phase is usually clearly discernible in the capillary tube. If necessary, the apparatus can be returned to the centrifuge and centrifugation repeated until all of the organic phase has accumulated in the capillary portion from where aliquots may be easily withdrawn with a suitable syringe for g.l.c. or t.l.c. analysis.

In our experience it is generally preferable to use heavier-than-water organic solvents such as chloroform and carbon disulphide for the micro-phase extraction of many drugs. However, in the analysis of samples with very low drug concentrations it is desirable to reduce background interference in chromatographic separations due to natural compounds present in biological extracts to a minimum and under these conditions it is often necessary to use the least polar extraction solvent that will still effectively extract the drug under consideration. With the possible exception of carbon tetrachloride, the polarities of the generally available high density organic solvents are not low enough for this purpose. We have used the apparatus in such situations with good results and have been particularly impressed with the results obtained using toluene or benzene for the extraction of a variety of drugs, typical examples being amitriptyline, chloroquine and pyrimethamine which have been extracted from alkaline aqueous solutions using toluene as micro-phase.

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