The recovery from water and from rat blood is reported in Table I. The lower recovery from blood suggests a possible protein-binding of the hydroxylated derivatives.

Preliminary studies indicate that blanks from extracts of rat blood or tissue do not show peaks that interfere seriously with a biological application of the gas chromatographic procedure.

The method described in this paper has the advantage over the previous methods of being simple and rapid. It also permits measurements of diazepam and its metabolites in the same sample.

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An accurate siphon for measuring fractions during column chromatography

A number of commercial fraction collectors employ a counterbalanced arm, which causes the turntable to rotate as it rises and falls. The arm is operated by the filling and emptying of a siphon attached to one end. This is a convenient system but the uniformity of the fractions produced depends entirely on the consistency of the siphon. The only form of siphon available commercially, as far as I know, is of type A1 (Fig. 1). Devices of this sort were described by NEDERBRAGT[1] and LIGON[2] but were first studied as an aid to column chromatography by BOVÉ[3]. Modifications intended for special purposes have since been reported[4,5] but BOVÉ's basic design has remained

unchanged. I have used several siphons of this type corresponding to the original specification[3] in essential details and these have all yielded relatively inconsistent results (see Table I).

There are two reasons for the poor performance of type A siphons. First, after each delivery, liquid will be left adhering to the walls of the siphon tube (d). This liquid tends to coalesce into a broken column which is partially displaced to the descending limb of the siphon tube as the main vessel (f) fills, and causes premature siphoning by exerting a small hydrostatic pressure downwards. The effective length of this column, and hence the error induced, varies from one delivery to another.

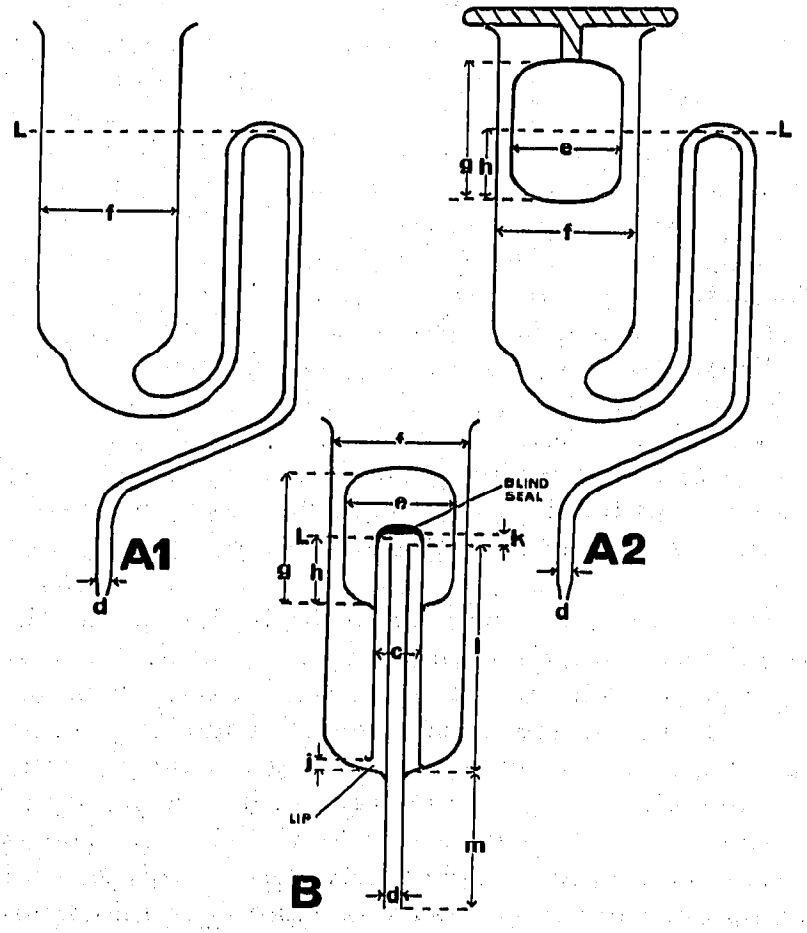

Fig. 1. Types of siphon investigated. In the text, each tube is identified by the letter used to indicate its diameter in these drawings.

This phenomenon is more noticeable with water than it is with liquids of lower surface tension such as acetone, and this is why acetone is siphoned slightly more consistently than water (Table I). This source of variation may be minimised by rigorous cleaning of the siphon, since the walls of a chemically clean siphon tube will drain away residual liquid so rapidly that it usually does not coalesce. In my experience, very rigorous cleaning agents are necessary; soaking in aqua regia (see Table I for conditions) is effective and does improve the consistency of type A siphons (Table I). Nevertheless, the 5 ml siphon tested, after cleaning in aqua regia, still

TABLE I

CONSISTENCY OF SIPHONS

For each test, the siphon was supplied with the solvent stated at a steady flow rate in the range 0.7–1.2 ml/min. Twenty consecutive fractions were collected and weighed to an accuracy of ± 5 mg. The maximum variation and standard deviation of each such group of results is given as a percentage of the average weight of the fractions delivered during that run.
Cleaning procedure: siphons were either (i) rinsed six times in the solvent being used or (ii) soaked for 24 h in 12 *N* hydrochloric acid–concentrated nitric acid (3 : 1, v/v), rinsed six times in tap water and then six times in the relevant solvent.

Type of siphon and nominal capacity	*Cleaning procedure*	*Solvent used*	*Maximum variation observed (20 deliveries)*	*Standard deviation (20 deliveries)*
A1, 5 ml	Rinsed	Water	±27.7%	±13.9%
	Aqua regia	Water	±13.0%	± 7.0%
	Rinsed	Acetone	±15.5%	±10.3%
A2, 5 ml	Aqua regia	Water	± 4.3%	± 2.7%
B, 1 ml	Rinsed	Water	± 1.2%	± 0.6%
B, 5 ml	Rinsed	Water	± 2.4%	± 1.0%
	Rinsed	Ethanol	± 0.3%	± 0.2%
	Rinsed	Acetone	± 1.9%	± 0.8%
	Rinsed	Xylene	± 0.6%	± 0.3%
B, 100 ml	Rinsed	Water	± 1.5%	± 0.9%

allowed a broken column of water to form twice during the first twenty operations, so such cleanliness only partially solves the problem. However, in the next paragraph, to simplify description, I shall temporarily assume that this source of variation has been eliminated.

The second source of error in type A siphons is the residual liquid left at the tip of the siphon tube. This will be bound by capillarity and form a liquid seal which must be displaced before siphoning can occur. The liquid in the main vessel does not therefore siphon over at level L but continues to rise until it exerts sufficient excess pressure to blow out the liquid seal. The level at which siphoning actually occurs varies from one operation to another, and this is the main source of inconsistency in clean siphons. This problem should not arise if the diameter of the siphon tube is increased towards its mouth as in some commercial siphons. However, this second source of error is unavoidable if the siphon tube contains much residual liquid from the previous delivery; *i.e.* the first source of inconsistency is always compounded with the second.

However, there is a very easy way of minimising the errors from both sources. If the cross-sectional area of the main siphon vessel is reduced, variations in the level at which siphoning occurs will cause smaller errors in the volumes delivered. For example, siphon A2 (Fig. 1), which has a cross-sectional area in the relevant region 33% of that of siphon A1, gives a standard deviation for water lower by a factor of 2.6 (Table I). Siphon A2 is identical to A1 except that a sealed tube (e), supported by a T piece, has been inserted into the main vessel. The difference between the external diameter of tube e and the internal diameter of tube f must be at least 0.15 cm if liquid is to run in freely. Though the reduction in cross-sectional area which may be

obtained in this way is limited, this is a convenient way of improving the performance of an existing type A siphon.

However, the main purpose of this account is to describe a new siphon which automatically blows residual liquid out of its siphon tube between each delivery and so avoids all the sources of inconsistency found in the conventional siphon. This modification, type B in Fig. 1, operates as follows. After each delivery, about two-thirds of the space between tubes c and d will be full of liquid and there will be the usual broken column present in the siphon tube, d. As the main vessel, f, fills up, liquid flows through the lip and displaces air from the top of c. The air, in turn, drives residual liquid down d and, by the time the level in the main vessel reaches the constricted region, tube d will be empty except for a single drop held at its tip by capillarity. The incoming liquid rises rapidly in the narrow space between e and f and its pressure is soon sufficient to blow out this last drop. More liquid is now free to pass into c, the level in the main vessel falls slightly, and siphoning will occur at level L. Siphoning ceases when air is drawn into c through the lip.

The blind seal at the end of tube c is essential to the success of this type of siphon. In its absence, organic liquids and sometimes even water, will continuously trickle down the internal wall of the siphon tube, instead of siphoning over as a solid column, and so defeat the whole purpose of the device. The blind seal prevents this by providing a plate of glass immediately above the entrance to the siphon tube. Liquid clings to this plate; its meniscus is therefore presented to the siphon tube from above, not from below as previously, and this initiates correct siphoning.

In order to prove the complete reliability of B type siphons, the 5 ml version was used to deliver 550 fractions of aqueous buffer and, later, 1104 fractions of acetone, which is particularly liable to cause siphon failure in the way just described because of its low viscosity and surface tension. Flow rates were 0.3 ml/min and 1 ml/min, respectively. The siphon operated perfectly throughout, and there was no failure of any kind.

Type B siphons, as expected, do give consistent results with both water and organic solvents (Table I). Indeed their performance with ethanol is remarkable since the residue left in the 5 ml siphon after each delivery (about 0.7 ml) cannot have varied by more than $\pm$ 15 μl during the test period. Consistency is maintained over long periods. The 5 ml siphon has been used to receive the aqueous effluent from an ion exchange column used for separating amines, each separation taking three days and requiring the collection of 300 fractions. During five runs, the relative position of the ammonia peak in the series of fractions only varied by $\pm$ 0.9% and that of the tyramine peak by $\pm$ 0.5 %. Moreover, the siphon would only have been responsible for part of these small variations. The most valuable feature of type B siphons is their ability to give such consistent results without special cleaning and, indeed, without attention of any sort. The mean volume delivered by one of these siphons should be independent of the liquid being used. Mean volumes delivered by the 5 ml siphon were: water, 4.91 ml; ethanol, 4.82 ml; acetone, 4.84 ml; xylene, 4.90 ml; variation, $\pm$ 0.045 ml, *i.e.* $\pm$ 0.9 %.

B type siphons are more compact, possibly slightly more robust, than the conventional sort and they deliver their liquid rather more rapidly. Emptying times for the siphons investigated, measured with water, were: 5 ml A1, 9 sec; 1 ml B, 2.5 sec; 5 ml B, 7 sec; 100 ml B, 35 sec.

The one disadvantage of B type siphons is that they retain a considerable volume of residual liquid after each delivery, most of it between tubes c and d. This residue is efficiently siphoned over during the next operation but, inevitably, represents a transfer of material from the first fraction to the succeeding one. The magnitude of this cross contamination is given in Table II. This factor is usually of little practical importance, but with small siphons (1 or 2 ml) and a column effluent containing comparatively sharp peaks, it will cause appreciable distortion of these peaks. Attempts to reduce the degree of cross contamination by progressively decreasing the volume between tubes c and d, lead, first, to inconsistent deliveries, and finally, to a siphon which will not operate at all. In some circumstances an A type siphon, which causes very little cross contamination, may be a better compromise.

Type B siphons delivering less than 1 ml are very difficult to construct and will probably never be useful, but there are no other obvious size restrictions. Siphons made to the dimensions given in Table III will usually deliver somewhat less than the nominal volume indicated. The delivery volume is most conveniently adjusted to the exact value required by blowing out the lower part of the side wall of tube f. In designing this type of siphon, the following limitations should be observed. The internal

TABLE II

CROSS CONTAMINATION OF FRACTIONS DUE TO USE OF SIPHONS

Each siphon tested was rinsed, then completely filled, with 1.0% aqueous potassium dichromate. The siphon was then supplied with pure water at a flow rate of 1 ml/min, and the dichromate present in each of the fractions was determined colorimetrically at 495 mμ. The figures given represent the concentration of dichromate in fractions 2, 3 and 4, taking the concentration of solute in fraction 1 (the original solution) as 100%.

Type of siphon and nominal capacity	*Percentage of solute transferred from fraction 1 to:*		
	Fraction 2	*Fraction 3*	*Fraction 4*
A1, 5 ml	2.9%	> 0.1%	—
A2, 5 ml	3.9%	> 0.1%	—
B, 1 ml	39.5%	18.8%	> 0.1%
B, 5 ml	14.2%	1.4%	> 0.1%
B, 100 ml	2.6%	> 0.1%	—

TABLE III

DIMENSIONS OF SIPHONS TESTED

The siphons were all constructed of borosilicate glass and dimensions are given in centimetres. Int = internal diameter; Ext = external diameter.

Type and nominal capacity	*c (Int)*	*d (Ext)*	*d (Int)*	*e (Ext)*	*f (Int)*	*g*	*h*	*j*	*k*	*l*	*m*
A2, 5 ml			0.18	0.80	0.98	4.0	2.0				
B, 1 ml	0.48	0.38	0.20	0.63	0.83	4.0	2.0	0.25	0.20	4.8	4.2
B, 5 ml	0.53	0.38	0.20	0.99	1.14	4.0	2.0	0.2	0.15	7.2	3.9
B, 100 ml	0.86	0.64	0.25	2.96	3.28	4.0	2.0	0.3	0.20	12.3	11.5

diameter of tube d should be in the range 0.2–0.25 cm; if smaller than this, liquid is held in the bore too strongly by capillarity and may not be blown out properly between deliveries; if larger there will be some risk of siphon failure due to liquid trickling continuously down the wall. A siphon tube of 0.4 cm internal diameter has been successfully used to obtain a high delivery rate when siphoning water, but a tube of this size is only suitable for special purposes. Dimension l may be adjusted to any convenient value and dimension m may be varied from 4 to 11 cm. The volume of the space between tubes c and d should be approximately 2.5 times the volume of the bore of tube d, to ensure that this is thoroughly blown out between deliveries. However, any increase in the ratio of these volumes will augment the amount of cross contamination caused by the siphon. Tube f may be of any convenient size but the difference between the internal diameter of tube f and the external diameter of tube e should not be less than 0.15 cm if liquid is to flow freely into the siphon. Increasing the clearance beyond this causes some loss of consistency. In siphons delivering less than 5 ml, tube c will be too large to fit inside e. The blind seal must then be made in tube e, at the appropriate level. Dimensions g and h are not critical, and the values given may be altered by $\pm$ 0.3 cm. Dimension k should be in the range 0.05–0.2 cm to ensure that the blind seal really induces correct siphoning. Finally, the lip should be an approximately square indentation in the wall of tube c, having an area of about 5 sq. mm. If the lip is too small, it will not allow air to be drawn into tube c at the end of the first delivery; from then on the siphon will be useless, because incoming liquid will be siphoned over continuously, instead of being delivered in accurately measured portions. A very large lip should also be avoided since, after siphoning has ceased, this allows the residual liquid from tube c to run back into tube f, and increases the extent to which the first fraction mixes with the succeeding ones.

This type of siphon is certainly well worth constructing, since it will give very consistent results with water and organic solvents, yet, unlike the conventional siphon, needs no special cleaning.

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