

● *Note*

AN INEXPENSIVE AND PRACTICAL AMBIENT-TEMPERATURE VACUUM STILL FOR BIOASSAYS

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INTRODUCTION

IN MEDICAL and academic radiation safety programs, researchers and clinicians often use biochemicals labeled with various radionuclides, including ^3H (tritium) and ^{14}C . While there is a vast amount of literature on the behavior of water in the human body, and on urinalysis for determination of exposure to ^3H in the form of water or H gas (Balonov et al. 1974; Brodsky 1983; NCRP 1985; Sanders and Reinig 1968), there is less information available concerning the bioassay of ^3H when it is in organic forms (Balonov et al. 1984; Brodsky 1983; Etnier et al. 1984; Gerber et al. 1986; NCRP 1979a; NCRP 1979b; Smith et al. 1983; Travis et al. 1984).

Labeled biochemicals have different retention functions than that of water. To better understand the dosimetry of ^3H -labeled biochemicals, we needed to determine the fraction of ^3H as water (or other volatile compound) and as non-volatile compounds (e.g., proteins) in urine and blood serum (Bohner 1986; Strom and Bohner 1986; Strom and Morse 1975). Note that these fluids are cell-free, aqueous solutions, so that we did not need to be concerned with intracellular water. We did not evaluate the suitability of the technique presented here for assay of materials containing cells.

We considered the azeotropic distillation method (Moghissi 1981; Moghissi et al. 1973) and rejected it for several reasons. The first and foremost reason was that some of our samples were as small as $35\ \mu\text{L}$, and recovery of volumes this small from the evaporator side of an azeotropic still was judged to be problematic. Sample sizes required for the azeotropic distillation method ranged from 20 to 200 g; ours were as small as 0.035 g. Second, we wanted to have complete recovery of *both* the volatile and non-volatile fractions, including biochemicals that might be soluble in the aromatic solvents benzene, tolu-

ene, xylene, or cyclohexane used in the azeotropic distillation method. In particular, we were concerned with leucine and its ketone metabolites, known to be soluble in benzene, toluene, xylene, and cyclohexane. Third, the higher temperatures in azeotropic distillation (69°C for benzene, 94.5°C for xylene, 81.4°C for cyclohexane) were judged to be unacceptable because they might result in the decomposition of tritiated biochemicals, perhaps into volatiles. Fourth, given the large number of samples we had to process, we wanted a technique that required less than the 2-4 h per sample required by azeotropic distillation (Moghissi et al. 1973). Finally, we did not want to introduce the additional steps of dealing with solvents and transfers of samples to and from glass vessels other than the liquid scintillation vials that would ultimately be used for sample quantitation, nor did we see a need for the expense of purchasing and disposing of azeotropic solvents, even such relatively safe ones (Moghissi 1981) as cyclohexane.

The remaining alternative was to use ambient-temperature vacuum distillation. Since we completed this work, an alternative reduced pressure distillation method has been published (Winshell and Ertl 1986). While that method is less capital-intensive, it involves more sample handling and more consumables. Furthermore, it is not clear from that publication what fraction of ^3H -water is actually recovered.

To satisfy our distillation needs, we designed, refined, and constructed a number of inexpensive, simple, and practical stills for ambient-temperature vacuum distillation of small volumes of bioassay fluids. This note describes those stills and results obtained from the method. The note does not include an intercomparison of our results with alternative methods, since our method showed quantitative recovery of tritiated water as well as activity in raw samples.

DESIGN CONSIDERATIONS

As implied earlier, one objective of our still design was to be able to distill samples with a minimum of sample handling. We settled on a design wherein the liquid to be distilled is placed in a liquid scintillation vial that serves as the evaporator, with another liquid scintillation vial serving as the condenser. This design eliminates sample transfers to and from the distillation apparatus, thereby avoiding potential loss of sample.

Another design objective was to produce the stills at little cost, using materials readily available in a typical radiation safety office, and using simple laboratory techniques. The resultant design performed satisfactorily in hundreds of distillations.

THE VACUUM SYSTEM

The vacuum system consists of a fore- or roughing-vacuum pump connected to a simple oil trap to prevent backstreaming of vacuum pump oil into the apparatus (Fig. 1). The oil trap is a 1 L thick-walled vacuum flask with a rubber stopper penetrated by a glass tube. The trap is connected to the vacuum pump using large-diameter thick-walled vacuum hose. A mechanical vacuum gauge (0 to 76 cm Hg) and a thermocouple vacuum gauge are connected to the oil trap.

The input to the oil trap is connected via 6-mm ID rubber hoses to glass tees that split the hose in two. Each fork is connected to another fork, resulting in four vacuum lines. Each rubber hose is terminated by a 5-mL disposable plastic syringe with the plunger removed and the open end cut off so that it can be inserted into the hose. The syringe tips are tapered Luer-Lok* types common to disposable syringes. The Luer-Lok connectors can be inserted in disposable three-way valves† used in intravenous tubing connections. The use of Luer-Loks provides quick connection and disconnection, and the three-way valves permit bleeding individual stills up to atmospheric pressure without affecting the rest of the vacuum system.

THE STILL

Each distillation apparatus consists of a 10-mm OD glass tube bent in a "U" shape, with plastic liquid scintillation vial caps at each end (Fig. 2). Each cap has a 10-mm (slightly more than $\frac{3}{8}$ " hole drilled in its center, and the cap on the condenser end has three additional small holes of 1.6 mm ($\frac{1}{16}$ " for the needle and two heater wires (Fig. 3). It is necessary to remove the cap liners (gaskets) and the glue that holds them in place. Since the cap liners are about 1 mm thick, it is necessary to remove 1 mm of plastic from the bottom of the cap to prevent it from coming in contact with the shoulder of the liquid scintillation vial, which may crack the vial.

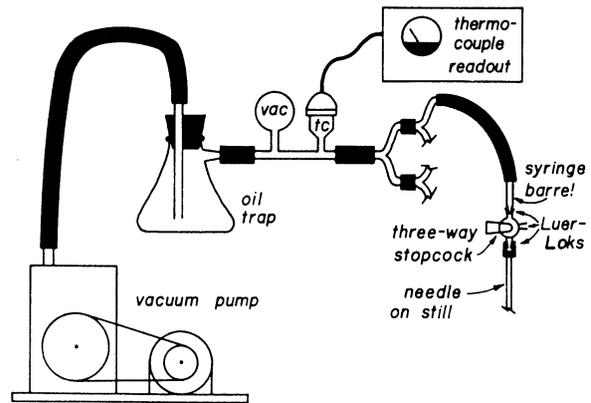


Fig. 1. A roughing-vacuum pump is connected to an oil trap and to mechanical ("vac") and thermocouple ("tc") vacuum gauges. The vacuum manifold terminates in four rubber hoses connected to disposable plastic syringe barrels. The Luer-Loks on the syringe barrels mate with disposable plastic three-way valves. The Luer-Lok on the valve is connected to the distillation apparatus.

The glass tube penetrates the evaporator cap less than 1 cm, to minimize the transfer of material by splattering if bubbles, which form during distillation, break in the evaporator. The glass tube penetrates 4 cm on the condenser side to deliver water vapor deep into the vial, where it has maximum contact time with the cold vial surface.

To prevent condensation in or on the glass tube, it is necessary to heat the tube electrically. The heater consists of about six turns of 26-gauge nichrome wire on the inside of the condenser vial and three or four turns above the vial. We use a low-voltage (0–12 V), constant-current (0–5 A) DC power supply to run the heaters, which are wired in series when several stills are in use. A power level of about 1 W suffices to keep the tube free of frost.

Evacuation is done through a 7.5 cm long, 18-gauge needle glued into the condenser end of the still, using quick-setting epoxy glue. The needle penetrates only a few millimeters into the vial, so that exhausting is done at the top of the vial. The long, narrow capillary-like needle is used to limit pumping speed and to minimize possible cross-contamination between stills. Slow evacuation is necessary to ensure that all water vapor is condensed rather than exhausted. Smaller-diameter needles, even significantly shorter ones, were found to pump too slowly to be useful. Needles that are much larger in diameter result in significant loss of sample.

After thorough cleaning and degreasing, still parts are assembled with quick-setting epoxy glue (Fig. 3). The glue is carefully mixed to minimize the formation of air bubbles that can pose decontamination or leakage problems. The heater wire is glued to the entire length of the tube around which it is coiled to maximize thermal conductivity. Failure of the glue due to excessive heat buildup may be observed when the apparatus is evacuated but not chilled in the alcohol-dry ice bath.

* Luer-Lok is a registered trademark of Becton Dickinson and Company, One Becton Dr., Franklin Lakes, NJ 07417-1880.

† Medex, Inc., "3-way stopcock," Hilliard, OH 43026.

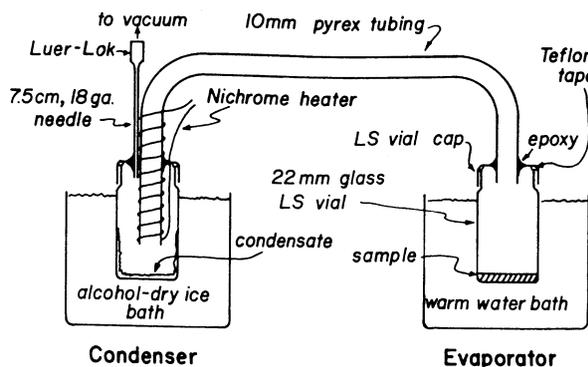


Fig. 2. The distillation apparatus consists of a length of pyrex tubing with liquid scintillation vial caps glued on each end. A 20-mL, 22-mm thread glass liquid scintillation vial is screwed on each end of the still. The bioassay sample to be distilled is placed in a water bath on the evaporator side. A nichrome wire heater prevents condensation on the still on the condenser side. The condenser sits in an alcohol-dry ice bath and is slowly evacuated through the 18-gauge needle. Distillation takes 10 to 30 min.

OPERATION

Two liquid scintillation vials (20 mL capacity, 22-mm threads, low-background glass) are used for each distillation run. The sample to be distilled is placed in one vial. Teflon[‡] thread-sealing tape, 12.7 mm ($\frac{1}{2}$ in.) wide and twice the vial circumference long (about 10 cm), is folded in half and wrapped around the top and sides of each vial, for two full turns. This results in four layers of Teflon tape on the top surface of the vial (Fig. 3). It is important not to get the tape on the threads, since the added diameter of the tape can cause the plastic vial caps to crack.

It is not necessary to screw the vials into the caps very tightly, since the Teflon tape flows to make a vacuum seal. We tried using O-rings instead of Teflon tape, but these were not successful in maintaining a vacuum seal.

When the vials have been attached to the still, it is lowered into temperature-controlled baths. The evaporator is placed in water at 20 to 25°C, and the condenser in an alcohol-dry ice bath at about -60°C. The condenser is allowed to cool for several minutes before applying vacuum. During this time, a vacuum line is connected to the Luer-Lok, and power supplied to the heater leads. With the valve closed, the vacuum system is started and pressure gauges checked. When the vacuum drops to 130 Pa (1 torr) or below, the valve is opened.

Within seconds, bubbling is observed in the evaporator vial, caused by the evolution of dissolved gas. If the evaporator vial is not in lukewarm water, the contents will soon freeze, and distillation (i.e., lyophilization) can take hours if this occurs. If the vial is maintained at room

temperature, however, the sample can be pumped to dryness in 5 to 10 min. We generally run distillations for 20 to 30 min to ensure dryness. The thermocouple vacuum gauge usually reads in the range of 40 to 130 Pa during distillations.

Typically, four stills are operated simultaneously using a single water bath and a single alcohol-dry ice bath. To prevent cross-contamination, each apparatus is brought to vacuum with the others isolated from the system. After each system has been evacuated, all valves are reopened.

After distillation, the dried, non-volatile residue in the evaporator vial is rehydrated for liquid scintillation counting. If the sample was urine, rehydration is done using 1 mL of dilute HCl at pH 2 to prevent precipitation of the dissolved solids. If the sample was blood serum or plasma, 1 mL of water is used. Liquid scintillation fluid is added directly to the vials and they are capped and counted.

Occasionally, violent bubbling is observed in the evaporator, resulting in droplets being carried into the apparatus. In such cases, it is necessary to rinse the still with small volumes of water or dilute HCl and include these in the residue vial. Blood serum and plasma samples that had been warmed to room temperature seemed to bubble less violently than those that were cold. The bubbling is apparently dissolved gases coming out of solution, rather than boiling.

The stills show consistent recovery of $100.1 \pm 1.5\%$ for nine quality assurance runs using tritiated water. When identical bioassay samples were distilled side by side, residue activities were generally within 5% of each other, that is, within the overall error of pipetting, dilution, and counting statistics. Redistillation of a rehydrated residue shows no significant quantity of volatile activity is trapped in the residue following distillation.

We compared the ratio of raw sample specific activity to the sum of distillate and residue specific activities in

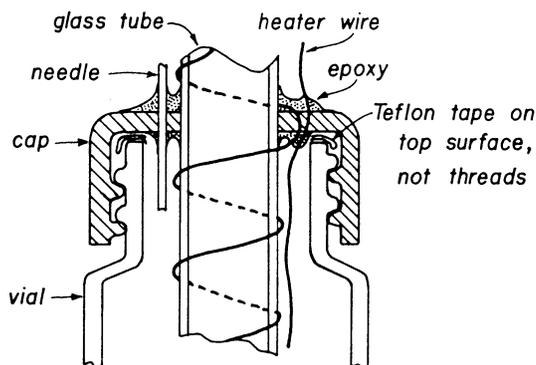


Fig. 3. The details of the condenser end are shown. Teflon thread sealing tape is applied to the top of each vial to make a vacuum seal. The quick-setting epoxy seals the vacuum feed-throughs and provides structural strength. The heater wire is glued to the glass tube at all points to enhance heat transfer to the glass tube.

[‡] Teflon is a registered trademark of E. I. du Pont de Nemours and Company, Inc., 1007 Market St., Wilmington, DE 19898.

Table 1. Results of distillations of urine and serum from a beagle dog and rats fed ^3H -leucine.^a

Animal	Feeding	N	(RAW)/(RESIDUE+DISTILLATE)				(RES./DIST.)	
			Avg.	Std. Dev.	Min.	Max.	Min.	Max.
Urine								
Beagle	Acute	94	1.012	0.081	0.780	1.182	0.038	16.3
Beagle	Chronic	58	0.989	0.057	0.800	1.172	0.086	21.2
Rat 1	Acute	29	0.981	0.067	0.783	1.145	0.049	31.6
Rat 3	Acute	45	1.015	0.112	0.754	1.354	0.038	14.9
Weighted Sub Totals		226	1.003	0.079				
Serum								
Beagle	Acute	71	0.940	0.079	0.756	1.157	0.46	2.28
Beagle	Chronic	17	0.916	0.047	0.829	1.000	0.78	1.59
Rat 1	Acute	20	1.037	0.077	0.927	1.265	0.32	0.54
Rat 3	Acute	31	0.969	0.090	0.710	1.114	0.32	1.28
Weighted Sub Totals		139	0.957	0.077				
Both Fluids								
Weighted Totals		365	0.985	0.078				

^a The first five columns containing numbers refer to the ratio of ^3H specific activity in the raw fluid to the sum of the ^3H specific activities in the residue and distillate of aliquots from the same sample (N is number of comparisons; Avg. is arithmetic mean; Std. Dev. is the standard deviation; Min and Max are minimum and maximum values, respectively). Specific activities ranged over four orders of magnitude. The last two columns give the range of residue to distillate ratios which the particular experiment covered. For example, the first urine sample collected from Rat 3 had 31.6 times as much activity in the residue as in the distillate, while a month later, there was less than 1/20 the activity in the residue as in the distillate, spanning a range of over 600 to 1. Sub Totals are column subtotals weighted by N.

226 urine samples and 139 serum samples, as shown in Table 1. The samples were from a beagle dog and rats fed ^3H -leucine, either in a single feeding or a series of chronic feedings. Absolute standardization for each sample was done by internal standardization.

For urine samples, the average ratio of (raw/[residue + distillate]) for all 226 trials was 1.003 ± 0.079 (Table 1). The ratio of activity in residue to that in distillate varied from 31.6 down to 0.38, or over 100-fold, in samples taken within hours of administration to months later. The ratios were always within one standard deviation of unity, with variability due to pipetting and counting sta-

tistics. There was a slight, statistically insignificant trend to lower (raw/[residue + distillate]) ratios with higher (residue/distillate) values in dog, but not in rat.

For serum samples, the average ratio of (raw/[residue + distillate]) activities for all 139 trials was 0.957 ± 0.077 (Table 1). The ratio of activity in residue to that in distillate varied from 2.28 down to 0.32, or over sevenfold, in samples taken within hours of administration to months later. The average for 88 dog samples was 0.935 ± 0.073 , while the average for 51 rat samples was 0.996 ± 0.085 . In retrospect, we believe that this may be a systematic difference, that is, the dog serum ratios may be systematically low, attributable to the fact that raw rat serum samples (usually 35 to 100 mg) were diluted with 1 mL of deionized water before counting, whereas the raw dog serum samples (usually 200 mg) were not diluted with water before counting. In serum distillations, there was no trend in the ratio of (raw/[residue + distillate]) activities with (residue/distillate) values in either species.

CONCLUSIONS

The simple, practical vacuum stills that we have designed, built, and tested can be made at reasonable cost in ordinary shop or laboratory facilities using materials readily available in the medical and academic environment. Operation requires dry ice or an equivalent cold bath, a low-voltage power supply, and a vacuum system. Sample handling and recovery problems are minimized by using liquid scintillation vials as part of the distillation apparatus. The stills are useful for determining what fraction of ^3H in a bioassay sample is found as water and what fraction is found as non-volatile materials. Sample recovery is consistently very good, as demonstrated by 375 trials of urine and serum distillations from rats and beagle dog that had been fed ^3H -leucine.

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