IN THE LABORATORY

An Apparatus for the Quantitative Separation of Volatile Substances by Fractionation and Distillation

W. RILEY MCGAUGHRAN and S. C. WERCH

Medical Research Division, Plough, Inc., Memphis, Tennessee

An apparatus that has proved useful for the quantitative separation of small quantities of volatile materials in complex pharmaceutical products is here described. As shown in Fig. 1, the apparatus is a combination unit composed of a fractionating column joined to a condenser by way of sealed glass connections and provided with standard ground-glass joints at the distillation and condenser ends. A small, sealed-in well at the top of the fractionating column, when filled with water, serves to increase the reflux at this point and offers a convenient place in which to insert a small thermometer for measuring the approximate distilling temperature. A 125- or 250-ml. iodine flask (⅋22 ⅋ neck) serves as the distillation flask, while a long-stemmed adapter and a long-necked receiving vessel (cooled with ice) are employed at the condenser end. Ceramic Berl Saddle column packing (6 mm.) is employed in the fractionating column.

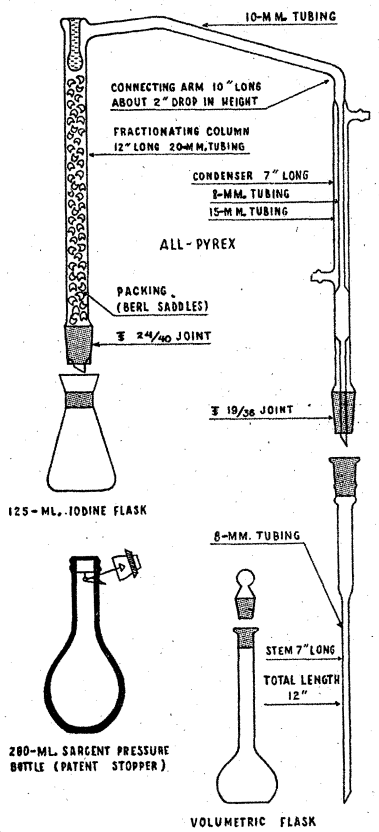

FIG. 1

Before operation of the apparatus, the flared mouth of the iodine flask is filled with water or some appropriate sealing fluid, and the lower tip of the adapter is submerged about ¼ inch in the solvent or reagent placed in the receiving vessel. The ground-glass joints, of course, should be wet before being connected.

The efficiency of the apparatus is largely dependent on the partial vacuum produced during distillation when an appropriate solvent is provided in the receiving flask. This vacuum, caused by the reduction of vapor pressure obtained at the surface of the solvent, is sustained until the adapter is disconnected. As long as ebullition is taking place at a fairly constant rate, an adequate partial vacuum is maintained. Bumping in the distilling flask, prevented mainly by the use of glass beads, etc., causes small variations in the partial vacuum, but the enlarged upper portion of the adapter acts as a safety valve in preventing the distillate from rising too high.

The apparatus has the following advantages: (1) Rapid and quantitative separation of volatile components is obtained. (2) Losses are at a minimum, due to the partial vacuum obtained during distillation, absence of joints in connecting arm, and air-tight connections at distillation and receiving ends. (3) Small amounts of volatile components can be recovered. (4) Standard taper connections permit the use of interchangeable flasks and adapters.

This apparatus has been successfully employed for over two years for the quantitative separation of alcohol, alcohol and ether, and chloroform from pharmaceutical products.

Stability of Penicillin in Glycerin and in Glycols

R. J. FERLAUTO and H. A. CLYMER

Smith, Kline & French Laboratories, Philadelphia

Penicillin stability in mixtures containing glycerin (*2, 4*), propylene glycol (*5*), or Carbowax (*1, 3*) (a solid, water-miscible polyethylene glycol) may be less than recent reports would indicate. Since these compounds are major components of such mixtures, penicillin stability in glycerin or glycols alone was determined in order to prognosticate, if possible, stability in preparations containing them.

The activity of commercial calcium (CaP), sodium amorphous (NaP), and sodium crystalline (NaP cryst.) salts[1] was determined, before and after storage at 5°, 23°, and 37°C., in glycerin, propylene glycol, and Carbowax,[2] by the Food and

[1] Pfizer, Heyden, and Commercial Solvents Corporation, respectively.

[2] Glycerin USP; propylene glycol and Carbowax 1500, Carbide and Carbon Chemicals Corporation.

Drug Administration agar diffusion technic for solutions (*6*), using the F.D.A. standard penicillin.

As indicated in Table 1, the order of stability at 5°C. is consistently NaP > NaP cryst. > CaP. CaP and NaP cryst.

TABLE 1

STABILITY OF PENICILLIN SALTS IN UNDILUTED VEHICLES

Vehicle	Penicillin salt	Storage temp. (°C.)	Percentage of activity after storage						
			2 hrs.	1 day	2 days	4 days	7 days	13 days	19 days
Glycerin	CaP	5	1	0					
	NaP cryst.	5	2.5	0.7	0				
	NaP	5	14.5		0.1				
Propylene glycol	CaP	5	1.5	0					
	NaP cryst.	5	4	0.05					
	NaP	5	81	67			21	6	
Carbowax	CaP	5	80	27		16	11	2	
	NaP cryst.	5	102	100		96	90	81	69
	" "	23	102	60		44	30	10	4.7
	" "	37	102	55		18	10	0	
	NaP	5	103	104		98	98	98	92
	"	23	103	100		96	63	38	28
	"	37	103	86		47	23	2.5	

were almost totally and immediately inactivated in glycerin or propylene glycol, while the rate of inactivation was much less in Carbowax; this was qualitatively true for NaP as well, although inactivation was slower than with the other salts.

In check experiments it was found that absolute (but not relative) inactivation rates varied with the commercial source

TABLE 2

STABILITY OF PENICILLIN SALTS IN FRACTIONS OF PROPYLENE GLYCOL DISTILLATION

Portions	Penicillin salt	Percentage of activity after storage at 5°C.							
		2 hrs.	1 day	2 days	10 days	16 days	29 days	38 days	65 days
I	CaP	80	38	15					
	NaP cryst.	93	91		53	14	8	4	
	NaP	98	100		92	90	81	83	68
II	CaP	50	7	3					
	NaP cryst.	98	101		69	35	23	11	
	NaP	95	100		92	92	91	89	82
III	CaP	0							
	NaP cryst.	2	0						
	NaP	31	20	5					

of glycols, and even with different lots from the same manufacturer. Propylene glycol was accordingly distilled, and penicillin stability determined in three portions: I (the first 25 per cent condensed), II (the next 50 per cent), and III (the residue).

All penicillin salts are more stable in I and II and less stable in the residue than in undistilled propylene glycol. In all fractions the stability order is that derived from Table 1.

Our experience is that penicillin stability varies not only with the vehicle and with the particular salt used, but also with the lot of vehicle, whether or not from the same manufacturer. Impurity may be responsible for the latter type of variation. Despite these fluctuations we have found sodium penicillin amorphous most stable and the calcium salt least stable in glycerin and glycols.

Information on penicillin stability is so meager, and in some cases the inactivation so rapid, that assay of the individual preparation is mandatory.

References

1. ANDREWS, G. C. *Bull. Hudson Cy. med. Soc.*, 1945, **27**, 6.
2. LEFFINGWELL, G., and LESSER, M. A. *Med. Rec.*, 1946, **159**, 291.
3. MOHS, F. E. *Arch. Surg.*, 1946, **52**, 466.
4. PARNABY, R. C. *Pharm. J.*, 1946, **156**, 55.
5. SENGER, F. L., WARRES, H. L., and RIFKIN, I. *J. Urol.*, 1946, **55**, 138.
6. ———. *Fed. Reg.*, September 8, 1945, 11478.

A Simplified Method of Preparing Active Extracts of B-Glucuronidase[1]

WILLIAM H. FISHMAN and PAUL TALALAY[2]

Departments of Surgery and *Biochemistry, University of Chicago*

The first procedures published (*3*, *4*) for the preparation of active extracts of β-glucuronidase were tedious and often proved to be unsatisfactory. By employing a new method (*1*) of obtaining the enzyme from beef spleen, potent highly purified extracts could be prepared with much less difficulty. However, in two steps of the process (acetone precipitation, evaporation of extracts in an air current) there was danger of loss of activity if a certain amount of care was not employed. Another disadvantage was the need for somewhat lengthy large-scale manipulations. For the past several years, one of us (F.) has routinely employed a simplified procedure for preparing small amounts of the enzyme from rat and mouse organs which does not have the disadvantages of the previous method noted above. Extracts of β-glucuronidase prepared in this way have been used in studying the kinetics of the hydrolysis of phenolphthalein glucuronide (*5*).

Recently, interest has been shown in the use of β-glucuronidase preparations in preference to strong mineral acid for hydrolyzing steroid glucuronides (*6*) in order to avoid destruction of steroid and prevent the production of artifacts (*2*). Accordingly, it was felt desirable at this time to report the method of preparation now in use and to present some data on the degree of purification achieved.

Six rats were killed by a blow on the head, and blood was permitted to drain from the carotids. The livers, kidneys, and spleens were rapidly dissected out. These were pooled separately and were homogenized with 100 cc. of cold water in a Waring blender or in a glass homogenizer, and the homogenate strained through cheesecloth (Step 1). The mixture was acidified to pH 5.0 with 1.0 N acetic acid and kept at 38° for 30 minutes. The proteins which flocculated out were removed by rapid centrifugation (20 minutes at 3,500 r.p.m.), and the supernatant separated and preserved. To this fraction was added an equal volume of saturated ammonium sulfate, the mixture then being centrifuged for 30 minutes. The super-

[1] Aided by a grant from the Otho S. A. Sprague Memorial Institute.

[2] Present address: Yale University School of Medicine, New Haven, Connecticut.