A COLLABORATIVE STUDY OF AN ASSAY PROCEDURE FOR CAMPHORATED TINCTURE OF OPIUM, U. S. P.*

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INTRODUCTION.

A comparative study of certain assay procedures for Camphorated Tincture of Opium by Bliss and collaborators (1) demonstrated the decided superiority of one method. The results of this study were presented as a "Report of the Group Committee on Revision, Sub-Committee 6, June 1931" (2). The procedure which yielded the most satisfactory results has been published separately (3).

A critical study of the methods employed by Buchbinder (4), Caines (5), Eaton (6), Kippenberger (7), Puckner (8), St. John (9), Warren and McClosky (10), Warthle (11) and the American Drug Manufacturers' Association, Sub-Committee on Alkaloids and Drug Standards (12), and the results obtained by collaborative work, herewith reported, led to the conclusion that the following method is the most satisfactory and practical procedure for this preparation of opium:

THE METHOD.

To 100 cc. of the sample add 2 cc. of approximately 0.5 *N* sulphuric acid and evaporate the mixture on the steam-bath to a volume of about 10 cc. Transfer the residue to a separator. Wash the evaporating dish twice with approximately 0.5 *N* sulphuric acid using 10 cc. each time and adding the washings to the separator. If necessary, wash the dish with several cc. of a mixture consisting of 85 volumes of chloroform and 15 volumes of alcohol, adding these washings to the separator also. Add about 9 Gm. of sodium chloride and carefully neutralize the solution by adding stronger ammonia by drops, finally adding 8 drops in excess. Add 30 cc. of a mixture consisting of 85 volumes of chloroform and 15 volumes of alcohol. Shake the mixture and then allow it to stand until a complete separation is obtained. Separate the immiscible solvent and run it into a separator (No. 2). Repeat the extraction of the alkaline solution with successive portions of 30, 20 and 20 cc. of the solvent mixture, collecting the extracts in separator No. 2. Test a few drops of the 4th extraction for alkaloids. Both in the initial and final extractions of morphine with organic solvent, test the residue remaining on evaporation of several drops of the 4th shake-out—after evaporation—with Marquis' Solution.[1] *If necessary, repeat the extractions until a negative test is obtained.* In that event, however, increase the quantities and volumes of all subsequent reagents so as to maintain the proportions prescribed.

Add 15 cc. of alkaline salt solution[2] to separator No. 2. Extract the morphine from the chloroform-alcohol solvent by shaking, using 3 successive portions of 15, 10 and 10 cc. of the alkaline solution, respectively, and collecting the extracts in separator No. 3. Wash the combined alkaline salt solution with 10 cc. of chloroform and discard the chloroform. Exactly neutralize the alkaline salt solution by adding hydrochloric acid drop by drop, finally adding 1 cc. in excess. Cool the solution under the faucet and shake it with 10 cc. of chloroform. Remove the chloroform to another separator (No. 4) and shake it with 5 cc. of saturated salt solution to which 3 drops of hydrochloric acid have been added. Discard the chloroform in the fourth separator and add the acid salt solution to the solution in the third separator.

Add stronger ammonia water to the third separator till the solution is just alkaline, and then add 8 drops in excess. Cool the solution under the faucet and extract the alkaloid imme-

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[1] *Marquis' Solution* (Sulphuric Acid-Formaldehyde): A mixture of 25 parts of concentrated sulphuric acid and 1 part of formaldehyde (40%).

[2] *Alkaline Salt Solution:* Dissolve 25 Gm. of sodium hydroxide in 1000 cc. of distilled water; saturate the solution with sodium chloride, and filter.

diately with successive portions of a mixture consisting of 85 volumes of chloroform and 15 volumes of alcohol. Filter each successive chloroform fraction into a beaker through a piece of cotton wetted with chloroform mixture and wedged into the neck of a small funnel. Discard the liquid in separator No. 3.

Evaporate the chloroformic solution on the water-bath to about 1 cc., but not to dryness. Add 10 cc. of neutral alcohol to the residue and heat to dissolve the alkaloids and to drive off the last traces of chloroform. Add 1 drop of methyl red T. S.; add 0.02 *N* sulphuric acid until the solution is acid with an excess of from 2 to 5 cc. At this stage look out for any undissolved specks. Heat again if necessary. Evaporate most of the alcohol, cool the residue, and add 15 to 20 cc. of recently boiled and cooled distilled water. Titrate the excess acid with 0.02 *N* sodium hydroxide which has been ascertained to be sufficiently free from carbonates to give a sharp end-point with methyl red T. S.

Each cc. of 0.02 *N* H_2SO_4 = 0.00571 Gm. $C_{17}H_{19}O_3N$.

COLLABORATIVE WORK.

Table I gives the collaborators' reports.

TABLE I.—COLLABORATORS' REPORTS.

Analyst.	Gm. Anhyd. Morphine per 100 cc.		Average.
R. L. Greenwood	(1)	0.0422	
	(2)	0.0440	
Ko. Suto	(3)	0.0407	
			0.04270
W. H. Blome	(1)	0.04191	
	(2)	0.04354	
	(3)	0.03831	
			0.04092
E. D. Davy	(1)	0.04030	
	(2)	0.03997	
	(3)	0.04000	
			0.04009
R. W. Morrison	(1)	0.04022	
	(2)	0.04030	
	(3)	0.04028	
			0.04027
N. T. Chamberlin	(1)	0.0388	
	(2)	0.0388	
	(3)	0.0382	
			0.03860
A. R. Bliss, Jr.	(1)	0.04030	
	(2)	0.04010	
	(3)	0.04026	
			0.04022

COLLABORATORS' COMMENTS.

R. I. Grantham, Director of the Analytical Control Laboratory, Sharp & Dohme.—"Mr. R. L. Greenwood and Mr. Ko Suto of this Laboratory have assayed the sample of Camphorated Tincture of Opium which you submitted for comparative study. The method appears to work satisfactorily, and neither of the analysts experienced any difficulties with the formation of emulsions."

W. H. Blome, Scientific Director, Fredgrick Stearns & Company.—"You will note that the first and second assays check very closely, while the third one departs somewhat markedly from both '1' and '2.' We feel that this method is

considerably better in most respects than the others, although it is rather long for use by others than manufacturers of large quantities of Paregoric."

Edward D. Davy, Professor of Analytical Pharmacy, Western Reserve University.—"I can see nothing wrong with this modification of the former process when applied to Paregoric."

R. W. Morrison, Instructor in Pharmacology, University of Tennessee, Memphis.—"Although a bit long, the method is practical."

N. T. Chamberlin, Assistant Professor of Pharmacy, Western Reserve University.—"The method seems very workable, and I believe will prove very satisfactory."

A. R. Bliss, Jr., Chief, Division of Pharmacology, University of Tennessee Memphis.—"The method has proved to be the most satisfactory and accurate method presented to date. On the whole, the results obtained with the method by seven analysts are unusually close. *The average for the seven collaborators is 0.04040.* The highest value obtained was 0.04400, and the lowest 0.03830; the average for these two values is 0.04110. The Opium used in manufacturing the Paregoric assayed 10.22%. The theoretical amount of anhydrous Morphine in 100 cc. of the Paregoric is, therefore, 0.04088 Gm."

CONCLUSION.

The method herewith reported, which is identical with the method presented at the U. S. P. Committee on Revision meeting of June 1931, excepting for several very minor changes in detail, is decidedly more satisfactory, accurate, practical and expeditious than all other methods reported to date.

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(3) Bulletin 21, U. S. P. XI, Sub-Committee on Proximate Assays—No. 6 (December 21, 1931), 76.

(4) Buchbinder, JOUR. A. PH. A., 6 (1917), 618.

(5) Caines, *Pharm. J.*, 118 (1927), 751.

(6) Eaton, *Ibid.*, 109 (1922), 231; *Bur. Chem. Bull.*, 137 (1911), 188; *Ibid.*, 152 (1911), 242.

(7) Z. Kippenberger, *Z. anal. Chem.*, 34 (1895), 307; *Ibid.*, 39 (1900), 290.

(8) Puckner, *J. Am. Chem. Soc.*, 23 (1901), 470.

(9) B. H. St. John, *Bur. Chem., U. S. Dept. Agric. Private Communication* through L. E. Warren, Drug Research Unit, Washington, D. C.

(10) Warren and McClosky, Report of the Assay Procedures for a Number of Drug and Pharmaceutical Preparations, Food, Drug and Insecticide Administration, Washington, D. C., page 77, 1930.

(11) Warthle, *Chem.-Ztg.*, 25 (1901), 290.

(12) "Proceedings A. D. M. A.," (1929), 171; *Ibid.*, (1930), 213.

According to *The British Medical Journal*, "on the occasion of the celebration of the tercentenary of the foundation of Dorpat University, fifty doctors, *honoris causa*, were created. These included Sir A. Smith-Woodward, of the Royal Society; Prof. James Young Simpson, of the University of Edinburgh; Prof. A. Birch-Hirschfeld, of Königsberg; Prof. G. Liljestrand, of Stockholm, and Prof. L. Martin, of Paris."

Prof. F. A. F. C. Went, of Utrecht, and Prof. F. F. Blackman, of Cambridge, have been elected corresponding members of the American Society of Plant Physiologists.