THE ASSAY OF SPIRIT OF PEPPERMINT.*

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Although Spirit of Peppermint has been recognized in the last six Pharmacopœias, it was not until the U. S. P. XI became official that a mandatory assay for the volatile oil content was provided.

Briefly described, this assay determines the oil content on a 5-cc. portion in a Babcock bottle by adding one cc. of kerosene and sufficient calcium chloride solution to bring the oil layer within the graduations on the neck, centrifuging, reading the volume of the oil layer, and deducting five divisions (equivalent to 1 cc.) for the kerosene. The remaining number of divisions is multiplied by 4.2 to obtain the volume of oil of peppermint in 100 cc. of the spirit.

This assay is an application of the "precipitation" principle early applied by A. S. Mitchell (1) to flavoring extracts. Various modifications of this method by Howard (2), LaWall (3), Randall, *et al.* (4) and others, have appeared in the literature.

The basic principles involved are (A) a volatile oil is quantitatively precipitated from alcoholic solution when mixed with a large excess of concentrated calcium chloride solution, (B) it is completely soluble in kerosene, (C) the volume obtained by dissolving the oil in the volatile solvent is additive, *i. e.*, it is the sum of the volumes of the two constituents. LaWall did not obtain the relationship set forth in (C), observed a 5 per cent shrinkage, and suggested (5) that the factor 4.2, rather than 4 be employed. This value was incorporated in the U. S. P. assay.

The official assay has been applied to a number of spirits in this laboratory, both commercial and standard, with the observation that the results obtained were invariably high. This led to the conjecture that the factor 4.2 might be too great. The assay, however, is vague in certain other respects, which might lead to variations in results. The Pharmacopœia provides no formula for the acidified calcium chloride solution, employs kerosene when a purified kerosene reagent is described in the text, permits the use of uncalibrated Babcock bottles, and does not specify the manner in which the upper meniscus of the oil layer should be read. A systematic study was undertaken, therefore, for the purpose of evaluating the influence of these variables.

Five hundred cc. of a standard spirit was prepared from U. S. P. alcohol, garbled peppermint leaves and an oil of peppermint having the following properties:

Solubility: One volume of oil in 4 volumes 70% alcohol
Specific Gravity: 0.9149 at $\frac{25°}{25°}$ C.
Optical Rotation in a 100-mm. tube: −23.23° at 23° C.
Refractive Index: 1.4620 at 20° C.
Esters, calculated as menthyl acetate: 7.04%
Menthol: 54.3%.

The calcium chloride reagent was prepared by adding an excess of No. 4 mesh technical calcium chloride to water, containing 50 cc. concentrated hydrochloric acid in approximately one gallon. The liquid was employed without filtering out the excess of salt. Purified U. S. P. kerosene reagent was used to dissolve the oil.

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A series of twelve portions of standard spirit were assayed using the aforementioned reagents and employing the U. S. P. technique. The values were computed using both the values 4.2 and 4 as factors, and appear in Table I.

TABLE I.—RESULTS OBTAINED BY U. S. P. METHOD.

Bottle.	U. S. P. Factor 4.2.	Factor 4.	Bottle.	U. S. P. Factor 4.2.	Factor 4.
894	10.63	10.12	9	10.42	9.92
895	10.54	10.04	888	10.58	10.08
896	10.58	10.08	889	10.58	10.08
897	10.84	10.32	890	10.54	10.04
898	10.84	10.32	891	10.79	10.28
899	10.84	10.32	Average	10.64	10.13
2	10.46	9.96			

Inasmuch as the standard spirit contains 10 per cent by volume of oil the results obtained using the U. S. P. factor 4.2 are obviously too great, the values with the theoretical factor 4 being much closer to the correct result. However, as these latter results average 1.3 per cent too high, the presence of another source of error is indicated. This led to an investigation of the reagents employed.

Saturated calcium chloride solution, acidified with hydrochloric acid is the description given by the Pharmacopœia of the precipitating reagent. Aside from the facts that the quality of the salt and the concentration of acid are not specified, this solution is objectionable on other grounds. To assure saturation at all temperatures an excess of the salt must be present and the mixture should be shaken prior to use. This results in a turbid fluid which, if filtration is attempted, frequently crystallizes in the stem of the funnel. Hence, the substitution of a calcium chloride solution of lower concentration and having a definite formula was made. This reagent is about 75 per cent saturated, contains about 1 per cent free hydrochloric acid and is adjusted to a specific gravity of 1.36. Comparative results of the two solutions appear in Table II.

TABLE II.—RESULTS OBTAINED USING ACIDIFIED $CaCl_2$ SOLN., SP. GR. 1.36.

Bottle.	U. S. P. Factor 4.2.	Factor 4.	Bottle.	U. S. P. Factor 4.2.	Factor 4.
894	10.71	10.20	9	10.58	10.08
895	10.79	10.28	9	10.54	10.04
896	10.79	10.28	5	11.09	10.56
897	10.88	10.36	8	10.50	10.00
898	10.75	10.24	Average	10.74	10.23
899	10.96	10.44	Average—Table I	10.64	10.13
2	10.58	10.08			

As the average values obtained by using the two reagents lie close together and apparently fall within the limit of experimental error, either may be employed. The greater convenience in handling led to the use of the modified solution in the balance of these experiments.

An investigation of the possible variations caused by kerosene was next undertaken. The following experiment was made to learn the effect of temperature on kerosene expansion expressed in Babcock divisions.

A standard calibrated Babcock bottle was filled to the zero mark at 0° C., the temperature was allowed to rise, and readings were made at definite intervals. The values, plotting Babcock divisions as abscissæ against centigrade degrees as ordinates, appear on the accompanying graph.

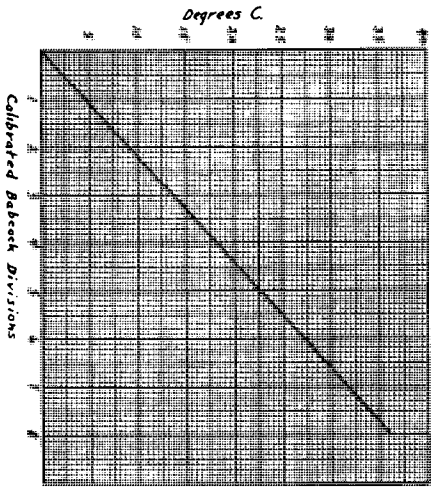

Fig. 1.

It was found that 50.8 cc. of kerosene expanded 7.9 divisions when the temperature was raised from 0° to 35.7° C. This is equivalent to an expansion of 0.0044 divisions per centigrade degree for one cc.—a completely negligible increment.

No reference is made as to how the reading of the upper meniscus should be made, and since this boundary has a definite thickness the following experiment was carried out.

Into each Babcock bottle was added one cc. of kerosene using a calibrated pipette, and sufficient calcium chloride reagent (specific gravity 1.36) to bring the oil within the graduated portion of the neck. The number of spaces occupied by the oil was determined by reading the lower curvature of the upper meniscus in one case and by recording the mean value of the upper and lower curvatures in the second.

TABLE III.—EFFECT OF READING LOWER PART AND MEAN MENISCUS.

Bottle.	Reading Obtained Using Mean Meniscus.	Reading Obtained Using Lower Meniscus.	Bottle.	Reading Obtained Using Mean Meniscus.	Reading Obtained Using Lower Meniscus.
888	5.05	5.00	893	5.13	5.07
889	5.07	5.01	3	5.16	5.09
890	5.06	5.01	5	5.15	5.10
891	5.15	5.10		——	——
892	5.08	5.03	Average	5.12	5.05

The values obtained indicate that reading the lower curvature of the upper meniscus gives more accurate results.

In order to determine whether or not the alcohol present in spirit of peppermint affects the observed volume of kerosene, 4.5 cc. of alchohol, 1 cc. of purified kerosene and acidified calcium chloride solution (specific gravity 1.36) were mixed and treated in the prescribed manner. Here, as in all subsequent work, the lower curvature of the upper meniscus was read.

TABLE IV.—RESULTS OBTAINED WITH KEROSENE AND ALCOHOL.

Bottle.	Volume in Babcock Units.	Bottle.	Volume in Babcock Units.
888	5.00	893	5.09
889	4.98	3	5.16
890	4.97	5	5.10
891	5.01		——
892	5.07	Average	5.05

The added alcohol does not appear to be responsible for any change in the observed volume of the kerosene layer.

In order to determine what effect peppermint oil would have on the observed volume a similar experiment was made omitting alcohol and including 0.5 cc. of oil. The results are recorded as observed readings and are also calculated to cc. of oil in 100 cc. spirit.

TABLE V.—RESULTS OBTAINED WITH KEROSENE AND PEPPERMINT OIL.

Bottle.	Babcock Units.	Cc. Oil/100 Cc. "Spirit." Factor 4.2.	Factor 4.
888	7.46	10.33	9.84
889	7.50	10.50	10.00
890	7.51	10.54	10.04
891	7.61	10.96	10.44
3	7.65	11.13	10.60
5	7.56	10.75	10.24
Average	7.55	10.70	10.19

The results here, as well as in preceding tables, indicate that if there is any deviation from the additive effect with regard to volume it is in the direction of expansion rather than contraction.

The study of kerosene behavior was concluded with an investigation intended to show whether different kerosenes gave different readings and whether the results with the purified and unpurified products were similar. Five commercial kerosenes were purchased from different sources, a portion of each was purified, and tests were made using both purified and non-purified oil.

TABLE VI.—RESULTS OBTAINED WITH DIFFERENT KEROSENES.

Kerosene (Commercial) No.	Factor 4.2.	Factor 4.	Kerosene (Purified No.)	Factor 4.2.	Factor 4.
1	10.64	10.14	1	10.69	10.18
2	10.74	10.23	2	10.61	10.11
3	10.66	10.15	3	10.64	10.14
4	10.70	10.19	4	10.70	10.19
5	10.64	10.14	5	10.67	10.16
Average	10.68	10.17		10.66	10.13

The use of different kerosenes, whether purified or not, does not apparently influence the results. However, since all the commercial kerosenes did not meet the U. S. P. requirement for color (specific gravity and distillation value were satisfactory), and since the Pharmacopœia describes a purified reagent it is felt that the purified product should be employed in the assay.

The one remaining important factor which is not recognized in the official assay is the variation in the graduation of Babcock bottles. In order to determine this effect, standard bottles (calibrated at the United States Bureau of Standards and at the Bureau of Chemistry of the Maryland State Health Department) were used throughout the investigation. These factors which varied from +0.55 per cent to −2.00 per cent, were not used in the earlier work but were later applied to the accumulated data, the comparative results appearing on page 630.

It will be noted that using the factor 4 and applying the calibration corrections yield results closely approximating theoretical figures. As a further illustration of this fact the data obtained on a number of commercial spirits employing the modi-

TABLE VII.—EFFECT OF CALIBRATION OF BABCOCK BOTTLES.

Average Values Using Factors 4.2 and 4 before and after Applying Calibration Correction Factors.

	Before.		After.	
	4.2.	4.	4.2.	4.
Table I	10.64	10.13	10.53	10.03
Table II	10.74	10.23	10.65	10.15
Table VI	10.70	10.19	10.38	9.87
Table VII				
(comm.)	10.68	10.17	10.49	9.99
(purified)	10.66	10.13	10.44	9.96

Average Values in Babcock Units before and after Applying Calibration Correction Factors.

	Before.		After.	
Table III	5.12	5.05	5.06	5.00
Table IV	5.05		5.00	

fied calcium chloride solution, purified kerosene, and reading the lower curvature of the upper meniscus are presented. Although the true values of these spirits must remain unknown, it may be generally assumed that the high values obtained using the U. S. P. factor and neglecting the calibration correction are fictitious.

TABLE VIII.

Bottle No.	Calibration Factor, %.	U. S. P. XI without Correction for Calibration.	U. S. P. XI with Correction for Calibration.	Factor 4 without Correction.	Factor 4 with Correction
888	−0.31	10.92	10.84	10.42	10.32
889	−0.56	11.26	11.09	10.72	10.56
890	−0.44	11.21	11.09	10.68	10.56
891	−1.25	11.93	11.51	11.36	10.96
892	−0.31	11.72	11.63	11.16	11.08
893	−1.13	11.76	11.38	11.20	10.84
894	−0.31	10.54	10.46	10.04	9.96
895	−0.06	11.00	11.00	10.48	10.48
896	−0.19	10.96	10.92	10.44	10.40
897	−0.75	11.13	10.88	10.60	10.36
898	−0.56	11.00	10.84	10.48	10.32
899	−1.25	11.34	10.92	10.80	10.40
3	−2.00	11.42	10.79	10.88	10.28
29	−0.52	11.09	10.92	10.56	10.40
9	+0.34	11.13	11.26	10.60	10.72
5	−1.74	12.64	12.05	12.04	11.48

As a result of these investigations it is believed that the official assay may be improved and rendered more accurate by adopting the following modifications:

(1) The substitution of the value 4 for the present factor 4.2;
(2) The use of calibrated glassware;
(3) The adoption of the modified calcium chloride solution and purified kerosene in place of the present reagents;
(4) Specifying that the oil column reading be made from the lower meniscus to the lower curvature of the upper meniscus.

CONCLUSIONS.

1. The official assay for Spirit of Peppermint yields results that are too high.
2. A modified assay has been devised employing specific reagents and cali-

brated glassware, by which results in close agreement with the theoretical values may be obtained.

REFERENCES.

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(4) Randall, W. W., and Penniman, W. B. D., *Ind. & Eng. Chem.*, 6, 926 (1914).
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THE PHARMACOLOGY OF PYRETHRUM FLOWERS.*[1]

BY HARRY ROSEN AND MARVIN R. THOMPSON.[2]

The literature pertaining to the chemistry and pharmacology of pyrethrum flowers is reviewed in the original thesis and by other recent writers (1) (2), and hence will not be repeated here. This report is intended to embrace only the experimental studies described in the thesis.

In the course of the experimental work it was noted that pyrethrum powder or solutions exposed to air deteriorated rapidly. It was also observed that roaches and flies apparently protected from contact with the powder but not from possible vapors, readily exhibited symptoms of pyrethrum action. The possibility of an active volatile fraction was therefore investigated.

EXPERIMENTAL.

Six hundred grams of insect flowers were steam distilled and 4 liters of distillate collected in 250-cc. fractions. Some of the early fractions on injection into the ventral lymph sac of frogs showed toxicity. These combined distillates were saturated with sodium chloride, extracted with petroleum ether, and the solvent allowed to evaporate at room temperature. The residue was taken up in 25 cc. alcohol and precipitated by the addition of saturated sodium chloride solution, a light brown semi-solid mass resulting. This also showed some toxicity to frogs but was not investigated further because of a very meager yield. Four more samples of insect flowers were likewise steam distilled, but the distillates showed no toxicity to frogs and were found to exert no action on isolated rabbit intestine. The frog and intestine test methods are described in detail elsewhere in this paper.

The remainder of each of these steam distillates was saturated with sodium chloride and divided into two portions. One portion was extracted with petroleum ether and assayed chemically and the other was extracted with purified kerosene and assayed by the Peet-Grady fly

TABLE I.—RESULTS OF STEAM DISTILLATE ASSAYS.

Sample	Assay of Drug before Distillation. Fly Method		Chemical Method. Total Pyrethrins.	Pyrethrin I.	Pyrethrin II.	Fly Method.		Assay of Steam Distillate. Chemical Method. Total Pyrethrins.	Pyrethrin I.	Pyrethrin II.
A	68%	Kill	0.81%	0.361%	0.449%	3%	Kill	0.0015%	0.001%	0.0005%
D	83%	Kill	0.80%	0.344%	0.456%	4%	Kill	0.0035%	0.0025%	0.0010%
F	88%	Kill	0.83%	0.365%	0.465%	5%	Kill	0.0036%	0.002%	0.0016%
J	84%	Kill	0.835%	0.365%	0.470%	6%	Kill	0.0031%	0.0017%	0.0014%

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[2] Abstracted from a thesis submitted in May 1936 by Harry Rosen to the Faculty of the Graduate School of the University of Maryland in partial fulfilment of the requirements for the degree of Doctor of Philosophy.