THE QUANTITATIVE DETERMINATION OF VOLATILE OILS IN VEGETABLE DRUGS.*

BY L. GOLDBERG,[1] R. K. SNYDER,[1] E. H. WIRTH[2] AND E. N. GATHERCOAL.[1]

Methods for the determination of the volatile oil content of vegetable drugs usually depend (*a*) upon extraction with a solvent; (*b*) upon distillation with water or steam or (*c*) upon a combination of the two methods. The literature in many cases, describes comparative studies of two methods, with the usual conclusion that the method giving the higher percentage result is the preferable one; but as these determinations were always run on samples of drugs, such a conclusion is inconsistent, since the volatile oil content of the drugs used was an unknown quantity. While one method may give results consistently higher than the other, the analyst never knows which result more closely approximates the actual amount of volatile oil present in the drug.

In order to make a critical study of various methods for the determination of the volatile oil content of vegetable drugs, purified and thoroughly oven-dried sawdust was impregnated with known quantities of volatile oil, of moisture, or of volatile oil and moisture. The impregnation was carried out in capped wide-mouth jars having a hole in the cap large enough to admit the nozzle of a fine-spray atomizer. The volatile oil or moisture was sprayed onto the sawdust with accompanying agitation of the jar to insure uniform impregnation. The weight of the dry sawdust was known and the atomizers were weighed before and after spraying. In using samples of this type the accuracy of any method studied may be gaged by the proximity of the final result to the actual amount of volatile oil, of moisture, or of both employed at the beginning. Artificially prepared samples of this type are, of course, not exactly similar to crude drugs, but they approximate them as closely as is experimentally possible. By using these samples each step in any method can be studied critically and any fallacies or manipulative errors can readily be determined. A number of methods have been studied in this manner.

VOLATILE ETHER EXTRACTIVE METHOD.

This method has been official for several decades and appears again in the U. S. P. XI (page 475). It consists, in brief, of (*a*) drying the drug over sulfuric acid for twelve hours; (*b*) extracting with absolute ether continuously for twenty hours; (*c*) evaporating the ether spontaneously; (*d*) drying the extract eighteen hours over sulfuric acid; (*e*) weighing and (*f*) heating gradually to constant weight at 110° C. The loss in weight is volatile oil.

Several sources of error may be present in this process: (1) It is doubtful whether the drying in (*a*) and (*d*) is sufficient. If water be present in the drug it would be extracted and added to the final loss in weight. (2) Even though absolute ether is used in the extraction process, a considerable amount of moisture may condense from the air when the ether is evaporated spontaneously (*c*). With resinous drugs there is a considerable amount of "scumming over" and the varnish-like skin may hold both moisture and ether beneath it. These will volatilize during the

* National Formulary Research Project F6 at the University of Illinois, College of Pharmacy, Chicago, Ill.

[1] National Formulary Laboratory.

[2] Associate Professor of Pharmacognosy, University of Illinois.

final heating and will thus be calculated as volatile oil. (3) There may be a loss of volatile oil in the desiccator (*d*) and also a loss during the spontaneous evaporation (*c*). (4) Some decomposition of constituents other than volatile oil may occur during the heating process (*f*).

EFFICIENCY OF DESICCATORS IN REMOVING MOISTURE.

Anhydrous sawdust in which moisture was uniformly incorporated was dried over calcium chloride and over sulfuric acid in both large (average capacity 2650 cc.) and small (average capacity 680 cc.) desiccators. The samples were weighed at time intervals to note the loss in weight. The moisture content of the sawdust, before placing in the desiccators, was determined on duplicate samples by heating at 110° C. to constant weight.

Sawdust Moisture Content: 10.8%.

Desiccants: Fresh.

Percentage of Total Moisture Lost:	Small $CaCl_2$.	Large $CaCl_2$.	Small H_2SO_4.	Large H_2SO_4.
In 24 hours	77.7%	78.6%	99.2%	87.5%
In 2 days	88.4%	85.1%	98.2%	86.5%

Sawdust Moisture Content: 10.4%.

Desiccants: 3 to 4 Weeks in Use.

Percentage of Total Moisture Lost:	Small $CaCl_2$.	Large $CaCl_2$.	Small H_2SO_4.	Large H_2SO_4.
In 24 hours	49.5%	54.1%	47.9%	47.4%
In 2 days	75.9%	69.7%	68.6%	67.9%
In 3 days	83.2%	75.9%	80.9%	75.7%

Sawdust Moisture Content: 4.85%.

Percentage of Total Moisture Lost:	Small $CaCl_2$.	Large $CaCl_2$.	Small H_2SO_4.	Large H_2SO_4.
In 24 hours	55.7%	61.9%	53.6%	63.9%
In 2 days	72.1%	70.1%	70.1%	76.3%
In 3 days	80.4%	82.4%	78.7%	86.6%

NOTES.

(1) The drying was not nearly complete at the end of twenty-four hours in the desiccators, except in one case: the freshly prepared, small H_2SO_4 desiccator. It may be assumed that a drug containing 5% to 10% of moisture is never fully dried in a desiccator in twelve hours.

(2) Neither the size of the desiccator nor the type of desiccant appear to materially affect the results. The average percentage of desiccation at the end of 24 hours over calcium chloride is 62.9%, and over sulfuric acid 66.6%; in the small desiccators 63.9%, and in the larger desiccators 65.7%.

(3) The fresh desiccants show a much greater drying power, especially during the first day, over the desiccants that had been in use several weeks.

LOSS OF VOLATILE OIL IN DESICCATORS.

Anyone familiar with the volatile ether-soluble extractive method has observed that when drying volatile oil drugs over sulfuric acid, there is a rather rapid darkening of the acid indicating the presence of organic matter; probably lost volatile oil. The following tables are illustrative of the amount of volatile oil lost from evaporating dishes standing in various desiccators at room temperature. The desiccators are the same as used in the previous work.

Oil of Eucalyptus (Sp. Gr. 0.905).

Per Cent of Total Volatile Oil Lost:	Small $CaCl_2$.	Large $CaCl_2$.	Small H_2SO_4.	Large H_2SO_4.
In 24 hours	94.5%	95.0%	94.9%	42.8%
In 2 days	95.2%	96.2%	97.4%	68.2%
In 5 days	95.2%	96.4%	98.0%	86.3%
In 7 days	95.0%	96.4%	98.2%	96.7%
In 8 days	95.8%	96.6%	98.8%	96.8%

Oil of Clove (Sp. Gr. 1.050).

Per Cent of Total Volatile Oil Lost:	Small $CaCl_2$.	Large $CaCl_2$.	Small H_2SO_4.	Large H_2SO_4.	Small $Mg(ClO_4)_2$.
3rd day	5.2%	5.6%	5.9%	6.1%	5.8%
4th day	5.6%	6.4%	9.0%	6.5%	6.4%
6th day	6.8%	7.7%	8.3%	7.9%	7.7%
7th day	7.1%	8.1%	8.7%	8.4%	8.2%
10th day	10.1%	11.1%	11.6%	11.3%	11.1%

Pinene (Sp. Gr. 0.895).

Per Cent of Total Volatile Oil Lost:	Small $CaCl_2$.	Large $CaCl_2$.	Small H_2SO_4.	Large H_2SO_4.	Small $Mg(ClO_4)_2$.
1st day	27.8%	32.4%	41.8%	86.4%	39.4%
4th day	64.9%	79.5%	86.5%	88.1%	92.1%
5th day	72.9%	86.9%	87.7%	88.3%	92.8%
7th day	82.0%	89.2%	88.5%	88.4%	93.0%
8th day	84.5%	89.9%	88.7%	88.4%	93.6%

Oil of Chenopodium (Sp. Gr. 0.950).

Per Cent of Total Volatile Oil Lost:	Small $CaCl_2$.	Large $CaCl_2$.	Small H_2SO_4.	Large H_2SO_4.
1st day	3.7%	4.0%	3.6%	4.0%
2nd day	6.6%	7.1%	8.5%	7.4%
5th day	12.3%	13.5%	22.8%	14.8%
6th day	13.9%	15.3%	26.4%	17.5%
7th day	15.6%	16.8%	29.6%	21.5%

NOTES.

(1) Volatile oils in evaporating dishes do not completely simulate drugs (volatile oil in cellular tissue), yet it is very evident that some volatile oil may be lost from the drug, during the preliminary drying of the drug in the desiccator before the ether extraction.

(2) The rate of evaporation of volatile oils in desiccators seems to be a function of the specific gravity; that is, the lower the specific gravity of the oil the more rapid the rate of evaporation. The rate of evaporation is, of course, a function of the vapor pressures of the oil constituents and the relation between the rate of evaporation and the specific gravity should be considered in the light of being a coincidence rather than fixed rule.

(3) The size of the desiccator and the nature of the desiccant appear to have little effect on the rate of evaporation. The larger desiccators with sulfuric acid are perhaps slightly more effective.

(4) It seems very evident that volatile oil must be lost during the desiccation of the ether extract after the ether has been spontaneously evaporated.

EXTRACTION OF VOLATILE OIL-SAWDUST MIXTURES BY THE OFFICIAL METHOD.

Known quantities of volatile oils absorbed by sawdust were extracted by the U. S. P. method for volatile ether-soluble extractive. After the ethereal solution was evaporated spontaneously and the extract dried over sulfuric acid for eighteen hours, the total ether-soluble extractive weighed much less than the original weight of the oil taken. After heating the total ether-soluble extractive at 110° C. to constant weight, the total loss in weight (volatile ether-soluble extractive) calculated on the original weight of the oil was noted.

	Original Weight of Oil Taken.	Loss during Evaporation and Desiccation.	Loss on Heating at 110° C.	Efficiency of Official Method.
Oil of Eucalyptus	0.2136 Gm.	0.2013 Gm.	0.0123 Gm.	5.77%
Oil of Peppermint	0.2821 Gm.	0.2350 Gm.	0.0471 Gm.	16.71%
Pinene	0.2457 Gm.	0.1279 Gm.	0.1178 Gm.	47.94%
Oil of Clove	0.2941 Gm.	0.0490 Gm.	0.2450 Gm.	83.30%
Oil of Chenopodium	0.2163 Gm.	0.0241 Gm.	0.1922 Gm.	88.85%

NOTES.

(1) The efficiency of the official method for determining volatile ether-soluble extractive is the percentage of loss in weight on heating at 110° C. as compared with the original weight of the oil.

(2) The loss in weight of volatile oil during evaporation and desiccation again bears a fair ratio to the specific gravity of the oil; the lower the specific gravity, the greater the loss. Here again, too much weight should not be placed upon this relationship.

(3) While the volatile oil-sawdust mixtures do not exactly simulate drugs (no resin being present and usual "scumming over" being absent) there is never-the-less definite evidence that considerable error may occur.

DISTILLATION METHODS.

D. A. B. Method.—The German Pharmacopœia VI was the first pharmacopœia to officially introduce a distillation method for the determination of volatile oils in drugs. In this method the drug material is distilled with water, the distillate (volatile oil and water) being caught in a separatory tunnel; NaCl is then added to the contents of the separatory funnel, and the oil removed by shaking with pentane. The pentane extracts are evaporated at low heat, the volatile oil residue dried in a desiccator for *half an hour* and weighed. Preliminary experiments show this method to be subject to errors similar to those found in the volatile ether-soluble extractive method: (*a*) insufficient drying and (*b*) loss of volatile oil in desiccator.

Criticisms of this method agreeing with our own findings have been published in the literature (1). The further investigation of this method has been deferred for the present.

Clevenger Method.—In 1927 Clevenger (2) introduced a volatile oil trap, so designed that the distilled volatile oil remains in the trap and the water is returned to the continuously boiling flask containing the drug and water mixture. That part of the trap retaining the oil is calibrated and volume readings can be made directly at any time during the progress of the distillation. The method has much to commend it, especially its simplicity. It is further advantageous in that the oil can be removed from the trap, and its constants (specific gravity, optical rotation, refractive index, etc.) determined.

The method is somewhat disadvantageous from the standpoint of the large sample of drug material that must be used. In order to eliminate experimental error a yield of from 1 to 2 cc. of oil should be obtained. Since many drugs contain less than 1% of volatile oil, this means that a sample of 100 Gm. or more must be used for one determination.

Experiments employing several volatile oils were conducted with this apparatus using 1 cc. or 2 cc. of oil, 50 Gm. of purified anhydrous sawdust and 200 cc. of water.

Modifications of the method were attempted, such as omitting the sawdust and simply mixing the volatile oil with the water; employing various concentrations of brine in place of water; and varying the type of condenser used. The final readings were usually about 10% lower than the amount of volatile oil taken.

Exp. No.	Quantity and Oil Used.	Sawdust.	Salt.	Final Reading.	Per Cent Efficiency.
(*a*)	1 cc. Eucalyptus	50 Gm.	....	0.90 cc.	90.0%
(*b*)	2 cc. Eucalyptus	50 Gm.	....	1.80 cc.	90.0%
(*c*)	2 cc. Peppermint	50 Gm.	....	1.80 cc.	90.0%
(*d*)	2 cc. Pepermint	50 Gm.	60 Gm.	1.85 cc.	92.5%
(*e*)	2 cc. Lavender	50 Gm.	....	1.80 cc.	90.0%
(*f*)	2 cc. Pinene	50 Gm.	....	1.80 cc.	90.0%
(*g*)	2 cc. Pinene	....	....	1.80 cc.	90.0%
(*h*)	2 cc. Pinene	....	....	1.90 cc.	95.0%
(*i*)	2 cc. Pinene*	....	60 Gm.	1.85 cc.	92.5%
(*j*)	2 cc. Pinene	....	....	1.80 cc.	90.0%
(*k*)	2 cc. Pinene	....	60 Gm.	1.80 cc.	90.0%
(*l*)	2 cc. Pinene	....	30 Gm.	1.80 cc.	90.0%
(*m*)	2 cc. Cinnamon	50 Gm.	....	1.85 cc.	92.5%
(*n*)	2 cc. Clove	50 Gm.	....	1.95 cc.	97.5%
(*o*)	2 cc. Clove	....	30 Gm.	1.90 cc.	95.0%
(*p*)	2 cc. Chenopodium	....	....	1.50 cc.	75.0%
(*q*)	2 cc. Chenopodium	....	30 Gm.	1.75 cc.	87.0%
(*r*)	2 cc. Chenopodium	....	30 Gm.	1.80 cc.	90.0%
(*s*)	2 cc. Chenopodium*	....	30 Gm.	1.80 cc.	90.0%
(*t*)	2 cc. Chenopodium*	....	30 Gm.	1.80 cc.	90.0%

* Water used was the saturated water remaining after a previous distillation.

NOTES.

(1) (*j*) and (*k*) were run with the usual type of reflux condenser; all others with the ball-type condenser.

(2) (*m*), (*n*) and (*o*) were run with the trap designed for oils heavier than water. With this type of apparatus the oil is transferred from the trap to a graduated cylinder. The trap is then washed with ether, the ether evaporated and the residual oil added to the graduated cylinder. The use of ether here may involve a slight source of error.

(3) Two possibilities of error are suggested, namely:

(*a*) The oil may remain in solution in the water; then the use of oil-saturated water and of brine should indicate an improvement in results and possibly they do.

(*b*) The last traces of oil may be non-distillable because of physical reasons, such as vapor density.

(4) The method is certainly a marked improvement over the present official method in that (*a*) it is simple in operation; (*b*) it consumes much less time; (*c*) it actually yields the volatile oil and its constants can be checked; and (*d*) it gives far more accurate results.

Mijnhardt Method.—In 1936 Mijnhardt published a method (1) employing a microtrap. In this case the trap is located within the neck of a Kjeldahl flask used for boiling the drug-water mixture. The trap is simply the lower part of the condenser, the oil being drawn down into the lower constricted portion of the trap after distillation. This constricted portion is graduated into 0.02-cc. units. With this method smaller samples (5 to 10 Gm.) of drug material may be used.

Several experiments employing various oils gave a loss of from 10% to 30% of volatile oil. When a brine mixture is used in place of water, care must be used regarding the concentration of the brine. If this is too high the boiling point will be

raised high enough to cause the water in the trap (which is located within the flask) to boil and thus ruin the results.

Results with the Mijnhardt apparatus were as follows:

Exp.	Quantity and Oil Used.	Sawdust.	Salt.	Final Reading.	Per Cent of Efficiency.
(*a*)	0.2 cc. Lavender	20 Gm.	....	0.14 cc.	70%
(*b*)	0.2 cc. Lavender	20 Gm.	60 Gm.	0.09 cc.	*
(*c*)	0.2 cc. Lavender	20 Gm.	30 Gm.	0.17 cc.	85%
(*d*)	0.2 cc. Pinene	....	....	0.15 cc.	75%
(*e*)	0.2 cc. Pinene**	....	....	0.16 cc.	80%
(*f*)	0.2 cc. Pinene	....	30 Gm.	0.18 cc.	90%
(*g*)	0.2 cc. Chenopodium**	....	30 Gm.	0.18 cc.	40%
(*h*)	0.2 cc. Chenopodium**	....	30 Gm.	0.10 cc.	50%

* The brine concentration in (*b*) was high enough to raise the temperature of the water in the trap above the boiling point and thus cause an error.

** Water used was the saturated water remaining after a previous distillation.

NOTES.

(1) To adapt this method to oils heavier than water, a known quantity of pinene is added to the drug, and subsequently subtracted from the total volume of oil in the trap. (Mijnhardt includes a volume shrinkage factor.)

(2) The method has certain favorable features but the losses are unduly large.

(3) Sometime after the completion of our work van Giffen published a paper (3) in which he points out errors in the Mijnhardt method which agree with our findings.

Wasicky Method.—In 1933 Wasicky (4) published a distillation method utilizing a flask for generating steam, in the upper compartment of which is a chamber containing the drug. In this method the drug is not in contact with the water. The distillate is caught in a separatory funnel at the lower end of which is a calibrated microburette. The oil is removed from the water in the separator by shaking with a carefully measured volume of dekalin, the whole inverted, the dekalin-oil mixture allowed to rise into the microburette and the volume read.

This method and apparatus have many features which commend it in a theoretical way, but we have been unfortunate in our failure to master the technique. The dekalin-oil mixture did not quantitatively rise into the microburette, but remained adherent to the sides of the separator. Repeated cleansing with sulfuric acid-dichromate cleaning solution failed to overcome the difficulty. We hope to take the matter up with Dr. Wasicky and learn to improve our technique.

Aside from manipulative difficulties it should also be mentioned that the apparatus is rather complex, somewhat costly and rather fragile.

DRYING METHODS.

Oven Method.—The U. S. P. XI gives directions for the determination of moisture in drugs containing ether-soluble constituents volatile at 100° C. (5) which the total loss in weight at 100° C. is determined on one sample; the volatile-ether extractive being determined on a second sample and subtracted from the total loss in weight upon heating, the difference being the moisture. A reversal of this process suggested itself and we have named it the oven method.

In this method a sample of the drug is placed in an oven and heated to constant weight at 100° C. This loss represents total volatile matter. The amount

of moisture is determined in a second sample by the toluene method of the U. S. P. XI. This figure subtracted from the total volatile figure represents the amount of volatile matter other than water.

A measured amount of volatile oil and water were carefully mixed with anhydrous sawdust and the above procedure was used for determining each. The following table illustrates the results obtained:

	Measured Quantity.	Determined Quantity.
Moisture	6.1 %	6.0% (Toluene method)
Oil of Clove	5.9 %	6.3%
Total	12.0 %	12.3% (Oven method)
Moisture	6.5 %	6.6% (Toluene method)
Pinene	5.06%	5.0%
Total	11.56%	11.6% (Oven method)
Moisture	6.1 %	6.0% (Toluene method)
Oil of Chenopodium	5.9 %	6.3%
Total	12.0 %	12.3% (Oven method)

NOTES.

As was anticipated, this method gave excellent results with the sawdust-volatile oil-moisture samples. It has much to commend it both from the standpoint of simplicity and from the absence of the sources of error so evident in other methods. When applied to actual drug evaluation there are, however, certain factors which may give rise to error. There is evidence in certain drugs of the presence of constituents (not volatile oils) which decompose at even as low a temperature as 100° C. In such cases the total loss in weight would represent more than just volatile oil and moisture.

OTHER METHODS.

Other methods appear in the literature but these invariably depend upon the same principles involved in the methods studied above, and are subject to the same errors. As this part of our work is brought to a close van Giffen (3) publishes a new method involving extraction with petroleum ether (under a reflux condenser) after which the petroleum ether is concentrated at 50° C., distilled with steam and the distillate shaken with petroleum ether, which is again concentrated at 50° C., dried with sodium sulfate and finally concentrated in a current of air, the residual volatile oil determined by weighing. While this method has not been investigated experimentally many sources of error suggest themselves.

SUMMARY.

1. A critical examination of the official method for volatile ether-soluble extractive reveals several sources of error, namely:

(*a*) The removal of the moisture from the drug kept in the desiccator for 12 hours apparently is difficult of accomplishment. Sawdust containing a known quantity of moisture and kept in various sizes of desiccators and with various desiccants showed an average percentage of desiccation of 65% after 24 hours and 83% after 2 to 5 days.

(*b*) Loss of volatile oil during the evaporation and desiccation of the ether extraction is certain. Volatile oils in dishes in various desiccators and over various desiccants showed an average loss of 33% in 24 hours and losses of from 10% to 98.8%, after several days. The rate of evaporation depends on the vapor pressure and possibly on the specific gravity of the oil.

(*c*) Even if absolute ether is used for the extraction process, moisture may condense from the air when the ether is spontaneously evaporated, collect in the extract and constitute a part of the calculated volatile extractive.

(*d*) Decomposition of constituents other than volatile oil during the heating process may occur.

2. Comparative studies of methods for volatile oil determination run on drug samples, and with the usual conclusion that the method giving the highest percentage of results is the preferable one, may be inconsistent, since the volatile oil content of the drugs used was an unknown quantity.

The official method run on samples of purified, anhydrous sawdust containing known quantities of volatile oil gave results showing from 5.77% to 88.85% efficiency. The quotient of efficiency is the percentage of loss in weight on heating at 110° C. as compared with the original weight of the oil taken.

3. Several distillation methods, namely, the D. A. B. Method (German Pharmacopœia VI), Clevenger Method, Mijnhardt Method and Wasicky Method were carefully tried out. The D. A. B. method has about the same faults as the U. S. P. method; the Mijnhardt method gave us a low efficiency; and we were not able to satisfactorily develop the technique of the Wasicky method. Both the Mijnhardt and Wasicky methods use a microburette and theoretically they seem to have good possibilities. The Clevenger method is accurate to at least 90%, is simple, easily read, rapid and yields the actual volatile oil in sufficient quantity to check its constants.

4. The simple oven method suggests itself as being of real practical value. It is simple in operation, saving of time, requires no complex apparatus and presents no manipulative difficulties. It certainly yields far more accurate results than the present official volatile ether-soluble extractive method. The total volatile matter is determined by heating at 100° and from this is subtracted the moisture determined by the toluene distillation method, the difference being volatile oil.

The only error which suggests itself involves the possibility of products present in drugs (other than volatile oil) which might decompose during the heating at 100° and thus cause the total volatile matter to represent more than just volatile oil and moisture. It is proposed to demonstrate the usefulness of this method by running it upon samples of drugs and checking its results against the Clevenger method, which appears to be accurate to 90%. If these results run uniformly about 10% higher than those obtained with the Clevenger method its usefulness will have been demonstrated. If on the other hand they run considerably higher than the Clevenger results, the loss in weight due to decomposition of non-volatile oil constituents in the drugs, will indicate its impracticability.

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(4) Wasicky, R., *Pharm. Presse*, Wissenschaftlich-praktisches Heft (May 1933). See also "Leitfaden für die Pharmakognostischen Untersuchungen im Unterricht und in der Praxis," page 190 (1936).

(5) U. S. P. XI, page 473, Paragraph 11.