REVIEW OF METHODS OF ASSAYING OIL OF BITTER ALMOND.

BY JANE L. HASTINGS.

True oil of bitter almond is obtained from the seeds of *Prunus Amygdalus amara* DeCandolle, after the fixed oil has been removed by expression. However, the essential oils from the kernels of apricots and peaches are identical for all practical purposes with the true almond oil. In fact, most of the so-called bitter almond oil of commerce is derived from apricots. The essential oil, as we know it, is not present in the kernels as such. It is formed by the action—in the presence of water—of emulsin, a natural ferment, on amygdalin, both being natural constituents of the seeds. By the reaction, benzaldehyde, dextrose and hydrocyanic acid are formed. The following equation expresses the reaction:

$$C_{20}H_{27}O_{11}N + 2H_2O = 2C_6H_{12}O_6 + C_6H_5.CHO + HCN$$

Amygdalin is a crystalline glucoside which has no odor of bitter almond and is not converted to the oil except by a hydrolytic agent, either water or by boiling with dilute acids.

The pure oil consists almost wholly of benzaldehyde. On account of this there has been a great deal of adulteration of the oil with artificial benzaldehyde. Benzaldehyde oxidizes to benzoic acid if allowed to stand in the air. Therefore it is necessary that bottles of the oil should be kept well stoppered and as full as possible. Schimmel & Company state that the addition of 10 per cent. of alcohol retards oxidation and alcohol in this proportion is sometimes added, not for adulteration but for preservation of the benzaldehyde, though the specific gravity of the oil is lowered thereby.

High specific gravity of the oil can usually be accounted for by the presence of a greater percentage of hydrocyanic acid than the 2 per cent. to 4 per cent. specification of the U. S. P. Schimmel & Company ("Schimmel & Company Report," April 1893) state that after the addition of 20 per cent. solution of HCN to a sample of oil of bitter almond of specific gravity 1.054, the gravity in two days had risen to 1.074. Oils containing up to 11 per cent. HCN have been known to reach a specific gravity of 1.096. The author has verified this fact in analyzing a commercial oil of which the specific gravity was 1.0688. The HCN content exceeded 6 per cent.

In studying oil of bitter almond, the author has been particularly interested in the benzaldehyde and hydrocyanic acid content. The methods given in the U. S. P. for the determination of both constituents of the oil are far from accurate as a consequence of indistinct end-points and the frequent formation of a precipitate before the end-point is reached. Gravimetric determinations, on account of the time required for complete precipitation and the subsequent drying of the precipitate, cannot be accomplished as quickly as the volumetric, but although the actual time spent by the analyst using the volumetric method is less, the results have to be checked as the worker feels uncertain of the end-point. Even then the results are not always satisfactory, as a rule, for the checks are not close enough to indicate the true value.

Where specifications for purity are given as in the U. S. P., it does not seem to be good judgment to put convenience ahead of accuracy. All volumetric methods available and modifications of them were tried out but in each case the checks obtained were not convincing as to the accuracy of a given method.

The following method is the one given in the ninth revision of the United States Pharmacopœia. The results of this method of analyzing four commercial oils are given in Table I.

TABLE I.

Sample.	Weight of oil.	Cc of N/10 $AgNO_3$.	Value of each cc $AgNO_3$ in grams HCN.	Grams of HCN.	HCN Per cent.
1	1.0769	7.40	0.0027	0.02001	1.85
	0.9271	6.64	0.0027	0.01836	1.93
2	1.0331	5.68	0.0027	0.01536	1.49
	0.4924	4.57	0.0027	0.01235	2.51
3	0.6073	6.88	0.0027	0.01859	3.06
	0.5309	7.05	0.0027	0.01909	3.60
4	0.6104	14.01	0.0027	0.03779	6.19
	0.6556	17.67	0.0027	0.04777	7.27

"Dissolve 15 Gm. of crystallized magnesium sulphate in enough distilled water to measure 100 mils, add 5 mils of this solution to 40 mils of distilled water, then add 5 mils of half-normal sodium hydroxide V. S. and two drops of potassium chromate T. S., and titrate the solution with tenth-normal silver nitrate V. S. to the production of a permanent reddish tint. Pour this mixture into a 100-mil flask containing about 1 gram of oil of bitter almond accurately weighed, mix well and titrate again with tenth-normal silver nitrate V. S. until a red tint, which does not disappear on shaking, is produced. Conduct this titration as rapidly as possible."

The first objection to the use of the foregoing method is the immediate formation of a precipitate. This, in itself, would not prevent the reading of an end-point if the precipitate coagulated nicely, but the solution remains cloudy and the precipitate becomes red before the surrounding solution does. Even with constant and vigorous shaking it is hard to be sure that the end-point taken is the true one. The color comes gradually and continues to disappear long after the point is reached which would be the end-point if a time limit of thirty seconds had been set. The results given in Table I are the nearest checks obtained from several consecutive determinations. In some cases the results had to be discarded entirely as the mixture darkened before any satisfactory reading could be made. The darkening was a result of the formation of silver hydroxide, possibly as a consequence of the concentrations of the mixture not having been worked out satisfactorily.

The following gravimetric method combines the best points of several suggested methods and works well. This method calls for little discussion since it presents no difficulty in manipulation. The results of the method tried on four commercial oils are given in Table II.

TABLE II.

Sample.	Weight of oil.	Weight of AgCN.	Equivalent weight of HCN.	Per cent. HCN.
1	1.7001	0.1398	0.02821	1.65
	0.5451	0.0404	0.008154	1.50
2	1.2519	0.0846	0.01707	1.36
	0.6401	0.0431	0.008699	1.36
3	0.3430	0.0561	0.011322	3.30
	0.6458	0.1028	0.020747	3.21
4	0.3085	0.1022	0.020626	6.68
	1.0510	0.3708	0.074825	7.12

One gram of the oil is weighed accurately in a tared, stoppered flask and dissolved in 15 cc of 95 per cent. alcohol. Ten cc of 10 per cent. ammonia water is added for the purpose of decomposing the phenyl-hydroxyacetonitrile which is formed in the oil by a direct reaction of benzaldehyde and hydrocyanic acid. 15 cc silver nitrate T. S. is added and subsequently the solution is acidified with nitric acid. The mixture is shaken until the precipitate has coagulated and it is then filtered on a tared Gooch crucible. The precipitate is washed with distilled water until free from silver nitrate and afterwards dried at 100° C. The crucible and contents are weighed, and the per cent. of hydrocyanic acid calculated from the following formula:

$$\text{Per cent. HCN} = \frac{\text{weight ppt.} \times 0.20182 \times 100}{\text{wt. oil taken}}$$

The gravimetric method is the most accurate and best for determining the hydrocyanic acid content.

The determination of the benzaldehyde content of oil of bitter almond is a more difficult problem. Much work has been done on the assay of the extract of the oil but little on the oil itself. Three methods were tried, the bisulphite, volumetric and gravimetric. The last-named method, which will be described further on, proved to be the only accurate one. The bisulphite method, checked against C. P. benzaldehyde, was discarded early in the experiment as the actual results were far from the theoretical values and there were no checks which could possibly be accepted as such.

In trying out the methods, C. P. benzaldehyde was used as the standard and the value of the method judged by the analysis of it. The volumetric method tried is the one given in U. S. P. IX, with the exception that 20 cc water was added before filtering the mixture. The method follows and the results are given in Table III.

TABLE III.

Sample.	Weight of oil.	Cc *N*/2 HCl for phenyl-hydrazine blank.	Cc of *N*/2 HCl added to oil plus phenyl-hydrazine.	Cc of *N*/2 KOH used in back titration.	Cc of *N*/2 HCl diff. between blank and sample.	Value of each cc. *N*/2 HCl in grams of benzal-dehyde.	Per cent. benzalde-hyde.
1	0.2990	21.22	36.39	20.48	5.31	0.05304	94.2
	0.4944	21.22	29.89	15.05	8.88	0.05304	95.26
	0.4782	22.10	24.91	11.73	8.92	0.05304	98.92
	0.5133	21.54	24.91	12.2	9.34	0.05304	96.51
	0.9213	21.54	24.91	18.4	16.44	0.05304	94.65
2	1.2370	23.09	31.70	28.68	20.07	0.05304	86.0
	0.6600	23.09	32.28	20.48	11.27	0.05304	90.57
	1.0879	22.79	28.97	25.60	19.42	0.05304	94.69
	0.3898	22.10	24.96	10.12	7.26	0.05304	98.65
3	0.7601	19.21	25.25	18.13	12.09	0.05304	84.36
	0.7546	22.6	20.2	8.42	10.82	0.05304	76.05
	0.7923	19.02	25.25	18.13	12.09	0.05304	80.93
	0.5583	19.21	25.25	15.06	9.02	0.05304	85.69
4	0.5555	22.10	24.91	11.84	9.03	0.05304	86.22
	0.4942	24.03	30.3	13.04	6.77	0.05304	72.66
5	0.7824	16.48	20.2	14.83	11.11	0.05304	75.31
	0.5859	20.2	18.31	5.05	6.94	0.05304	62.82
	0.7837	21.54	24.91	13.16	9.79	0.05304	66.19

"Dissolve about 3 mils of freshly redistilled phenylhydrazine in 60 mils of alcohol and titrate 25 mils of this solution, which must always be freshly prepared, with half-normal hydrochloric acid V. S., using methyl orange T. S. as indicator. To about 1 Gm. of the oil of bitter almond, accurately weighed, add 25 mils of the phenylhydrazine solution just prepared and allow it to stand for thirty minutes. Add a drop of methyl orange T. S., and acidify the mixture by adding a measured excess of half-normal hydrochloric acid V. S. Filter the mixture and wash the precipitate with small portions of distilled water until the washings cease to redden blue litmus paper. Then titrate the excess of hydrochloric acid in the filtrate with half-normal potassium hydroxide V. S. and subtract the number of mils of the half-normal hydrochloric acid V. S. consumed from the number of mils of the half-normal hydrochloric acid V. S. used in titrating the 25 mils of phenylhydrazine solution; the difference multiplied by 0.053 gives the weight of benzaldehyde."

In the method just described there is no end-point. The solution, before any indicator is added, is yellow and the change from yellow to red in the blank is very gradual. An arbitrary color change may be taken as an end-point on the blank but unless the titration is taken back and forth with half-normal hydrochloric acid and potassium hydroxide, there can be no certainty in the choosing of an end-point on the sample.

In using this method, it is always necessary to check the blank, either by adding a known excess of acid and titrating back or by titrating a fresh blank. The addition of water before filtering helps somewhat, but there is still a tendency toward cloudiness so that the final end-point is often obscured.

The values given in Table III are the results of consecutive determinations and are arranged in the order in which they were carried out in the laboratory. When the work was begun, it was thought that the end-point might be gradual because the wrong indicator had been used, so several others were tried. Thymol blue (p_H 1.2 to 2.8) was the only one which gave any satisfaction at all, but the results obtained with it were no more accurate than those obtained with methyl orange so that there was no apparent advantage in changing the indicator.

The results given in Table III are only those of determinations carried out uniformly in every respect. Variations were tried but as the results obtained showed no more accuracy than the others, no record of the results appeared necessary. Table III bears out the foregoing criticisms and indicates that the method is so inaccurate as to make it of little practical value.

The third and last method tried was the gravimetric method in which the benz-

TABLE IV.

Sample.	Weight of oil.	Weight of benzylidene-phenylhydrazone.	Weight of benzaldehyde.	Per cent. benzaldehyde.
1	0.1563	0.2791	0.15094	96.57
	0.2320	0.4179	0.2260	97.41
2	0.1152	0.1945	0.10519	91.31
	0.1448	0.2438	0.13185	91.06
3	0.2380	0.3823	0.20675	86.87
	0.1730	0.2812	0.15208	87.90
4	0.1817	0.2530	0.13683	75.31
	0.1072	0.1524	0.08242	76.88
5	0.1014	0.1349	0.07296	71.95
	0.1191	0.1575	0.08515	71.52

aldehyde is precipitated as benzylidenephenylhydrazone by phenylhydrazine. The method follows and the results of its use are given in Table IV.

The reagent for precipitation is made up as in the determinations of benzaldehyde in extract of bitter almond. Three cc of glacial acetic acid is added to 40 cc of water and mixed thoroughly. Two cc of freshly redistilled phenylhydrazine is added to this and the mixture again shaken.

Weigh accurately 0.1 to 0.15 Gm. of oil of bitter almond in a tared stoppered flask; dissolve in 10 cc of alcohol and add 15 cc of the phenylhydrazine mixture. Allow the mixture to stand in a cool, dark place for several hours. Add 200 cc of water and filter on a prepared Gooch crucible which has been previously weighed. Wash the precipitate freely with water and finally with 10 cc of 10 per cent. alcohol. Dry to constant weight in a vacuum desiccator over sulphuric acid or in a vacuum oven at 70° C. Weigh the Gooch and precipitate and calculate the amount of benzaldehyde in the sample according to the following formula:

$$\text{Per cent. benzaldehyde} = \frac{\text{weight of ppt.} \times 0.5408 \times 100}{\text{wt. of oil}}$$

It is most important in this method that the phenylhydrazine be freshly redistilled and be kept in a cool, dark place after distillation until ready for use. If the oil is dissolved in too much alcohol, the precipitate does not form well but this is easily remedied by the addition of a small amount of water. In case a larger amount of the oil is weighed, the amount of the phenylhydrazine mixture added should be increased 10 cc for each 0.1 Gm. of oil in excess of 0.15 Gm. This method is practically the one used by Denis and Dunbar (*Jour. Ind. and Eng. Chem.*, 1909) in analyzing almond flavoring extract. They found that it gave 97 to 99 per cent. of the theoretical yield and that benzoic acid or nitrobenzene did not affect the results. Results in Table IV show that 96 to 98 per cent. of the benzaldehyde may be determined by this method and checks may be made if desired.

SUMMARY.

1. The volumetric method of determining the hydrocyanic content of oil of bitter almond is unsatisfactory because of the impossibility of judging the end-point.

2. The gravimetric method for hydrocyanic acid works well, giving close checks.

3. The volumetric method for determining the benzaldehyde in oil of bitter almond is inaccurate, giving results from 94.2 to 98.9 per cent. on C. P. benzaldehyde.

4. The gravimetric method for benzaldehyde gives from 96 to 98 per cent. of theoretical value and is therefore more accurate.

BIBLIOGRAPHY.

E. J. Parry, "The Chemistry of Essential Oils," Scott, Greenwood & Sons, London, 1908.

A. H. Allen, "Commercial Organic Analysis," Vol. III and IX, P. Blakiston's Son & Company, Philadelphia, Pa.

Schimmel & Co., "Semi-Annual Reports," Schimmel & Co., Miltitz near Leipzig.

E. Gildemeister and Fr. Hoffmann, "The Volatile Oils," John Wiley & Sons, New York, 1913.

Association of Official and Agricultural Chemists, "Methods of Analysis," Second Edition, 1919.

W. Denis and P. B. Dunbar, *Journal of Ind. and Eng. Chem.*, I, 256, 1909.

LABORATORIES OF LEHN & FINK, INC.,
BLOOMFIELD, N. J.