

T H E S I S

on

THE QUANTITATIVE DETERMINATION OF CARVONE  
IN ESSENTIAL OILS

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by

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## INTRODUCTION

The study of a method for the determination of carvone in the official<sup>1</sup> volatile oils was begun after attempting to determine the carvone content of oil of caraway by the method outlined in the United States Pharmacopoeia. The U. S. P. method is not satisfactory as the neutralization of the sodium hydroxide, formed by the hydrolysis of carvone sodium sulphite, cannot be accomplished in the size flask prescribed in the official monograph. Assay by the U. S. P. IX<sup>2</sup> method is also unsatisfactory as the accuracy is not greater than one per cent. In this method one hundredth part of a cubic centimeter must be estimated and multiplied by ten in order to arrive at the volume of carvone present.

A study of the literature revealed that no simple accurate method has been developed for the determination of carvone in essential oils. The U. S. P. IX gives the following monograph for the assay of carvone in oil of caraway: introduce 10 mils of the oil into a 200 mil flask with a long graduated neck (cassia flask)

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1. Oils listed in the "United States Pharmacopoea" and "The National Formulary."
  2. "Ninth Revision of the United States Pharmacopoeia," p. 289.

by means of a pipette, add fifty mils of a saturated solution of sodium sulphite, which has been carefully rendered neutral to phenolphthalein T. S. by means of acetic acid, heat the mixture in a bath containing boiling water and shake the flask repeatedly, neutralizing the mixture from time to time by the addition of a few drops of dilute acetic acid. When no coloration appears, upon the addition of a few more drops of phenolphthalein T. S. and heating for fifteen minutes, cool, and, when the liquids have separated completely, add sufficient of the sodium sulphite solution to raise the lower limit of the oily layer within the graduated portion of the neck and note the volume of residual liquid. This measures not more than five mils, indicating the presence of not less than fifty per cent by volume of carvone.

The U. S. P. X<sup>1</sup> has modified the preceding monograph in the following manner: the size of the flask has been changed from 200 mils to 100 mils and a five per cent solution of sodium bisulphite has been substituted for dilute acetic acid to neutralize the sodium hydroxide.

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1. "Tenth Revision of the United States Pharmacopoeia," p. 253.

Most of the methods that have been suggested are based on the formation of either a semicarbazone or a carvoxime.

Reilly and Drumm<sup>1</sup> used semicarbazide hydrochloride for the determination of carvone. The semicarbazone, which is formed in the reaction, is insoluble and may be separated and weighed. Corrections must be made for a slight solubility and the method is reported as yielding ninety-seven per cent of the theory.

Alden and Nolte<sup>2</sup> used hydroxylamine hydrochloride for the same purpose as did Walker<sup>3</sup> a few years later. The error in both cases is described as being several units per cent.

Kremmers<sup>4</sup> has introduced several modifications of the hydroxylamine hydrochloride methods which have increased their accuracy but the reported error is approximately two per cent.

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1. Reilly and Drumm: "Determination of Carvone in Dill Oil," *Analyst* 53:209-11.
  2. Alden and Nolte: "Estimation of Carvone in Volatile Oils." *Pharm. Archives*, 1899.
  3. Walker: "Estimation of Carvone in Etherial Oils." *Pharm. Centr.*, 1900.
  4. Kremmers: "Jour. Soc. Chem. Ind.," 1904.

These methods and their modifications involve complications and are not particularly accurate, for the compounds formed are slightly volatile and arbitrary corrections are necessary to account for the losses apparently occurring from the manipulation of the various processes.

All of the methods advanced up to the present time are open to the following criticisms: the shorter methods are inaccurate; the longer methods are involved and are not suitable for use in the United States Pharmacopoeia, and, in many cases, are but little more accurate than the shorter ones.

## EXPERIMENTAL APPROACH

The structural formula<sup>1-2</sup> of carvone and its sulphite addition products suggested three possible methods for its quantitative determination in essential oils. These methods were: estimation from absorbed halogen, estimation from calcium combined as carvone calcium sulphite, estimation from the sulphite combined as carvone calcium sulphite. The third method was finally adopted as the most feasible.

ATTEMPTED HALOGEN METHODS. It was believed possible to estimate carvone quantitatively from the halogen absorbed at its three double bonds or gravimetrically from a possible insoluble addition product. Bromine and iodine were the halogens used in the assays attempted in this phase of the problem. Koppeschaar's<sup>3</sup> solution was used first and then a similar solution containing iodide and iodate. It was found that the amount of halogen entering into combination depends

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1. Schmidt, Dr. Julius: "Text of Organic Chemistry."

2. "Lehrbuch der Organischen Chemie."

3. U. S. P. X, p. 498.

upon several factors: time of reaction, temperature, and the presence of an immiscible solvent.

Temperature was found to have the greatest effect on the amount of halogen entering into combination with carvone. Koppeschaar's solution when allowed to act for a period of forty-five to sixty minutes adds approximately two atoms of bromine if the reaction is carried on at room temperature. Increasing the temperature to 100 degrees, apparently, will add successively three, four and five atoms depending on the time the reaction is allowed to proceed. A solution of iodine, similar to Koppeschaar's reagent, gives in general the same results.

When an immiscible solvent was added to dissolve the carvone and the halogen the results were found to be lower than when the determination was carried out in a water solution, under the same conditions of time and temperature. Solutions of iodine or bromine in chloroform or carbon tetrachloride gave disappointing results. The solutions were too volatile to be standardized and the addition product of the reactions was soluble, which prevented its removal from the solution for gravimetric estimation.

When an aqueous solution of either bromine or iodine are added to carvone, care being observed to keep the conditions exactly the same for both samples, the calculated percentage of carvone will check with surprising accuracy. The difficulty, however, is to regulate the conditions to secure a carvone addition product containing a definite number of halogen atoms. It is the belief of the writer that with a properly standardized procedure that this method could be used for the quantitative determination of carvone. Insufficient time prevented an extensive study of the problem in this manner.

The following data is a portion of that obtained from attempted determination of carvone with bromine and iodine:

Reagent	Known Wt. Carvone	Exp. Wt. Carvone	Per Cent Recovered
A. Koppeschaar	0.0974	0.0791	81.21
B. Koppeschaar	0.1137	0.0946	83.20
C. Koppeschaar	0.1334	0.1322	99.10
D. Koppeschaar	0.1383	0.1350	97.61
E. Iodine-iodate	0.1295	0.11105	85.75
F. Iodine-iodate	0.1562	0.1339	85.72

Samples A and B were heated on a water bath for a period of forty-five minutes with a known volume of standard reagent. The flasks were fitted with a reflux condenser and sealed with a curved tube containing potassium iodide solution to prevent escape of bromine. The flasks were cooled after heating the specified length of time; the condenser was washed with distilled water; and the washings and the potassium iodide solution used to seal the condenser was added to the contents of the flask. The amount of bromine absorbed was then determined by titration with standard thiosulphate solution, using starch indicator.

Samples C and D determined as above except that five cubic centimeters of chloroform were added after the titration was started. This procedure gives a clearer endpoint.

Samples E and F were heated on a water bath for a period of sixty minutes with acid iodide-iodate solution.

In all cases the calculations were based on the assumption that four atoms of iodine combined with one molecule of carvone.

CALCIUM METHOD. The reaction between sodium sulphite and carvone in the U. S. P. method will not

go to completion unless the sodium hydroxide formed by the hydrolysis of the carvone sodium sulphite is neutralized with sodium acid sulphite. Hence, it appeared advisable to revise the assay scheme to secure a more rapid and less laborious method to bring the reaction to completion. It was believed that an atom of calcium, rather than one of sodium, in the addition compound with sulphite would result in the formation of a water insoluble compound and that the speed and completeness of the reaction would be increased.

A calcium acid sulphite solution, containing an excess of sulphurous acid, was added to a known weight of carvone in an iodine flask. The flask was stoppered firmly and heated on a sand bath at a temperature between seventy and eighty degrees until the carvone entered into the solution which was about two hours.

A precipitate formed in the flask but upon examination it proved to be calcium sulphite, and additional tests demonstrated that the carvone calcium sulphite compound is water soluble. After ascertaining the carvone formed a water soluble addition product with calcium acid sulphite, the possibility suggested itself that a determination of the combined calcium might be used as a means of estimating the carvone.

To estimate the calcium in combination with the carvone it was necessary to remove that present in the solution as calcium acid sulphite. Oxalic acid was added to the solution and a copious precipitate formed, which was removed by filtration, the carvone calcium sulphite hydrolyzed by ammonia water, and the liberated calcium precipitated as calcium oxalate. This was collected, washed, dried and ignited. The amount of calcium oxide obtained, however, was much too large to have been combined with the carvone. The varying results of several such determinations showed that not all the calcium combined as acid sulphite was being removed as the oxalate from the acid solution.

A second solution was carefully neutralized with ammonium hydroxide, using phenolphthalein indicator, and then precipitated with ammonium oxalate. The calcium oxide obtained from several trials by this method was too low, indicating hydrolysis of the calcium carvone sulphite in alkaline solution. The pH value of phenolphthalein is about nine, and over neutralization suggested itself. A new series of trials was started using methyl orange indicator, pH value approximately four. This change in pH was not sufficient to prevent hydrolysis as the results again ran low.

The carvone calcium sulphite compound is apparently not affected by the oxalic acid treatment, even after the temperature has been raised to the boiling point. This fact was verified by determining the optical rotation of a known dilution of the solution before and after treatment with oxalic acid. After corrections for dilution are applied there is apparently no change in the rotary power of the compound. The reversal of the optical activity of the carvone in calcium sulphite solution was a very interesting fact and should be studied as another means of quantitative determination.

Under the conditions of the experiment it was impractical to find the acidity of the solution and attempt to correct for dissolved calcium after the oxalic acid precipitation.

An attempt was made to remove the calcium acid sulphite by converting it to a carbonate. The calcium acid sulphite solution of carvone was heated to boiling and barium chloride added to remove the excess sulphite. This precipitate was removed by filtration and the filtrate treated with ammonium carbonate. Again the acid condition of the solution had increased the

solubility of the barium and calcium salts to such an extent that the results of the analyses were high, although much better than in the previous method.

SULPHITE-SULPHATE METHOD. The determination of calcium was surrounded by so many difficulties that it appeared more feasible to attempt a determination of the combined sulphite rather than the calcium. The principal steps in the method of analysis may be briefly listed as follows: carvone calcium sulphite was formed by warming a known weight of carvone with a sulphurous acid solution of calcium sulphite, removing the excess sulphite by oxidation with iodine in the presence of barium chloride, filtered, and the carvone calcium sulphite compound hydrolyzed with alkali and the liberated sulphite oxidized to sulphate and weighed as barium sulphate.

A solution of carvone was made with calcium acid sulphite reagent\* and made up to volume. Aliquot parts were placed in beakers, heated to the boiling point, and an excess of barium chloride added to precipitate the excess sulphite, and filtered. The filtrate was

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\* Strength of all reagents used listed in appendix.

titrated with iodine until a blue color, using starch as an indicator, was obtained that persisted for a period of about two minutes. The sulphate formed was precipitated as barium sulphate by the excess of barium chloride in the solution. The filtrate from this precipitation was then made alkaline by adding solid potassium hydroxide and twenty-five cubic centimeters of hydrogen peroxide. This solution was heated to boiling and boiled gently for twenty minutes to complete the destruction of the carvone compound and to oxidize the sulphite to sulphate. As the oxidation proceeds the solution changes from colorless to brick red. The solution was acidified with hydrochloric acid to dissolve the hydroxides of calcium and barium and filtered through an ashless filter and the barium sulphate weighed. The results were comparable but low based on the assumption that one sulphite radicle combines with one molecule of carvone.

Another series of determinations was made adding acetic acid to the solution before titrating with iodine for it seemed likely that hydrolysis took place when the sulphite was completely removed. The results obtained from this modification were much better and comparable

between themselves; however, they were low, averaging about ninety-four per cent.

A third series of determinations were started and the acetic acid added before the sulphite was precipitated with barium chloride. In this set of samples the solution was heated to the boiling point and removed from the flame to prevent the loss of the acid added. The results from this series of determinations were comparable between themselves and averaged around ninety-eight per cent.

Acetic acid having suggested itself for the prevention of hydrolysis as it was used in the U. S. P. IX for neutralization of the hydroxide formed in the determination of carvone, hydrochloric acid was tried out but the results were not satisfactory. Varying amounts of acetic acid were then tried and from the limited amount of work done the optimum amount appears to be about four cubic centimeters.

LABORATORY PROCEDURE. Approximately one gram of carvone was weighed and placed into a two hundred fifty cc. iodine flask. One hundred cubic centimeters of calcium acid sulphite reagent added and the flask, tightly stoppered, placed on the sand bath. Digestion was allowed to proceed until the carvone was all

dissolved, which took about three hours if the flask was shaken occasionally. When the solution was complete the stopper was removed and a part of the sulphur dioxide was driven off as shown by the precipitate of calcium sulphite in the flask. The reason for this was to prevent the formation of a precipitate after it was made up to volume. The solution was filtered into a two hundred fifty cubic centimeter volumetric flask and the flask and precipitate washed with distilled water until it was up to volume.

A fifty cubic centimeter aliquot was measured and placed in a six hundred cubic centimeter beaker and four cubic centimeters of dilute acetic acid were added. The solution was brought to a boil and twenty-five cubic centimeters of barium chloride were added. The solution was filtered while hot; the filtrate did not need to be entirely free from precipitate for the purpose was to remove the excess of sulphite so it would not require iodine for its oxidation. Iodine was then added until a blue color was obtained, from starch indicator, which persisted at least two minutes. During this titration an excess of barium chloride was necessary to remove the sulphate formed. The solution was then filtered. A Buechner funnel with paper on top

of an asbestos mat was found to be the most satisfactory for this filtration as the barium sulphate is largely amorphous.

The filtrate is made alkaline by adding solid potassium hydroxide and twenty-five cubic centimeters of hydrogen peroxide. The solution is then boiled gently for fifteen minutes or until the maximum red color develops. Hydrochloric acid is then added until all of the hydroxides of barium and calcium are dissolved, the solution boiled gently for about five minutes and filtered through an ashless filter paper. The precipitate is then ignited, weighed and calculated on the basis of one molecule barium sulphate for each molecule of carvone.

Oil of caraway is run in the same manner except that the sample taken may be increased to one and a half grams.

DATA. Carvone: From the Organic Chemistry Division, Eastman Kodak Company.

<u>Sample</u>	<u>Wt. of Carvone</u>	<u>Theoretical Wt. BaSO<sub>4</sub></u>	<u>Experimental Wt. BaSO<sub>4</sub></u>	<u>Per Cent Carvone</u>
No. 1	0.24898	0.38717	0.3813	98.48
No. 2	0.22384	0.34806	0.3462	99.46
No. 3	0.28142	0.4376	0.4302	98.30
No. 4	0.28142	0.4376	0.4324	98.81
No. 5	0.28142	0.4376	0.4579	104.63

<u>Sample</u>	<u>Wt. of Oil Caraway</u>	<u>Theoretical Wt. BaSO<sub>4</sub></u>	<u>Experimental Wt. BaSO<sub>4</sub></u>	<u>Per Cent Carvone</u>
No. 1	0.2565	0.3988	0.2120	53.13
No. 2	0.3568	0.5548	0.2993	53.94

## CONCLUSIONS

The results of the work done with halogens seems to indicate that under standardized conditions the absorption of a halogen could be used to measure the amount of carvone, but it would be a difficult process to standardize.

The determination of carvone by measurement of the calcium would be possible under two conditions: the removal of the excess calcium sulphite, or the removal of the calcium sulphite to the point where a definite amount was known to exist in the solution.

The sulphite-sulphate method appears to be more accurate than most of the gravimetric methods now in use. Kremmer's hydroxylamine hydrochloride method shows an error of two per cent and is considered the most accurate work that has been done. The average of the first four results of the sulphite-sulphate method show an error of one and twenty-five hundredths per cent. The fifth result listed was omitted in the average, for it is undoubtedly high and would give a false impression of the accuracy of the method.

From the limited work done on the oil of caraway, the method will, without question, give good results in

the determination of carvone in the oil. Further work on the application of the process to the various essential oils should be carried out, but lack of time prevented the work being done by the author.

## APPENDIX

CALCIUM ACID SULPHITE REAGENT: Eight grams of calcium sulphite for each hundred cubic centimeters of distilled water is put into solution by passing sulphur dioxide through the solution.

BARIUM CHLORIDE: An approximately one normal solution.

ACETIC ACID DILUTE: An approximately six per cent solution.

IODINE SOLUTION: A five per cent solution of iodine in ten per cent potassium iodide.

HYDROGEN PEROXIDE: A three per cent solution.

HYDROCHLORIC ACID: Full strength.

ALL REAGENTS MUST BE FREE FROM SULPHATES AND SULPHITES.

## BIBLIOGRAPHY

- Alden, F. W. and Nolte, S.  
Estimation of Carvone in Volatile Oils 1899  
Pharm. Archives, 2:81-91.
- Walder, J.  
Estimation of Carvone in Etherial Oils 1900  
Pharm. Centr., 41:613-616.
- Sadtler,  
Method of Sadtler 1904  
Jour. Soc. of Chem. Industry, 23:304
- Simmons, W. H.  
Perfumery Essence 1926  
Oil Record, 17:334-342.
- Reilly, J. and Drumm P. J.  
Determination of Carvone in Dill Oil 1928  
Analysist, 53:209-211.