

Analysis of Certain Volatile Oils in Aerosol Formulations

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The analysis of several volatile oils was made utilizing both the chemical method and the method of gas chromatography. Pure samples and mixtures of volatile oils were subjected to these methods of analysis. In addition, samples of volatile oils contained in an aerosol preparation were determined. The existing methods of analysis were modified to accommodate the aerosol package. While gas chromatography is useful, it has no real advantage over the existing chemical analysis. However, in the analysis of mixtures of volatile oils gas chromatography is essential.

THE CHEMICAL evaluation of most essential oils depends primarily upon the determination of some functionally active group considered to be characteristic of that component of the oil which is the most abundant or most essential constituent present (1-6). By determining the percentage of this specific component present in the pure oil, one can utilize this information to determine the amount of the volatile oil present in an unknown preparation. With mixtures of volatile oils containing components with the same functional groups, this type of analysis becomes more complex, and other methods of analysis must be utilized.

Aerosols are becoming increasingly more important in the pharmaceutical field since many therapeutically active agents are packaged in this manner. They show promise of becoming a most important mode of administration for topical use as well as for inhalation therapy. Analytical procedures must be developed so that the aerosol formulation may be analyzed accurately. Many aerosol proprietaries contain varying amounts of essential oils. The aerosol vaporizer and many of the topical aerosols containing perfumes and other volatile oils are examples of the possible application of the analytical procedure presented in this paper.

This investigation is concerned with the possibility of utilizing gas chromatography as a means of circumventing the difficulties encountered in attempting to establish the percentages of volatile oils in those mixtures having similarly chemically reactive groups and also to determine the possibility of ascertaining the percentage of certain volatile oils when formulated into an aerosol package.

EXPERIMENTAL

Determination of Purity of Oils.—An official (3, 5, 6) or other acceptable assay (4) was carried out on each oil to determine quantitatively the amount of essential constituent present. The volatile oils included cinnamon oil U.S.P.; spearmint oil N.F.; benzaldehyde N.F.; water white imported camphor oil (Magnus, Mabee, and Reynard, Inc.); benzaldehyde-spearmint oil mixture; and spearmint oil-camphor oil mixture.

Since the volatile oils will contain varying amounts of the essential components, it was necessary to determine initially the quantity of carbonyl-containing components in each of the respective pure oils and mixtures. The results of these determinations appear in Table I and represent the purity for each of the oils used; the values given in the table represent the average of three determinations.

Preparation of Aerosol Samples.—These samples were prepared by accurately weighing about 10-12 Gm. of the oil and about 30 Gm. of absolute ethanol and placing the solution into a metal aerosol container. The container then was sealed with a standard aerosol spray-type valve. About 25 Gm. of propellant 12 (dichlorodifluoromethane) then was added by the pressure method (7) utilizing a gas-tight buret. The total weight of the sample was approximately 65 Gm.

Analysis of Aerosols by Chemical Methods.—The aerosols were analyzed chemically by employing the Stillman-Reed method (2) with certain modifications to accommodate the aerosol product.

The contents of the container were discharged into 75 ml. of hydroxylamine hydrochloride solution by slowly bubbling the contents of the aerosol into the solution to allow for maximum solubility of the oil and prevent loss of oil with the propellant. The device employed in this technique is illustrated in Fig. 1. Sufficient sample was expelled to obtain the equivalent of about 1 Gm. of oil. The transfer tube (polyethylene) was then washed with an additional 25 ml. of reagent to collect any oil retained by the tubing. To determine the weight of a given sample, the aerosol container was weighed accurately before and after removal of the sample. For each of these carbonyl-bearing constituents present, one equivalent of hydrochloric acid was liberated, and the free acid was titrated with a standardized base to a light-green end point, employing bromphenol blue as the indicator. It was desirable to couple this visual means of detection with potentiometric means (8), since the visual end point is rather

Received August 4, 1964, from the Department of Pharmaceutical Sciences, St. John's University, Jamaica, N. Y.

Accepted for publication November 2, 1964.

Presented to the Scientific Section, A.P.H.A., New York City meeting, August 1964.

Abstracted in part from a thesis submitted by Carl B. Rifino to St. John's University, Jamaica, N. Y., in partial fulfillment of Master of Science degree requirements.

The authors express their thanks to Mr. O. Gaglione, Instructor, Department of Pharmaceutical Chemistry, for his assistance during this investigation.

TABLE I.—ANALYSIS OF VOLATILE OILS BY GAS CHROMATOGRAPHY AND CHEMICAL METHODS

Volatile Oil	Compt. Detected	Method Employed	Carbonyl-Containing Compd., w/w %	
			Chemical Method	Gas Chromatography
Cinnamon oil	Total aldehydes ^a	Sulfite-bisulfite (6)	84.10	...
	Total aldehydes ^a	Stillman-Reed (2)	85.50	...
Spearmint oil	Carvone	Sulfite-bisulfite (5)	65.16	67.50
	Carvone	Stillman-Reed (2)	65.50	
Benzaldehyde	Benzaldehyde	Stillman-Reed (2)	97.34	100.00
Camphor oil	Camphor	Aschan (4)	2.27	3.40
	Camphor	Stillman-Reed (2)	4.18	

^a Total aldehydes consist of cinnamaldehyde and other trace aldehydes.

difficult to ascertain. Results of these assays appear in Table II and represent the average of three determinations.

Gas Chromatography.—The apparatus employed for the analysis of volatile oils by gas chromatography was a Perkin-Elmer model 154B Vapor Fractometer equipped with a thermal conductivity cell (9). The column consisted of stainless steel tubing containing Carbowax 1500 supported on acid-washed Celite. Liquid samples were analyzed by injecting the oil (10 μ l.) with a Hamilton syringe into the liquid injection port.

To determine the oil content of the aerosol containers, the temperature of all the components was lowered to about -40° utilizing a dry ice-acetone bath. The aerosol container and its contents as well as the Hamilton gas-tight syringe, beakers, etc., were all lowered to this temperature. This

procedure prevented loss of propellant and oil. The contents then were expelled into the cooled beaker. A sample of 10–15 μ l. was injected with the gas-tight syringe directly into the gas chromatograph. The conditions for this analysis are included on the chromatograms (Figs. 2–5).

Utilizing the method of multiplying the height of the peak by the width at half-height (9), it was possible to determine the percentage of ketone or aldehyde present in the aerosol, which in turn represented a certain concentration of oil in the sample. The results of these determinations are shown in Tables I and II.

Mixtures of Essential Oils.—Certain mixtures of two essential oils, containing two components with the same reactive functional group, were subjected to chemical analysis and gas chromatography. The results of these determinations are shown in Table II.

DISCUSSION

It was necessary to make certain alterations for the chemical analysis of the volatile oils in their aerosol packages because the accepted method of analysis proved to be inadequate. The Stillman-Reed method proved to be the most versatile because it overcame the inadequacies of the others—namely (a) a large sample (10 ml.) of oil is required for analysis by the sulfite-bisulfite method (10), which is impractical for aerosol analysis; (b) the latter method requires small increments of reagent for a satisfactory reaction rate; and (c) the Aschan method is impractical for aerosol analysis.

The evaluation of volatile oils by gas chromatography has been reported by many investigators (11–13).

The utilization of gas chromatography in this study was limited to the determination of only the aldehyde or ketone content of the volatile oils. No attempt was made to identify the other components present since the chemical assays themselves require only an estimation of the carbonyl content to ascertain purity.

The identity of the ketone or aldehyde peaks in the chromatogram was established by comparing

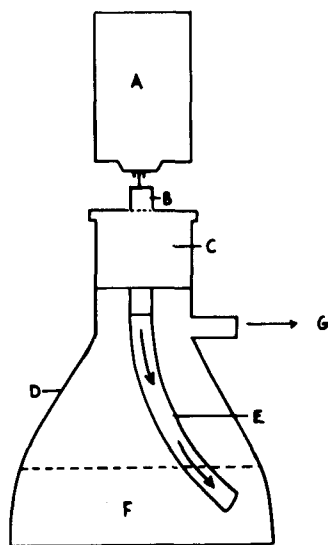


Fig. 1.—Aerosol sampling device. Key: A, aerosol container; B, transfer valve with hypodermic needle; C, rubber stopper; D, vacuum flask; E, plastic tubing; F, solvent or reagent; and G, vapor outlet.

TABLE II.—ASSAY OF AEROSOLS CONTAINING VOLATILE OILS BY STILLMAN-REED METHOD AND GAS CHROMATOGRAPHY

Aerosol Sample	Oil Added, w/w %	Stillman-Reed		Gas Chromatography	
		Carbonyl Found, w/w %	Oil Equiv., w/w %	Carbonyl Found, w/w %	Oil Equiv., w/w %
Spearmint oil	16.29	11.02	16.82	10.72	16.45
Camphor oil	15.55	0.87	41.16	0.37	17.42

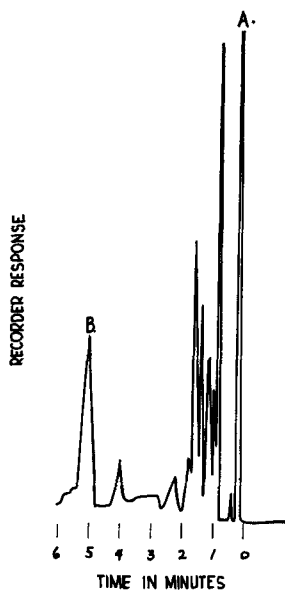


Fig. 2.—Analysis of camphor oil by gas chromatography (8 v.; col. K; column temperature, 176°; 33 ml. helium/min.). Key: A, propellant 12; B, camphor.

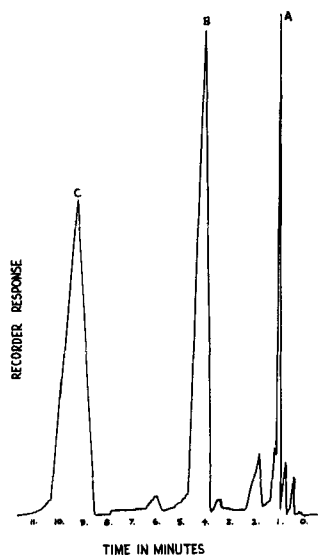


Fig. 4.—Analysis of benzaldehyde (40%) spearmint oil (60%) by gas chromatography (8 v.; col. K; column temperature, 176°; 34 ml. helium/min.). Key: A, propellant 12; B, benzaldehyde and C, carvone.

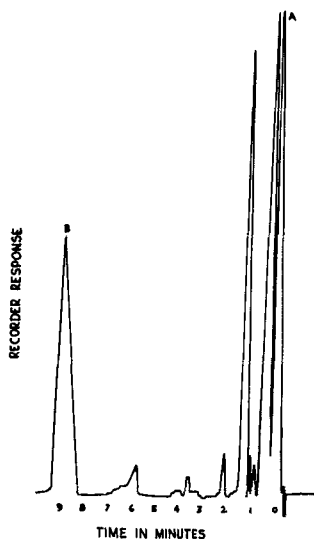


Fig. 3.—Analysis of spearmint oil by gas chromatography (8 v.; col. K; column temperature, 175°; 37 ml. helium/min.). Key: A, propellant 12; B, carvone.

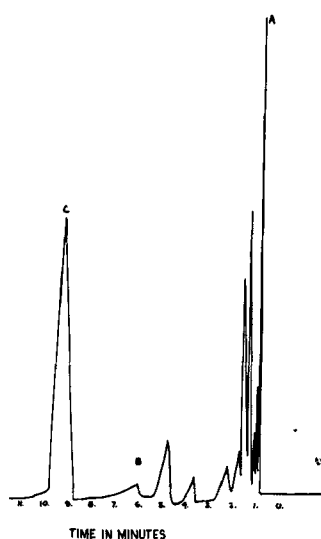


Fig. 5.—Analysis of spearmint oil (30%) camphor oil (70%) by gas chromatography (8 v.; col. K; column temperature, 176°; 34 helium/min.). Key: A, propellant 12; B, camphor; and C, carvone.

TABLE III.—ANALYSIS OF CERTAIN VOLATILE OIL MIXTURES BY STILLMAN-REED METHOD AND GAS CHROMATOGRAPHY

Oil Mixture	w/w %		w/w %	
	Stillman-Reed	Gas Chromatography	Calcd. Oil Equiv.	Theoret. Oil Equiv.
Benzaldehyde-spearmint oil (40:60% v/v)	85.20 ^a	76.25 ^b	Benzaldehyde, 47.53 Spearmint oil, 52.47	47.09 52.91
Spearmint-camphor oil (30:70% v/v)	22.53 ^a	20.03 ^c	Camphor oil, 70.67 Spearmint oil, 29.33	69.69 30.31

^a Total carbonyl. ^b Represents 43.75% w/w benzaldehyde and 32.50% w/w carvone. ^c Represents 2.53% w/w camphor and 17.50% w/w carvone.

the relative retention times and by the enrichment method.

In addition to these methods, two chromatographic columns of different polarities, Carbowax 1500 and silicone oil DC 550, were employed to insure that the separation of all the oil's components was complete. The results obtained by the utilization

of one column were verified upon employing the other.

In gas chromatography, numerical evaluation of a component's peak area is manifested in the units of mole per cent. It is necessary to establish some common link between the mole per cent concentration of this method and the w/w per cent concentra-

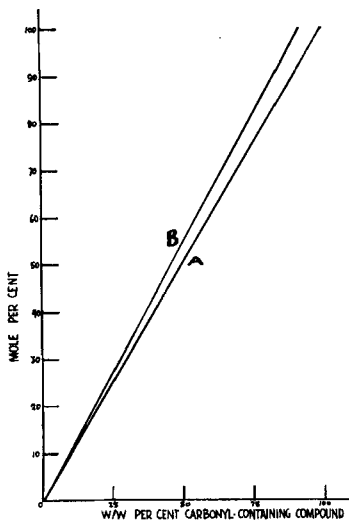


Fig. 6.—Calibration curves for spearmint oil and benzaldehyde. Key: A, carvone; B, benzaldehyde.

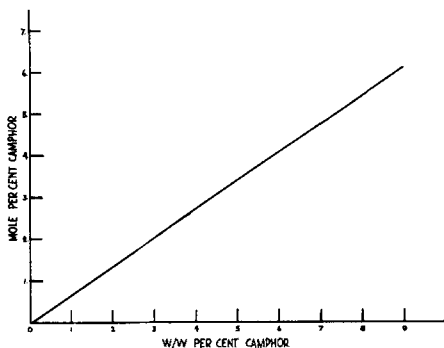


Fig. 7.—Calibration curve for camphor oil.

tion of the chemical methods. This was accomplished by employing alcoholic solutions of the pure ketone or aldehyde, whose concentration was determined accurately on a weight per cent basis, and by the subsequent determination by gas chromatography of the mole per cent concentration. A range of concentrations were employed, and a calibration curve was drawn. (See Figs. 6 and 7.)

A comparative evaluation of the chemical and gas chromatographic analyses of the oil mixtures reveals that the latter is far superior. Utilization of the chemical method for oil mixture analysis enables the analyst to determine the total carbonyl content only when tailor-made situations are constructed. In this study, it was necessary to know exactly the amount of each oil contained in a given mixture. In this manner, it was possible to determine the total carbonyl content. This information, however, was employed to determine the theoretical value as well as the calculated value of the total carbonyl content of the mixture. The latter was accomplished by determining the weight contributed to the molecular weight of a compound by the carbonyl group, and this step eliminates the need to determine the average molecular weight of the compounds in question.

No attempt has been made in this paper to suggest the gas chromatographic method of analysis for cinnamon oil because there has been evidence to suspect that the results obtained were inaccurate. Unfortunately, a reliable separation of this oil could not be obtained by gas chromatography under the conditions of this investigation.

SUMMARY AND CONCLUSIONS

While gas chromatography is accurate, extremely sensitive, and reliable, it has no great advantage over certain chemical methods for the evaluation of many essential oils and their aerosol formulations, except for the time factor and convenience of analysis.

In mixtures of volatile oils, however, the situation is much different. The real advantage of gas chromatography as an analytical tool in essential oil analysis is that the composition of this mixture can be determined accurately. If a mixture, whose composition is unknown, contains oils having the same functional group, it is impossible to determine the percentage of each oil in the mixture by chemical analysis. With gas chromatography, having once established the characteristic chromatogram for the compounds with the same functional groups, it becomes a relatively simple matter to establish the percentage of each such group and in turn translate this into the corresponding percentage of each oil in the mixture.

Even though no attempt has been made to identify the peaks other than those of a ketone or aldehyde of the essential oils, it should be seen readily that the other peaks formed may be analyzed, and they would enable the analyst to determine the concentration of each component of a formulation during one process.

Finally, while it has been shown that the percentage of volatile oil, formulated into an aerosol package, can be determined by modifying the chemical assay for the pure oil itself, gas chromatography offers a better method for determining not only the percentage of one compound in the formulation but also is capable of determining percentages of different compounds in that same formulation.

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