Quantitative Determination of Volatile Components in Pressurized Aerosols by Gas Chromatography

By SHELDON COHEN

Gas chromatography has been used for analysis of aerosol propellants and active ingredients. The propellant analysis generally involved introduction of a previously vaporized sample into the gas chromatograph. Such methods become impractical when dealing with small aerosol packages such as those used for inhalation therapy. The purpose of this work was to develop methods for the accurate sampling and analysis of the propellants and cosolvents of such packages. These methods may also be extended to other volatile components of aerosol packages. Two sampling techniques were developed. These include the use of gas-tight sampling devices to either prepare solutions of the aerosol contents or to load a small-volume, high-pressure, liquid syringe. The volatile components were separated on a porous polystyrene bead column and the composition was calculated from peak areas.

PRESSURIZED AEROSOLS used for inhalation therapy generally are packaged in small containers fitted with metered valves. Accurate sampling of such packages and analysis of the volatile constituents, contained therein, are difficult, since the composition of a small volume of such mixtures in the liquid phase can change considerably by fractionating as it volatilizes. The aerosols may contain a wide boiling range of solvents, from propellant 12, b.p. −29.8°, to some of the glycols with boiling points in excess of 200°. To further complicate matters, the metered valve does not provide a convenient access to sample for such analyses.

Prior to the development of gas chromatography, aerosol propellants were analyzed by several methods such as mass spectrometry, infrared spectrophotometry, liquid density measurements, and vapor pressure determinations (1). Gas chromatography has been utilized for the past decade for the analysis of halogenated hydrocarbons such as used in aerosols (1, 2, 4, 7) and for the analysis of other ingredients in pharmaceutical aerosols (3, 6).

Percival (1) and Thonet (7) used variations of gas sampling systems to obtain samples of aerosol propellants in the vapor state prior to introduction into the gas chromatograph. The components were subsequently separated on di-*n*-octyl phthalate or alumina columns in the former work and on a di-*n*-butyl maleate column in the latter. The use of metered valves makes these methods impractical. There is also the possibility of condensation of the higher boiling constituents in the vapor sampling system and absorption of propellants by rubber "o" rings normally found in gas sampling valves.

Lysyj (4) used a parallel dual column system to analyze a mixture of air, carbon dioxide, propellant 11, propellant 12, and ethylene oxide used in a sterilizing process. The high boiling constituents were separated on a polyglycol column and the low boiling constituents on a dibenzyl ether column. Separate detectors were used to monitor each column simultaneously. The system was intended primarily as a rapid in-process control of a mixture in the gas phase.

Jenkins and Amburgey (2) chilled aerosol samples and dissolved them in large volumes of solvent. They used 0.5–0.8 Gm. of aerosol in 10 ml. of cyclohexane. The components were then separated on a decyl phthalate column. Such a system runs the risk of sample evaporation and fractionation during handling. If water is to be determined there is also the possibility of atmospheric water vapor condensing in the sample.

The methods, to be described here, were developed for the accurate sampling and analysis of the volatile constituents of small pressurized packages used in inhalation therapy. These methods take advantage of some of the newer hardware for handling samples and of porous polystyrene beads as column packing (5) for improved separations of aerosol components.

EXPERIMENTAL

Equipment—An F&M model 810-12 gas chromatograph using a thermal conductivity detector was used. The columns used were Porapak types Q, N, R, and S, 80/100 mesh packed in 2.5 m. (8 ft.) long, 5 mm. ($^3/_{16}$-in.) stainless steel columns. The column temperature was programmed starting at 120° and holding for 1 min. and then heating at 15°/min. to 240°, and holding as needed. The injection block temperature was 210°, and the de-

Received December 4, 1967, from the Riker Laboratories, Northridge, CA 91324

Accepted for publication March 19, 1968.

Presented at the Third Annual Western Regional Meeting of the Industrial Pharmaceutical Technology Section, APhA Academy of Pharmaceutical Sciences, Los Angeles, Calif., October 1967.

The author wishes to thank Mr. T. H. Graves for his able assistance in preparing this manuscript.

tector temperature was 190°. The carrier gas used was helium at a flow rate of 65 ml./min. A 1-mv. Servo recorder equipped with a disk integrator was used.

Hamilton type 701N 10-μl. syringes were used to inject samples into the gas chromatograph in the solution method. A Pressure-Flo high-pressure liquid syringe (Precision Sampling Corp.) of 10 μl. capacity was used to inject samples in the direct method. The gas-tight syringes were Pressure-Lok (Precision Sampling Corp.). These come equipped with valves for containing pressurized samples and were used to prepare sample and standard solutions, and for loading the Pressure-Flo syringe. Sizes convenient for the amount of material being transferred should be used. (High-pressure materials are more easily handled in smaller syringes.) Plastic-coated aerosol bottles that could be fitted with crimp-on type multiple-dose stoppers were used as sample and standard solution containers.

Sampling—A commercial sampling system (Alltek Associates) was used for samples in metal containers. In this system a threaded plate is affixed to the metal container with hose clamps. Smaller hose clamps were substituted for those supplied with the system in order to accommodate the smaller containers. A threaded fitting, closed at one end with a replaceable rubber septum is engaged in the plate threads. The end of the fitting towards the container had a sharpened tube capable of puncturing the container and a rubber gasket around this tube to prevent leakage from the container after puncture. The fitting is twisted into the threads, thus puncturing the can and affixing to it the penetrable rubber septum, through which samples may be withdrawn with gas-tight syringes. In order to prevent septum rupture, it was found advisable to use a septum-retaining nut with an aperture smaller than that of the retaining nut supplied with the system.

For samples in glass containers, a sampling system (Fig. 1) was constructed that enabled the cap to be punctured with an 18-gauge hypodermic needle (C). Leakage at the puncture is prevented by a rubber washer (D). The hub of the needle is fitted with a small rubber septum (B) held in place by a male Luer-Lok plug (A) through which a 1.6 mm. ($^1/_{16}$-in.) hole has been drilled. The sample may then be withdrawn through this septum with a gas-tight syringe.

Samples that do not lend themselves to the use of these devices may be cooled to a temperature sufficiently low to minimize loss and then opened and transferred to a plastic-coated aerosol bottle cooled to the temperature of the sample. The bottle is immediately sealed with a multiple-dose cap through which a sample may be withdrawn with a gas-tight syringe. This operation is conveniently carried out inside an open chest-type freezer to minimize sample loss and moisture condensation. This last method runs a greater risk of sample change than the previous two.

Care must be taken to assure that a minimum change in composition is caused by the sampling procedure. Since the composition of the aerosol will change as it volatilizes, the system must be leak proof, a minimum volume of space should be added by the sampling system to the container, and the volume of space left by withdrawn samples must be considered.

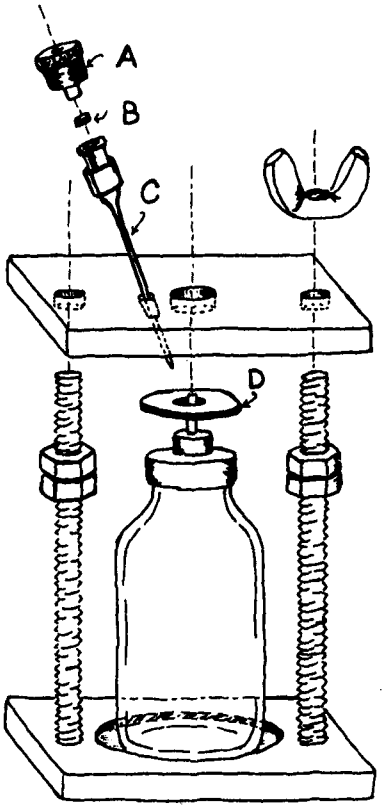

Fig. 1—Sampling system for glass containers.

Solution Method of Analysis—Ethylene glycol monomethyl ether, chloroform, or methylene chloride are suitable solvents for most samples. The choice may be determined by considering the retention time of the solvent relative to those of the aerosol components and by considering the other materials present in the solvent. For example, chloroform normally contains ethanol as a preservative and ethylene glycol monomethyl ether normally contains some water. Ethylene glycol monomethyl ether was found most convenient to use.

Twenty-five milliliters of the solvent is pipeted into a 60-ml. plastic-coated aerosol bottle. A multiple-dose cap is crimped in place and its safety seal removed. The 60-ml. bottle and its contents are weighed; 1.25–2.50 Gm. of the aerosol sample is drawn into a 2-ml. gas-tight syringe from the sample container, which has been prepared by one of the methods described under *Sampling*, and the syringe valve is shut. The sample is transferred from the gas-tight syringe, through the septum, into the tared 60-ml. bottle, the gross weight of the bottle is determined, and the weight of sample taken is calculated.

The standards are prepared in the same manner as the sample. All standard materials that boil near or below room temperature are handled in gas-tight syringes. Less volatile materials can be handled in ordinary syringes. Propellants for the standard solution may be stored in pressurized cylinders and transferred from an inverted cylinder to a gas-tight syringe through a rubber septum

fitted to the cylinder outlet. A 9.6 mm. (3/8 in.) diameter rubber septum can be fitted to 6.4 mm. (1/4-in.) flared tube fitting such as normally found on such cylinders. Alternately the propellants may be stored in the plastic-coated aerosol bottles sealed with multiple-dose caps. One solution may contain several standard components of known amounts. When preparing such a solution the least volatile materials should be added first. It is important that the standard solution contains the identical volume of solvent as the sample solution contains.

The sample solution, the standard solution, and several Hamilton syringes are placed in a freezer at −20°. Just prior to use, a solution and syringe are removed from the freezer, 5–8 μl. of the solution is immediately loaded into the syringe and injected into the gas chromatograph. The recorder chart drive is turned on and the temperature program is initiated simultaneously with the injection. The chromatograph is attenuated for maximum response on scale for each peak. Each component in the sample solution is run at the same attenuation as in the standard solution. A chromatogram of the solvent is run in the same manner as the other solutions. Care should be exercised to avoid bubbling the solutions in the Hamilton syringes. The injections should be made in a reproducible manner. Figure 2 illustrates a chromatogram obtained by this method.

From the integrator record the areas of each component of the sample solution and the standard solution are determined. The attenuation factor can be ignored for any component as long as it is the same in each chromatogram. The areas are corrected geometrically for any base line or integrator drift. The ratios of the area of each component to that of the solvent are calculated. These ratios are corrected if necessary for the blank solvent. For each individual component in the sample:

$$\% \text{ component (w/w)} = \frac{100\ (R_A)(W_S)}{(R_S)(SW)}$$

where, R_A = ratio of the area of the component peak to that of the solvent peak in the sample solution, R_S = above ratio in the standard solution, W_S = grams of component in the standard solution, SW = sample weight (Gm.) of the aerosol in the sample solution.

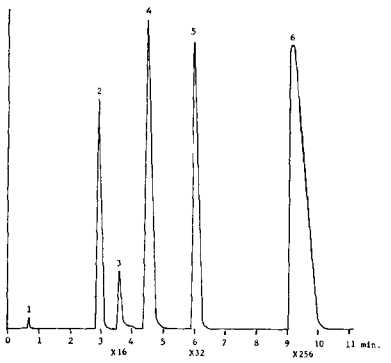

Fig. 2—Chromatogram of aerosol components on a Porapak R column. 1, air; 2, propellant 12; 3, water; 4, propellant 114; 5, ethanol; 6, ethylene glycol monomethyl ether.

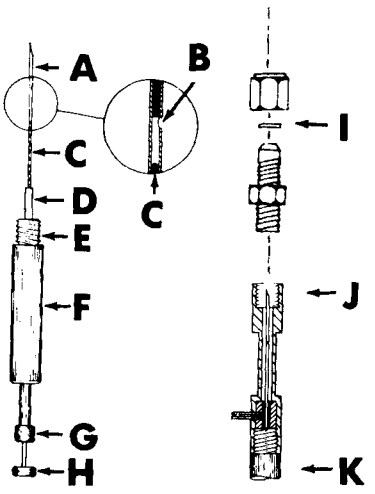

Fig. 3—Pressure-Flo syringe system.

Direct Method of Analysis—Samples analyzed by this method should be limited to those that will not impair the performance of the Pressure-Flo syringe with any residue. Using a 6.4-mm. flared tube to 3.2-mm. (1/8-in.)M.P.T. adapter (Fig. 3) a 6.9-mm. diameter rubber septum (I) is fitted to the sampling end (J) of the filling adapter supplied with the syringe. The retaining nut holding the septum in place should have a reduced aperture to prevent septum rupture. The Pressure-Flo syringe is fitted to the other end (K) of the adapter. The sample is injected through the rubber septum, into the adapter with a gas-tight syringe, preferably of 1.0 ml. capacity, Manual pressure is maintained on the plunger of the gas-tight syringe throughout the filling operation. The manufacturer recommends 25 p.s.i. pressure in excess of the sample vapor pressure to assure a reproducible sample. Unless elaborate equipment is available for determining this, the best approach is a few trial runs to develop an operator feel for the amount of pressure necessary.

The plunger handle (H) of the Pressure-Flo syringe is slowly pumped several times and left in the withdrawn position. This retracts the plunger (C) and allows the sample to enter the hollow needle through the port (B). If a volume of less than 10 μl. is desired using the 10-μl. syringe, a shim can be made so that the plunger is left in a reproducible partially withdrawn position. The needle handle (G) is now withdrawn, drawing the needle (A) into the sealing tube (D), thus entrapping the sample. The Pressure-Flo syringe is then removed from the adapter and a small Teflon sleeve is slipped over the sealing tube (D). The sleeve prevents the sealing tube from puncturing the injector septum of the chromatograph. The sealing tube should protrude slightly beyond the Teflon sleeve, enabling it to slightly penetrate the injector septum. The sealing tube is firmly butted against the injector septum and the needle handle (G) is rapidly depressed, driving the needle through the injector septum and allowing the sample to vaporize in the injection port. The plunger handle (H) is immediately depressed, pumped several times, and left in the depressed position to remove any residual vapor from the hollow needle. The needle handle (G) is with-

drawn, disengaging the syringe from the septum. The recorder chart drive is turned on and the temperature program is initiated simultaneously with the injection. The chromatogram is attenuated for maximum response, on scale, for each peak.

A mixture of the components found in the sample is accurately prepared. The composition should approximate that of the sample. This is done by weighing the components, in order of increasing volatility, into an evacuated plastic-coated aerosol bottle sealed with a multiple-dose cap. Gas-tight syringes are used where needed. To prevent composition changes in the standard mixture, the head space in the container should initially be limited to less than 10% of the liquid volume and should not exceed 40% as the standard mixture is used. This should prevent changes in composition from exceeding 0.5% relative. The standard mixture is treated in the same manner as the sample. The chromatogram is run at the same attenuations used for each component in the sample. Figure 4 illustrates a chromatogram obtained by this method.

From the chromatogram of the standard mixture, the relative detector response for each component is determined as compared to one arbitrarily selected for reference (that is, relative detector response = unity).

$$R = \frac{A/C}{A_r/C_r}$$

where, R = relative detector response for a given component, A = peak area for the component in the standard, C = percent composition of the component in the standard, A_r = peak area of the reference component in the standard, C_r = percent composition of the reference component in the standard. Then for each component in the sample:

$$\% \text{ composition} = 100\,\frac{A/R}{S}$$

where, S = sum of the ratios, A/R, for all the components in the sample. This calculation provides the composition in terms of the percent of the volatile portion of the sample.

DISCUSSION

Because of limitations of the Pressure-Flo syringe, only highly volatile systems could presently be analyzed by the direct method. The direct method offers applicability for rapid analysis and process control of systems which contain components of known identity. Samples containing suspended solids may be analyzed by the direct method if they can be filtered through a Swiney adapter from a gas-tight syringe. If extensive pretreatment is required, it may be better to use the solution method. The solution method offers general applicability for analysis of systems of unknown components having a wide boiling range. Nonvolatile solids in the sample would not be expected to interfere with the solution method, however, these would accumulate in the injection block of the gas chromatograph. This should be cleaned out at intervals, frequent enough to prevent interferences with the gas chromatograms due to adsorption and decomposition. The experience in this laboratory with ingredients such as epinephrine, isoproterenol, and phenylephrine has shown no complications with these when the injection block was cleaned at intervals of several days.

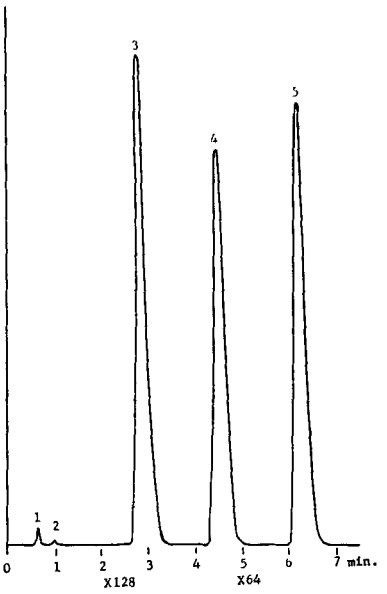

Fig. 4—Chromatogram of aerosol components on a Porapak Q column. 1, air; 2, water; 3–5 propellants 12, 114, and 11, respectively.

Both methods give results of comparable accuracy ±0.5% for most components in a multicomponent analysis. The results for several accurately prepared samples are given in Table I. Ethanol was found to give slightly higher errors up to +0.9%. When greater accuracy is desired for a selected component, it may be obtained by altering the system

TABLE I—ANALYSIS OF AEROSOL COMPONENTS

Components	% Composition (w/w) Taken	Found	Discrepancy
Solution Method			
Propellant 12	48.6 (50.6)	48.3	−0.3
Propellant 114	24.9 (24.1)	25.3	+0.4
Propellant 11	26.5 (25.2)	26.6	+0.1
Total	100.0	100.2	+0.2
Propellant 12	50.4	49.9	−0.5
Propellant 114	24.5	24.9	+0.4
Propellant 11	25.1	25.3	+0.2
Total	100.0	100.1	+0.1
Propellant 12	20.6	20.1	−0.5
Propellant 114	44.8	45.4	+0.6
Ethanol	32.6	33.5	+0.9
Water	2.0	2.1	+0.1
Total	100.0	101.1	+1.1
Propellant 12	20.6	20.1	−0.5
Propellant 114	44.9	44.9	0.0
Ethanol	32.5	33.2	+0.7
Water	2.0	2.1	+0.1
Total	100.0	100.3	+0.3
Direct Method			
Propellant 12	50.3	50.7	+0.4
Propellant 114	24.3	24.0	−0.3
Propellant 11	25.4	25.3	−0.1
Total	100.0	100.0	0.0

of analysis to obtain greater response and resolution for the selected component. The conditions for analysis of multicomponent systems are often necessarily compromises.

The parenthetical values given for the composition of the first example represent the actual amounts taken. Twenty grams of this solution was in a container that had 61 ml. of head space. The actual composition of the solution then in the container was calculated from physical data of the components.

The relative retention times (to that of propellant 11) for some of the components studied are given in Table II for several Porapak columns. The dimensions of the columns, temperature program, and other parameters are those described in the experimental portion. The values for the propellants are essentially the same for all columns. The values for the other components, which are more polar, vary with the columns. A column can be selected that will completely resolve most systems encountered. With a completely unknown system, the Type S column is useful, the only difficulty encountered being a very slight overlap of the propellant 12 and the water peaks. Type Q columns will not resolve propellant 114 and ethanol. Type R columns will only partially resolve propellant 11 and ethanol; type N columns will only barely resolve propellant 11 and ethanol and will also have a very slight overlap of the water and the propellant 114 peaks.

Other overlaps may be encountered if the column is overloaded causing broadening of a peak. The Porapak columns have very limited capacity and will display overloading by a lessening of the peak retention time and increased band width.

Table II—Relative Retention Times of Aerosol Components

Porapak Type	Q	R	S	N
Propellant 12	0.44	0.41	0.46	0.47
Propellant 114	0.74	0.69	0.74	0.74
Propellant 11	1.00	1.00	1.00	1.00
Propellant 113	1.25	1.26	1.25	1.21
Ethanol	0.74	0.96	0.89	1.03
Water	0.18	0.52	0.34	0.60
Ethylene glycol monomethyl ether	1.35	1.54	1.43	1.61

SUMMARY

Gas chromatography has been the method of choice in recent years for the analysis of aerosol propellants. The advent of the porous polystyrene beads has improved the separation and accuracy of these analyses. The methods presented here provide accurate and convenient sampling and analysis of small aerosol packaged pharmaceuticals. These methods may be extended to other sizes and products.

REFERENCES

(1) Percival, W. C., *Anal. Chem.*, **29**, 20(1957).
(2) Jenkins, J. W., and Amburgey, J. M., *Proc. Sci. Sect. Toilet Goods Assoc.*, No. 31, May 1959.
(3) Lawless, G. B., Sciarra, J. J., and Monte-Bovi, A. J., *J. Pharm. Sci.*, **54**, 273(1965).
(4) Lysyj, I., *Gas Chromatography Instr. Soc. Am. Symposium, June* **1961**, Brenner, N., Callen, J. E., and Weiss, M. D., Eds., Academic Press Inc., New York, N. Y., p. 499 (1962).
(5) Hollis, O. L., *Anal. Chem.*, **38**, 309(1966).
(6) Rifino, C. B., Monte-Bovi, A. J., and Sciarra, J. J. *J. Pharm. Sci.*, **54**, 413(1965).
(7) Thonet, T. A., *Aerosol Age*, **4**, 20(1959).

Keyphrases

Aerosols—volatile components, analysis
Propellants—aerosol analysis
Diagram—sampling apparatus
Sampling techniques—aerosols
GLC—analysis