Gas Chromatography and Titrimetry for the Analysis of Certain Medicinal Amines in Aerosols

By GREGORY B. LAWLESS, JOHN J. SCIARRA, and ANTHONY J. MONTE-BOVI

A comparison was made of various methods which could be utilized to determine quantitatively various medicinal amines used in a pressurized dosage form. The medicinal amines investigated during this study were atropine N.F., butacaine, ephedrine (anhydrous) N.F., tuaminoheptane, and propylhexedrine U.S.P. The conditions necessary for the chromatographic analysis were established initially using liquid samples of the medicinal amines dissolved in acetone, nitrobenzene, ethanol, and/or chloroform. Once these conditions were established, the aerosol formulation containing the medicinal amine was analyzed by gas chromatography. A procedure for sampling the aerosol was developed, so that those medicinal aerosol products containing amines also could be analyzed by several existing methods of analysis. A titrimetric method was used for each aerosol as a means of comparison for the gas chromatographic analysis. Atropine and butacaine aerosols could not be determined by gas chromatography. Tuaminoheptane and propylhexedrine were assayed by gas chromatography from a nitrobenzene solution, while ephedrine was assayed from an acetone solution. The results of this investigation indicate the effectiveness of these two methods and show their application to the analysis of aerosol products.

GAS CHROMATOGRAPHY has emerged as an effective analytical tool for the quantitative determination of many medicinal substances (1–6). Fales and Pisano investigated biologically active amines, such as histamine, serotonin, and synephrine (7), through the use of gas chromatography. Separation and identification of sympathomimetic amines was the area of study for Brochmann-Hanssen and Svendsen (8). It was possible to identify several of these amines directly; however, in closely related compounds, derivatives had to be synthesized to effect separation. Ephedrine and pseudoephedrine were treated in this manner.

Porush *et al.* (9, 10) reported methods concerned with the analysis of various aerosol products. Several of the physical testing methods for aerosols, such as particle size and the analytical control procedures necessary in the production of aerosols, were investigated.

The purpose of this investigation was to devise an analytical procedure by which aerosols containing certain medicinal amines could be analyzed by conventional methods as well as gas chromatography (11, 12). Gas chromatography was chosen as the agent for the analysis of these amines because of its ability for separation and identification. Until now, this separation took considerable time and was often quite troublesome, if at all possible (13–17).

The major difficulty associated with the analysis of aerosol products is concerned with the problem of obtaining a representative sample. Since the contents are under pressure, the usually encountered sampling techniques cannot be employed. Care must be exercised in taking the aliquot so that a complete evaluation of the contents of the medicinal containing aerosol can be obtained.

The importance of developing newer methods of analysis for the packaged aerosol becomes vital as the use of this dosage form becomes more widespread (18).

The amines which were the subject of study include atropine N.F., butacaine, ephedrine (anhydrous) N.F., tuaminoheptane, and propylhexedrine U.S.P. These were chosen because of their widespread use and possible application for administration by inhalation techniques.

EXPERIMENTAL

The instrument used was an Aerograph model A-350B equipped with a thermal conductivity cell detector. The columns were stainless steel spirals, 6 to 8 ft. in length, and having an inner diameter of 3 mm. The inert support was Chromosorb P, 100 to 140 mesh. Silicone rubber SE-30 was used as a solid support in a concentration of 1.15%.

Preparation of Aerosol Solutions.—The aerosols subjected to analysis were formulated by the pressure fill procedure in the following manner. About 2.2 Gm. of ephedrine (anhydrous) N.F., was weighed accurately and dissolved in 10 ml. of propellant 142b (chlorodifluoroethane). The aerosol then was prepared in a compatibility tube, and the weight

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of each component was determined during formulation. About 4.7 Gm. of tuaminoheptane was weighed accurately and placed into a compatibility tube. Ten milliliters of propellant 12 (dichlorodifluoromethane) was added, and the aerosol tube was weighed to determine the weight of the propellant used. About 1.0 Gm. of atropine N.F. was weighed accurately and placed in a compatibility tube containing approximately 11.0 Gm. of absolute alcohol which had been weighed accurately. Ten milliliters of propellant 12 (dichlorodifluoromethane) was added, and the aerosol was weighed to determine the weight of the propellant. Propylhexedrine aerosol was prepared by placing about 4.0 Gm. of this substance, which had been accurately weighed, into a compatibility tube. Ten milliliters of propellant 12 (dichlorodifluoromethane) was added; then the aerosol was weighed to determine the weight of the propellant used.

Each of the above amines was assayed initially to determine its purity prior to its use in the aerosol formulations. The quantities indicated in the aerosol formulations represent 100% pure amine or its equivalent.

Volumetric Analysis of Aerosols.—The various medicinal aerosols used in this investigation were assayed by a volumetric method and gas chromatographic method. The medicinal amine was dissolved directly in the propellant and, in those cases where the amine was insoluble, a mixture of the propellant and a cosolvent. This produced a clear solution which was subject to analysis. Where possible, the methods of analysis given in the "United States Pharmacopeia," "The National Formulary," or the "Official Methods of Analysis of the Association of Official Agricultural Chemists" were used. These methods then were modified where necessary to accommodate the aerosol package.

A representative sample was obtained from the formulated aerosol which according to the procedure employed a 500-ml. filter flask, a piece of clean, flexible inert plastic tubing[1] about 20 mm. in length, a one-hole rubber stopper (No. 7), and a connection with a nozzle attached, as shown in Fig. 1.

Since the aerosols in this investigation did not contain a dip tube, it was necessary to invert the container to obtain a sample of the liquid phase. The aerosol was inverted, and the valve was placed on the nozzle of the sampling device (Fig. 1). By pushing down on the aerosol container, the contents will flow through the nozzle of the sampling device, through the plastic tubing, and into the filter flask. For the analysis of the amine, the solution present in the filter flask consisted of a known excess of a standardized acid. A magnetic stirrer was used to insure an even distribution of the amine in the acid. The total weight of the sample was determined by weighing the aerosol container before and after removal of the sample.

After the sample had been dispensed into the filter flask, the nozzle and tubing were washed with neutral ethanol, followed by distilled water to insure that no amine was adhering to these parts. The propellant was allowed to vaporize and escape through the open arm of the filter flask. The amount of amine then was determined by means of a residual titration (19–23). These results are shown in Table I.

Analysis of Aerosols by Gas Chromatography.—To obtain the proper elution of the amines from the column of the gas chromatograph, certain conditions must be met. These conditions were determined for each amine by evaluating them initially as liquid samples in acetone, ethanol, and chloroform. It was later established that for ephedrine, acetone was the best solvent under these conditions, and that nitrobenzene gave the best results for tuaminoheptane and propylhexedrine. The proper conditions for the gas chromatographic analysis of these amines are listed in Table II.

The aerosol package presents a problem insofar as obtaining a sample, since the standard techniques cannot be employed. The sampling technique employed in this investigation was similar to the one used for the volumetric analysis of aerosols. The method consisted of expiring the aerosol into the appropriate solvent, which then was analyzed. The aerosols to be analyzed were formulated in a manner similar to those prepared for volumetric analysis.

In the procedure for analysis, the aerosol was weighed initially, then an aliquot of the liquid phase was allowed to expire slowly into a 10-ml. volumetric flask containing the solvent of choice. The means by which the sample was transferred was a section of stainless steel tubing tapered at one end to fit inside the valve opening of the aerosol. By pushing down on the aerosol, its contents were released through the tubing and into the solvent. The section of tubing was washed with solvent to remove any amine adhering to it. The volumetric flask was brought to volume with more solvent. The aerosol was reweighed, and the difference in weight was

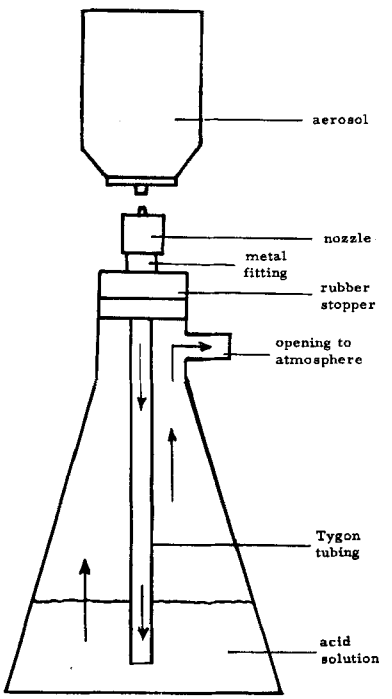

Fig. 1.—Sampling device for volumetric analysis of aerosols.

[1] Tygon, U. S. Stoneware, Akron, Ohio.

TABLE I.—VOLUMETRIC ANALYSIS OF MEDICINAL AMINES IN AEROSOLS

Medicinal Amine	Wt. of Sample, Gm.	Wt. of Amine in Sample,[a] Gm.	Acid Added, meq.	Base Added, meq.	Amine Recovered, Gm.
Ephedrine					
1	2.7672	0.4999	4.4720	1.4504	0.4992
2	2.7771	0.5017	4.4720	1.4480	0.5012
3	2.6282	0.4748	4.4720	1.6012	0.4742
Tuaminoheptane					
1	1.3622	0.3631	9.7520	6.5959	0.3670
2	1.3389	0.3569	9.7520	6.6550	0.3601
3	1.3731	0.3660	9.7520	6.5762	0.3693
Propylhexedrine					
1	1.8373	0.4163	12.1900	9.4776	0.4211
2	1.8285	0.4143	12.1900	9.4874	0.4197
3	1.8263	0.4138	12.1900	9.4924	0.4189
Atropine					
1	6.2950	0.2517	3.3440	2.4159	0.2577
2	3.8367	0.1534	3.3440	2.8022	0.1566
3	6.5480	0.2618	3.3440	2.4221	0.2664
Butacaine					
1	5.4218	0.3879	0.1270	...	0.3894
2	5.7718	0.4137	0.1360	...	0.4154
3	5.6295	0.4035	0.1321	...	0.4050

[a] Calculated on the basis of initial analysis of each amine for purity.

indicative of the weight of the aliquot used. Using a microliter syringe, an accurately measured amount of the solution containing the amine then was injected into the gas chromatograph for analysis.

The area under the peak of the amine from the chromatogram will be representative of the amount of amine present in the injected sample. From the calibration data for each amine, the amount of amine in the injected sample was determined.

To obtain the calibration data, a known amount of the amine was injected into the gas chromatograph, and the area of the peak eluted was determined. By comparing the peak area against concentration of amine, a calibration curve was obtained. The concentration used to prepare the calibration curves was within the range of the concentration of the amine in the aerosol to be analyzed. From the calibration data, the amount of amine present in the sample was determined. Utilizing this information, the amine present in the 10-ml. volumetric flask can be calculated. Three samples of each aerosol were analyzed, and the results of the analysis appear in Table III. Figure 2 illustrates the chromatogram obtained for the propylhexedrine aerosol. Similar chromatograms were obtained for the other amines.

TABLE II.—CONDITIONS FOR GAS CHROMATOGRAPHIC ANALYSIS OF AEROSOLS

Amine	Column[a] Temp., °C.	Detector Temp., °C.	Injector Temp., °C.	Flow Rate, ml./min.
Ephedrine aerosol	171	220	260	30.0
Tuaminoheptane aerosol	120	170	189	30.0
Propylhexedrine aerosol	120	170	189	30.0

[a] The column used for all determinations was 1.15% SE-30 (General Electric Co., methyl silicone) on Chromosorb P (Johns Manville Corp., diatomaceous earth).

DISCUSSION

Aerosol products containing certain medicinal amines can be assayed accurately by means of the titrimetric and gas chromatographic method. Tables I and III indicate the results of these determinations; both methods yielded acceptable results. In all cases, over 99.5% of the amount of amine present could be detected. All of the amines studied could be determined by means of a residual titration, except for butacaine, where a direct titration was necessary. These systems were titrated to a methyl red end point. A comparison of the theoretical amount of amine present and the actual amount is shown in Tables I and IV and is indicative of the acceptability of this procedure.

In the volumetric analysis of the aerosols, the major difficulty encountered involved the sampling procedure. The method of obtaining a representative aliquot of the aerosol quantitatively requires that the release of the aerosol product into the known excess of the standardized acid be done slowly to avoid vigorous agitation of the acid solution. If

TABLE III.—GAS CHROMATOGRAPHIC ANALYSIS OF AEROSOLS

Amine	Sample Expired, Gm.	Amine in Sample Expired, Gm.	Amine in Sample Injected, Gm.	Av. Peak Area of Injection, sq. in.	Amine Recovered,[a] Gm.	Retention Time, min.
Ephedrine	7.4568	1.3470	1.347×10^{-3}	3.7525	1.350×10^{-3}	2.9
Propylhexedrine	11.5973	2.6276	3.941×10^{-4}	3.0682	3.920×10^{-4}	5.4
Tuaminoheptane	11.4536	2.8710	2.871×10^{-4}	1.3825	2.893×10^{-4}	2.2

[a] Average amount of the amine recovered from the injected sample.

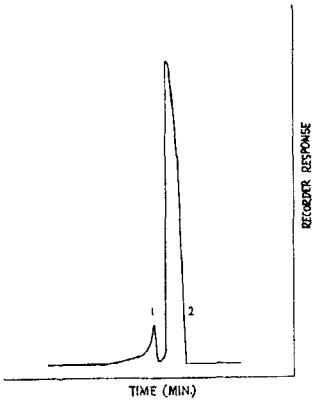

Fig. 2.—Propylhexedrine–nitrobenzene chromatogram. Key: *1*, propylhexedrine; *2*, nitrobenzene.

TABLE IV.—COMPARISON OF RESULTS FROM GAS CHROMATOGRAPHIC ANALYSIS AND VOLUMETRIC ANALYSIS[a]

Aerosol	Volumetric Analysis,[b] %	Gas Chromatographic Analysis,[b] %
Ephedrine	99.87	100.32
Propylhexedrine	101.23	99.47
Tuaminoheptane	100.95	100.76
Atropine[c]	102.07	...
Butacaine[c]	100.39	...

[a] Amount recovered calculated upon initial analysis for purity of each amine. [b] The per cent recovery is the average of three determinations. [c] The proper conditions for gas chromatographic analysis were not established during this investigation.

the aliquot is expired too rapidly, the escaping propellant will cause the solution to bubble vigorously, resulting in a loss of some of the amine and/or the standardized acid through the opening of the filter flask. It is also essential that all of the amine left adhering to the plastic tubing, connection, and nozzle be removed before the analysis is completed.

The volumetric analysis which gave the least accurate results was that of atropine aerosol. This assay gave results which were 2.07% higher than the quantity known to be present. The analysis showing the greatest degree of accuracy was that of ephedrine aerosol, which differed from the known amount of ephedrine by 0.13%.

The gas chromatographic analysis of the medicinal amines in aerosols was performed on the aerosols containing approximately the same concentration of amine as those analyzed by volumetric analysis. The major difficulty encountered in gas chromatographic analysis, as with the volumetric analysis, is the method of sampling and the technique involved in obtaining this sample. The aerosol was expired through a piece of stainless steel tubing into a 10-ml. volumetric flask containing the solvent specific for this analysis. It is imperative that the aliquot be expired slowly into the solvent. Vigorous agitation of the solvent may cause it to bubble out of the volumetric flask, thus losing a portion of the amine from the aliquot. After the aliquot has been expired and the volumetric flask brought to volume, a sample of this solution is removed with a microliter syringe and can be injected into the instrument for analysis.

Table III shows the weight of the aliquot expired from the aerosol and the amount of medicinal amine present in this aliquot. Three samples of each aliquot were injected, and the peak area for each injection was calculated. The amount of amine in the samples injected and the peak area from each of these injections, along with the average peak area, appear in Table III. By comparing the average peak area, from the three samples of each amine injected, the average concentration of the amine in the injected samples was determined from a standard. The amount of amine present in each of the injected samples and the actual amine recovered from these samples appear in Table III.

The data in Table III show the accuracy of each gas chromatographic analysis. The least accurate of these analyses was tuaminoheptane, which differed from the known by 0.76%, while the analysis showing the greatest degree of accuracy was ephedrine. The ephedrine aerosol analysis differed from the known amount present by only 0.32%.

From Table IV, a comparison of the results of the volumetric analysis and the gas chromatographic analysis can be made. Both methods seem to produce comparable results with these specific amines. Once a satisfactory sample of the aerosol product is obtained, it can be subjected to either procedure.

SUMMARY AND CONCLUSIONS

A sampling technique has been developed and found useful in analyzing medicinal amines contained in aerosol products. These medicinal amines can be determined by existing titrimetric and gas chromatographic methods.

Ephedrine, tuaminoheptane, and propylhexedrine were determined satisfactorily by both methods, while atropine and butacaine were limited to the titrimetric method. Conditions for the analysis of atropine and butacaine by gas chromatography could not be determined during this investigation.

The technique involved in the sampling procedure for both these methods of analysis is difficult and tedious. This is the major drawback to both of these analytical procedures. However, this technique does yield good results and can be employed for this analysis.

This method of sampling does not allow for the evaluation of the propellant present. In aerosol analysis, it is essential that the nature of the propellant and the amount present be established. Since the quantity of propellant will determine the amount of medicinal delivered in any given volume of aerosol formulation, the amount present must be known to insure delivery of the proper dose from a metered valve. Further studies are under way to include this determination and the analysis of the aerosol as a complete package.

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Availability of Drugs in the Presence of Surface-Active Agents II

Effects of Some Oxyethylene Oxypropylene Polymers on the Biological Activity of Hexetidine

By WITOLD SASKI and S. G. SHAH*

A growth inhibition study of hexetidine (bis-1,3-β-ethylhexyl-5-amino-5-methyl hexahydropyrimidine) was carried out employing *Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus,* and *Streptococcus faecalis* as test organisms. Minimum and total inhibitory concentrations of hexetidine for the named organisms were determined. The oxyethylene oxypropylene polymers Pluronic F 68, L 64, and L 62 had neither antibacterial nor antifungal power *per se*. The activity of hexetidine in the presence of these surface-active agents at their critical micelle concentrations (CMC's) was decreased. Its activity was enhanced considerably in the presence of Pluronic F 68 and L 64 in the concentrations lower than their CMC's. Pluronic L 62 did not enhance the activity of hexetidine at any concentration. No antifungal activity of hexetidine either alone or in combination with Pluronic F 68 against *Aspergillus niger* was observed.

RESULTS OF A STUDY by Alexander and Trim (1) on the effect of surface-active substances upon penetration of hexylresorcinol into the hog round worm have thrown some light on the mechanism of the transport of this drug through the *Ascaris* cuticle. Sodium cholate, sodium oleate, and cetyl trimethyl ammonium bromide (CTAB), in very dilute solutions, accelerated the penetration of hexylresorcinol; the maximum biological activity occurred at the critical concentration for micelle formation. When concentration of the surfactants was increased beyond their respective critical micelle concentrations (CMC's), the activity of the drug decreased, falling ultimately to zero. Billard and Dieulafé (2) found that the toxic effect of curare, injected intraperitoneally into guinea pigs, could be augmented by the addition of low concentrations of soap and decreased by higher concentrations. Frobisher (3), examining the germicidal activity of phenol/sodium oleate mixture against *Bacillus typhosus*, found an optimum soap concentration for a given phenol concentration.

Several publications, referred to in the previous paper (4), reported synergistic effects with ionic surface-active agents in low concentrations when used together with various antiseptics. Likewise, many reported inactivation of preservatives due to the presence of nonionic surfactants (5). However, there is a dearth of data concerning the possible enhancement of the drug action by the nonionic surfactants. The authors set out to explore the effects of some oxyethylene oxypropylene polymers known as Pluronic F 68, Pluronic L 64, and Pluronic L 62[1] in varying

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[1] Provided by the Wyandotte Chemical Corp., Wyandotte, Mich.