CHROM. 5654

SAMPLING SYSTEM AND ANALYSIS OF BASIC COMPONENTS OF SPRAYS BY LIQUID CHROMATOGRAPHY

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SUMMARY

A sampling system for chromatography is described by means of which compressed samples can be sampled and transferred into a chromatograph without previous pressure release. The system was used for the sampling of sprays from their original vessels without mechanical damage to their containers. The advantage of the use of modern liquid chromatography for the analysis of the basic components of sprays is demonstrated. Hexane was used as the mobile and Porapak Q and T as the stationary phases for the separation of Freon, isopropanol and N,N-diethyl-*m*-toluamide. The dependence of column efficiency and resolution on the flow-rate of the mobile phase is discussed. The reproducibility of the determination of the basic component with the sampling equipment used is a weight concentration of 26.6 ± 0.78%; *i.e.*, ± 3% relative. Changes in the composition of the analyzed mixture were not found when the volume of the vessel was decreased to 10% of its original volume.

INTRODUCTION

When a series of chromatographic analyses are carried out, the problem arises of transferring samples from high pressure containers into a chromatograph without qualitative and quantitative changes occurring in their compositions. In many cases, sampling from the liquid phase of a sample is also necessary. Many analyses of the sprays of pharmaceuticals, cosmetics, dyes and other chemicals (*e.g.* insecticides) have recently become necessary in addition to traditional analyses, such as the analyses of the propane–butane fraction and impurities in liquid carbon dioxide. Sprays of these types usually consist of the basic components and propellants (usually Freons) and additives for providing perfumes or for inhibiting undesired reactions. In most sprays, the basic components are of low volatility or are non-volatile. Sampling from

the gaseous phase is thus inefficient or gives incorrect results for the sample composition.

Apparatus that allows a selected volume to be sampled from the liquid phase of the sample kept at the original pressure and to be transferred into a liquid or gas chromatograph has been developed and tested. As the number of products (especially in the pharmaceutical and cosmetics industries) on which random checks should be made increases, the sampling equipment was designed in such a way that the container would not be damaged.

Liquid chromatography gave good results for the analysis of the basic components of the spray that we chose as an example of the application of the sampling equipment. The substances, the analysis of which would otherwise have to be carried out by gas chromatography (GC) with the temperature programmed up to 200°, were analysed by liquid chromatography in a relatively short time. The possibility of using liquid chromatography for other analyses of this type is suggested, especially group separation of compounds.

EXPERIMENTAL

The sampling equipment used for a liquid chromatograph is shown in Fig. 1. The sampling block 7 is fixed between the nozzle with the needle guide 1 (Fig. 1) and injection port 10 to which a chromatographic column 11 is fixed. The original spraying head of the vessel containing the spray was sealed to an inlet tube 9 with an epoxide resin. Silicone rubber sealing was protected against the mobile phase and sample by non-swelling Viton.

Prior to sampling, the inlet tubes and chamber were washed with a sample. The vessel containing the spray had to be heated during the sampling. For sampling from a full vessel containing 80 g of spray, heating for 2 min in a beaker with 150 ml water at 40°, for an injection block temperature of 20°, was necessary. For injection into the gas chromatograph, equipment of a similar design was used. The sampling block 7, screwed to the heated injection port, was cooled with water. If a syringe with a piston in the needle was used (Hamilton 7001 and 7005), heating of the vessel was not necessary.

The apparatus shown in Fig. 2 was used for analysis by liquid chromatography. Analytically pure *n*-hexane, used as the mobile phase, was degassed by passage through a water bath heated to 55–60°. An MC 300 piston pump (Mikrotechna N.E., Prague) was used. Pressure pulses were balanced with a damping system of dynamic resistances (capillaries of length 50 cm and I.D. 0.2 mm) and capacities (metallic manometric tubes)[2]. The inlet pressure of the column was varied in the range 0.2–25 atm, depending on the length and packing of the columns and the flow-rate of the mobile phase. The composition of the eluate was recorded with a capacitance detector[3].

GC analyses were carried out in a Perkin-Elmer F 11 gas chromatograph with a capillary column of length 150 cm and I.D. 1 mm, packed with Porapak P, 80–100 mesh, at 80°.

Spray samples were injected by means of the sampling systems described, and other substances in the classical manner with Hamilton 7001 and 7005 syringes with the piston in the needle and volumes of 1 and 5 μl, respectively. *n*-Decane was used for measuring the dead volumes of the columns in liquid chromatography. Standard

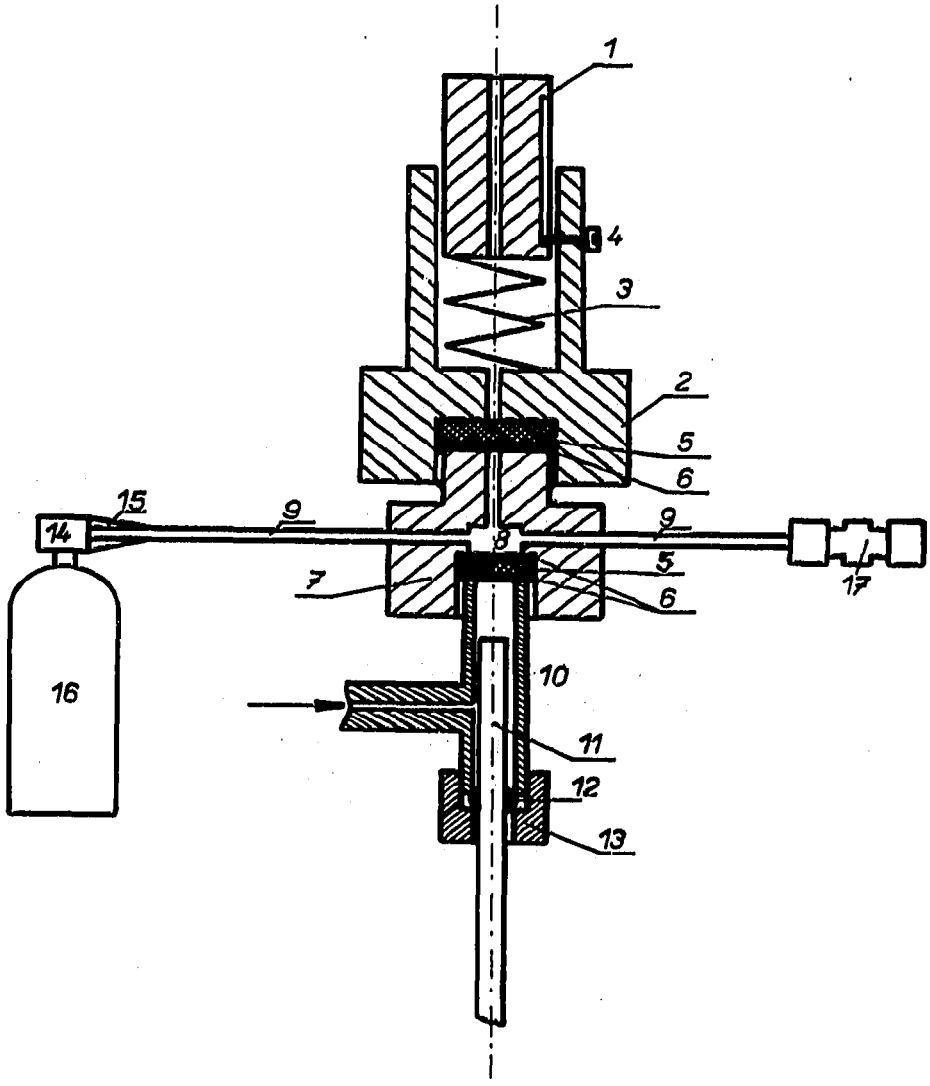

Fig. 1. Schematic section through the sampling equipment. 1 = Needle guide; 2 = nozzle body; 3 = spring; 4 = safety screw; 5 = silicone rubber sealing; 6 = protective Viton sealing; 7 = sampling block; 8 = chamber for sampling; 9 = inlet and outlet tubes; 10 = injection port; 11 = chromatographic column; 12 = sealing; 13 = flange nut; 14 = spraying head; 15 = epoxide resin; 16 = spray vessel; 17 = stopper cap consisting of a metal nut and a metal coupling.

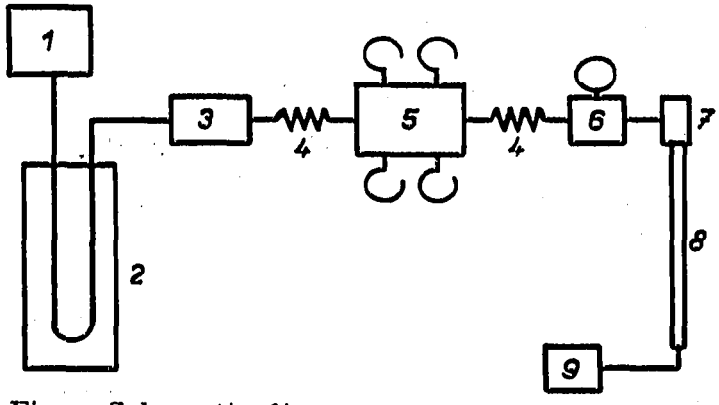

Fig. 2. Schematic diagram of the whole apparatus. 1 = Reservoir of the liquid phase; 2 = degassing bath; 3 = pump; 4 = capillary; 5 = block with manometric tubes; 6 = manometer 7 = sampling block; 8 = column; 9 = detector.

TABLE I

PROPERTIES OF COLUMNS USED

Column No.	*Porapak type*	*Batch No.*	*Mesh*	*Packing (g)*
1	T	595	100–200	0.781
2	Q	399	120–150	0.651
3	Q	399	120–150	0.656
4	Q	399	120–150	0.710
5	Q	668	150–200	0.602
6	Q	668	150–200	0.624

solutions (isopropanol and N,N-diethyl-*m*-toluamide) were prepared by weighing.

Straight stainless-steel columns of length 50 cm and I.D. 0.2 cm were used either separately or connected in series. Porapak Q and T were used as column packings. The columns were not tempered. The columns used are listed in Table I.

RESULTS AND DISCUSSION

The difference in the temperatures of the vessel containing the sample and the sampling block is necessary for filling the whole space with the liquid. The temperature of the ampoule was increased during the sampling by simple immersion in a glass beaker containing heated water. In the gas chromatograph, the injection port of which must usually be preheated, the sampling block was cooled by a stream of tap water. In this case, in order to balance the temperatures of the block and needle (*ca.* 3 min is sufficient), the syringe needle had to be left inserted in the guide for reliable sampling. If a non-cooled needle is used for sampling, incorrect sampling can occur owing to the partial evaporation of the sample in the needle. When sampling the liquid into a non-cooled part of the syringe (*e.g.*, glass capillary), the vessel with the sample must be heated even during cooling of the sampling block. After long use of the microsyringe, a leak was found (bubbles leaked around the piston in the detector needle) and it had to be replaced. The guide[1] of the needle 1 (Fig. 1) in the sampling block proved to be indispensable for sampling at higher pressures (*ca.* 15 atm and above). When sampling without the guide under these pressures, a portion of the sample with the mobile phase leaked round the rubber stopper.

TABLE II

COEFFICIENTS OF VARIATION FOR VARIOUS VOLUMES OF THE SAMPLED MATERIAL

Sampled volume (µl)	*Theoretical[a] value for sampling syringe (%)*	*Isopropanol*		*N,N-Diethyl-m-toluamide in pressure sampling system (%)*
		Without pressure (%)	*In pressure sampling system (%)*	
2	1.1	2.1	3.9	3.6
3	0.6	0.5	2.8	3.9
4	0.4	0.6	2.1	8.4
5	0.4	2.6	3.7	

[a] Extrapolated from values in Ref. 4.

The reproducibility of the sampling with and without the use of the sampling block was considered to be the basic characteristic of the sampling block designed. The spray containing N,N-diethyl-*m*-toluamide, isopropanol and Freons (Krka, Yugoslavia) as basic components was injected through the injection port. In order to compare the results of the sampling, isopropanol was selected as it can be sampled without the risk of sample evaporation or inadequate washing of the syringe owing to the high viscosity of the liquid sampled. The results are presented in Table II. The extrapolated value of the theoretical coefficient of variation[4] for the sampling syringe is given in the second column, and the experimental value of the coefficient of variation of isopropanol calculated from the area under the curve in the liquid chromatogram is given in the third column. The results obtained by using the sampling block described above are presented in the fourth and fifth columns. The values of the coefficient of variation of isopropanol sampled with the other basic components of the spray, especially with excess (*ca.* 60% w/w) of Freon, are listed in the fourth column. Increased values for N,N-diethyl-*m*-toluamide (column 5) are attributed to the asymmetric character of the chromatographic curve of this compound and thus also to the less exact evaluation of the curve.

In order to prove the general applicability of the sampling block in GC, the coefficients of variation of the heights of the Freon peaks and those of isopropanol were measured. The coefficients of variation for 1 μl of the sampled solution are 3.7% for Freon and 2.3% for isopropanol; those for 2 μl of the sampled solution are 3.3% and 3.7%, respectively. The difference between this sampling block and that used in liquid chromatography is in the cooling of the body (7 in Fig. 1) with a stream of water. The injection port of the chromatograph was heated to *ca.* 250°, so that the analysis was not affected by a cold injection of the sample.

As the method of sampling described allows the liquid phase of the compressed sample to be sampled, the change in the contents of the basic components (N,N-diethyl-*m*-toluamide and isopropanol) depending on the sample content in the ampoule was investigated. Five samplings and analyses were carried out with the gradual venting of the liquid phase from the ampoule. No systematic change in the composition of the basic components was found on gradually decreasing the weight of the sample from the original 80.5 g to 8.5 g. The sampling block described can thus also be used for checking samples that have partially run out.

In order to determine the optimum conditions of the analysis, the reduced heights equivalent to a theoretical plate (HETP), the resolution of the basic components of the spray and the times necessary for analyses on Porapak Q and T (columns Nos. 1 and 2, Table I) were compared. While the reduced HETP did not differ for both types of Porapak (Fig. 3), differences in the resolution and, especially, in the total time of the analysis (Table III) were observed. Freon and isopropanol were resolved better on the column with Porapak T; isopropanol and N,N-diethyl-*m*-toluamide on the Porapak Q column. The time of the analysis was three times longer on the Porapak T packing than on Porapak Q at the same flow-rates. Considering the total analysis time and the resolution of the second and third components, the use of Porapak Q was shown to be more advantageous. As pressure drops were low when working with columns 0.5 m long, longer columns packed with Porapak Q, 120–150 and 150–200 mesh (Table IV), were used.

Porapak Q, 150–200 mesh, was selected for the analysis of the basic components

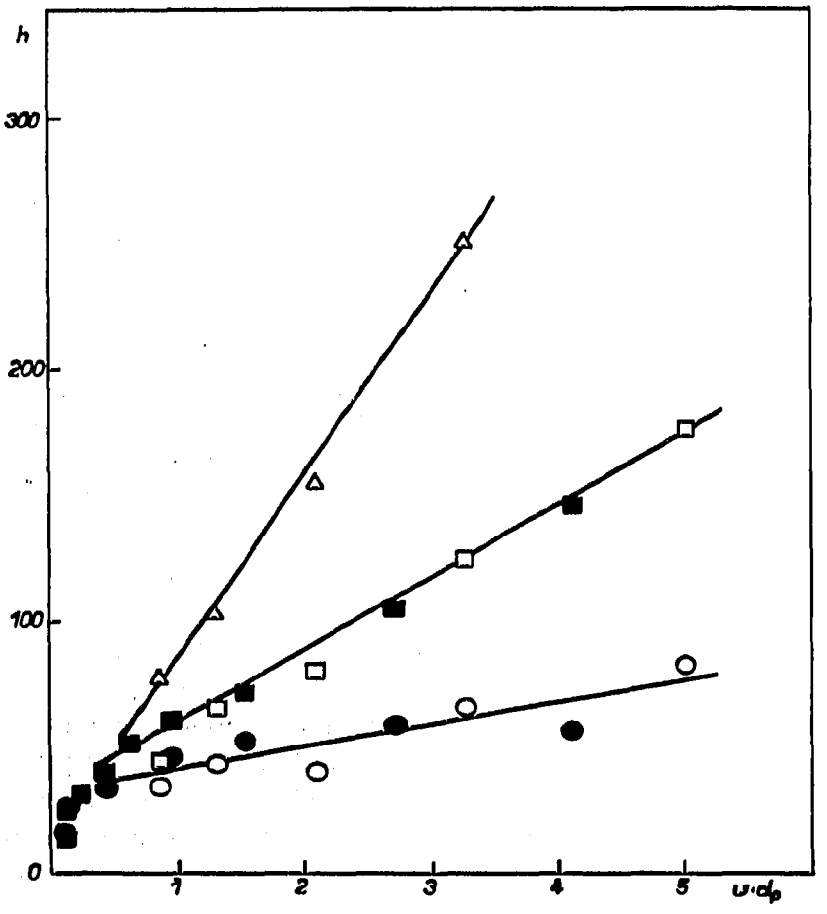

Fig. 3. The dependence of reduced HETP (h) on $u \cdot d_P$ (u = a linear flow rate of the mobile phase; d_P = a particle diameter). Open symbols = Porapak T; closed symbols = Porapak Q. ○, Freons; □, isopropanol; △, N,N-diethyl-*m*-toluamide.

of two different spray samples. The analysis can be carried out, with good resolution of all three components (Fig. 4), by using a total column length of 100 cm and a flow-rate of the mobile phase of 3.60 cm/sec for 11 min. The method of absolute calibration was used for the quantitative evaluation of the chromatogram. The results in Table V agree with those given by the manufacturer.

TABLE III

ANALYSIS OF SPRAYS ON PORAPAK T AND Q

Flow rate, u (cm/sec)	$R_{1,2}$[a]		$R_{2,3}$[a]		*Time of analysis (min)*		*Pressure drop (atm)*	
	Column 1	*Column 2*	*Column 1*	*Column 2*	*Column 1*	*Column 2*	*Column 1*	*Column 2*
3.62	1.59	0.810	0.488	0.556	15	5	7.7	10.0
2.38	1.95	0.914	0.705	0.608	21	7	5.2	6.6
1.52	2.25	1.11	0.922	0.940	33	11	3.6	4.5
0.922	2.54	1.11	1.13	1.12	45	16	2.3	2.9

[a] 1 = Freon; 2 = isopropanol; 3 = N,N-diethyl-*m*-toluamide.

TABLE IV

ANALYSIS OF SPRAYS ON LONG COLUMNS WITH PORAPAK Q

Total length of column (cm)	*Columns used*	*Flow rate, u (cm/sec)*	$R_{1,2}$[a]	$R_{2,3}$[a]	*Time of analysis (min)*	*Pressure drop (atm)*
150	2,3,4	2.22	1.73	1.40	20	18
100	2,3	2.22	1.34	1.14	15	12
100	5,6	4.12	1.68	1.05	9	24
100	5,6	3.60	1.76	1.16	11	21
100	5,6	2.22	1.96	1.37	18	11.8

[a] 1 = Freon; 2 = isopropanol; 3 = N,N-diethyl-*m*-toluamide.

The selection of Porapak for analyses with a mobile liquid phase, which is not new[5], was shown to be advantageous for the determination of basic spray components which differ substantially in polarity. It allowed their good separation in a relatively short time without the need for the gradient technique or flow- or temperature-programming. The relatively poor efficiency of the packing, which could not be improved by the packing technique, was a disadvantage. The inlet pressure began to increase after about 15 h when operating with a high inlet pressure (above 20 atm).

The good and rapid separation of compounds with large differences in polarities

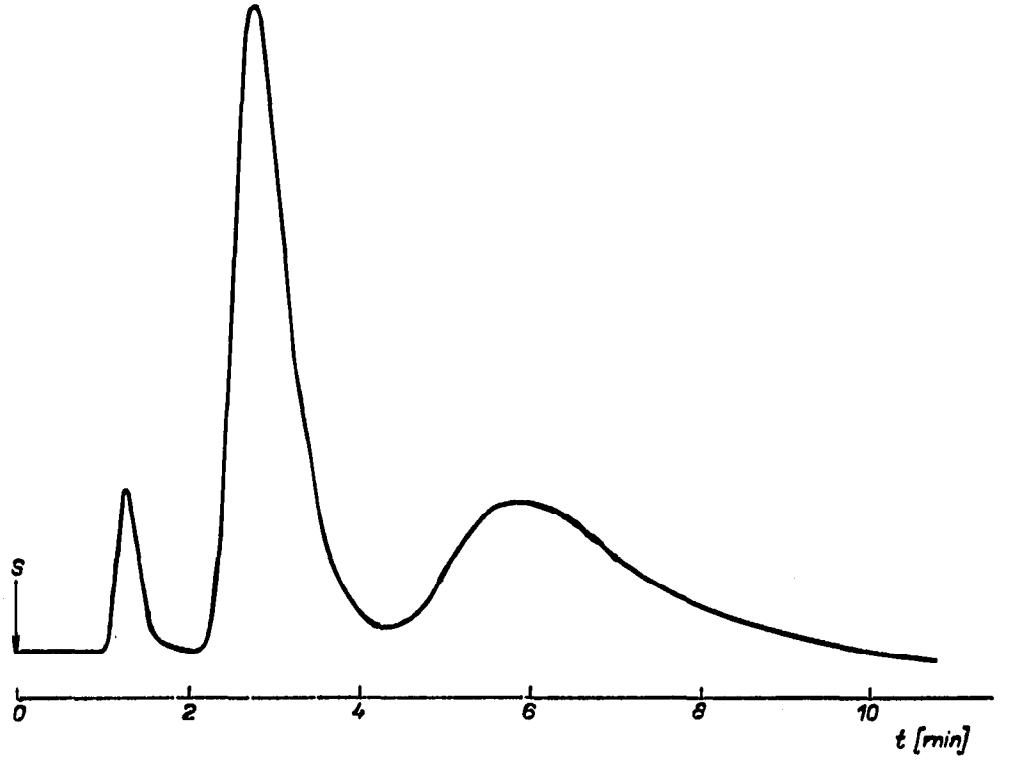

Fig. 4. Chromatogram of the basic components of the spray. Conditions: column 100 cm long, I.D. 2 mm, packed with Porapak Q, 150–200 mesh; linear velocity of the mobile phase, 3.6 cm/sec; injection, 1.5 μl. Retention sequence of the components: freons, isopropanol, N,N-diethyl-*m*-toluamide.

TABLE V

ANALYSIS OF VARIOUS SRPAY SAMPLES

Sample	*Content, % w/w*	
	N,N-diethyl-m-toluamide	*Isopropanol*
A	13.7	26.7
B	12.9	25.1
Value given by manufacturer	13.2	26.6

that was obtained suggested that the use of Porapak should be advantageous for other analyses of this type. The result may also be influenced by the selection of various types that differ in surface polarity. Their use will, however, necessitate operation with lower inlet pressures and with lower linear velocities than are usually possible in modern liquid chromatography. Higher column efficiencies may rather be obtained by decreasing the linear velocity of the mobile phase than by increasing the column length.

ACKNOWLEDGEMENT

Our thanks are due to RNDr. K. TESAŘÍK who carried out the gas chromatographic analyses for this work.

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