https://ntrs.nasa.gov/search.jsp?R=19820010985 2020-03-21T10:20:25+00:00Z
View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by NASA Technical Reports Server

NOTICE

THIS DOCUMENT HAS BEEN REPRODUCED FROM MICROFICHE. ALTHOUGH IT IS RECOGNIZED THAT CERTAIN PORTIONS ARE ILLEGIBLE, IT IS BEING RELEASED IN THE INTEREST OF MAKING AVAILABLE AS MUCH INFORMATION AS POSSIBLE

NASA-CR-167513

(NASA-CR-167513) ANALYSIS OF VOLATILE METABOLITES IN BIOLOGICAL FLUIDS AS INDICATORS OF PRODROMAL DISEASE CONDITION Final Report (Houston Univ.) 25 p HC A02/MF A01 CSCL 06E G3/52

N82-18859

Unclas 09155

ANALYSIS OF VOLATILE METABOLITES IN BIOLOGICAL FLUIDS AS INDICATORS OF PRODROMAL DISEASE CONDITION

Contract No: NAS 9-15882

FINAL REPORT

January, 1982

Principal Investigator: Dr. A. Zlatkis

Department of Chemistry

University of Houston

Houston, Texas

INTRODUCTION

To maintain a healthy population with a fully active life-span, it is important that methods be developed for the early detection of disease. The recognition of the complexity of the diagnosis of disease has led to a demand from the physician for more detailed information on the chemical composition of essential fluids and body tissues. The concept of profiling as a diagnostic aid extends from the asumption of Garrod (1), made in 1908, that pathological states could possibly be reflected in characteristic changes in the concentration of certain constituents in biological fluids. This assumption also forms the cornerstone of Pauling's concept of "orthomolecular medicine" (2,3).

Of particular interest to this laboratory has been the development of techniques for the analysis of the organic volatile fraction from biological fluids. The volatile profile cannot be defined as a single class of substances, rather it is a broad spectrum of materials of different polarities characterised by having a boiling-point in the low to medium range (up to approximately 300°C) and the fact that the compounds are suitable for gas chromatography without derivatization. The organic volatile profiles are very complex mixtures of metabolic byproducts, intermediates, and terminal products of enzymatic degradations composed mainly of alcohols, ketones, aldehydes, pyrazines, sulfides, isothiocyanates, pyrroles, and furans.

The concentration of organic volatiles in biological fluids covers a wide range with many important components present at trace levels. The complexity of the organic volatile fraction re-

quires the use of capillary columns for their separation. The nature of biological fluids prevents the use of direct chromatographic injection techniques as, apart from the sensitivity problem, the large amounts of high relative molecular mass biological molecules and inorganic salts destroy the capillary columns used for the separation. The low sample capacity of capillary columns limits the volume injected to a few tenths of a microlitre, which is a problem in trace analysis unless isolation of the organic volatile fraction from the biological matrix can be performed in a selective manner.

No single sampling procedure is ideal for the isolation of the organic volatile fraction from all biological fluids. The headspace sampling technique (Figure 1) with condensation of the organic volatiles in a cryogenic trap (3-7) or by collection on a porous polymer (8-21) is well suited to the analysis of 24-h human urine samples where sample volume is not a problem. The minimum sample volume for profile analysis is 25.0 ml of urine because of the low efficiency of removing the organic volatiles from the matrix arising from the slow equilibration between organic molecules in the urine sample and the gas phase above the fluid. Sampling times are long and the profile obtained is representative of the concentration of the volatiles in the sample rather than a complete purging of the volatiles. Headspace techniques have also been used for the analysis of serum (22-24), tissue homogenates (22,25), and spinal fluid (12) where sample volume limitations are a problem in developing adequate profiles for diagnostic purposes. For the analysis of small sample volumes (1-5 ml) gas-

phase stripping techniques can be employed (26), but the same observations as applied to the dynamic headspace methods are also true of this technique. When applied to serum, gas-phase stripping techniques (Figure 2) failed to reveal the complete complexity of the organic volatile fraction and the sample requirement of the order of a few milliliters was too high for some problems, e.g., animal studies with small rodents (22, 26). This prompted the investigation of solvent extraction techniques as a means of isolating the organic volatile fraction from urine (27-29), plasma (26, 29, 30), tissue homogenates (26), blood (31), breast milk and amniotic fluid (29). The profiles obtained by solvent extraction are selective as the extraction efficiency is dependent on the partition coefficient of the solutes and the area of contact between the solvent and sample. The limitations of the solvent extraction technique are the need for very pure solvents, evaporation of the solvent is usually accompanied by a loss of compounds of low boiling-point and the fact that no solvent will extract to equal extents the complex range of volatile compounds present in biological fluids. The transevaporator sampling apparatus has been used for the analysis of urine, serum, amniotic fluid, breast milk, and saliva with a sample volume requirement of 5-500 µl.

SAMPLE CONCENTRATION WITH THE TRANSEVAPORATOR

The transevaporator was designed for the micro analysis of trace volatiles in an aqueous solution by selective concentration and removal of water. The apparatus is shown in Figure 3 and can be used to provide in two steps both a modified headspace and an extraction profile on a single sample ranging from 5-500 µl. The sample in a syringe is slowly injected on to the Porasil E

micro-column, which is then attached to the transevaporator by a pre-shrunk PTFE sleeve (sample end down). The remainder of the apparatus is assembled with a water-cooled micro-condenser included between the transevaporator and the Porasil E micro-column. The modified headspace sample is then collected by attaching a Tenax trap to the transevaporator using either a ground-glass joint or a shrinkable PTFE sleeve. The sample transfer is effected by passing helium at a flow-rate of 16 ml min^{-1} through the apparatus for 5-10 mins. The Tenax tube is then removed and stored at $-10^{o}C$ in a screw-capped PTFE-lined culture tube until analysed. The elution micro-column is removed and attached to a second trans-evaporator without a condenser. The extraction profile is collected on a glass bead column attached to the transevaporator. For extraction, 0.5-1.0 ml of solvent (pentane, diethyl ether, 2-chloropropane) is added by syringe to the conical tip of the centrifuge tube, and the apparatus reassembled. The helium carrier gas was switched on and adjusted to a flow rate of 0.5 ml min^{-1} or less so that the solvent travelled slowly up the Porasil E column (1-2 min) and was eventually transferred entirely to the glass bead column. The elution micro-column is then removed, the apparatus reassembled and the helium flow rate increased to 16 ml min^{-1} to remove excess of solvent (5-10 min). The organic volatiles trapped on either Tenax or glass beads are thermally desorbed in a desorption chamber maintained at $280^{o}C$ by purging with helium at 7 ml min^{-1} for 10 min. The volatiles are trapped in a U-shaped stainless steel precolumn (30 cm x 1.0 mm i.d.) coated with the same stationary phase as used for the analytical column and immersed in a liquid nitrogen Dewar flask. The pre-

column is attached to the analytical column (first) via a Swagelock fitting, connected to the carrier gas inlet (injector) by a second fitting, a carrier gas flow rate of 2-5 ml min^{-1} established and the liquid nitrogen trap removed. When the inside diameter of the precolumn was greater than that of the analytical column, the sample was transferred to a second 15-cm precolumn of the same inside diameter as the analytical column by immersing the second precolumn in a liquid nitrogen Dewar flask and heating the first to 200°C with a small electrical current from a d.c. power supply or by use of a heat gun. When the analytical column is made of metal, the first 15 cm of it can be wound into a spiral and used as the second precolumn. To start the analysis, the liquid nitrogen Dewar flask was removed and the temperature program initiated.

The organic volatile fraction was separated on either a nickel capillary column (100 m x 0.25 mm i.d.) coated with Witconol LA-23, or with a 106 m x 0.25 mm i.d. glass column coated with Silar 10C. The sampling procedure is designed to be independent of the gas chromatograph used and any gas chromatograph that is compatible with the use of capillary columns can be used for the analysis.

DATA HANDLING

As a result of the complexity of the organic volatile fraction and problems associated with collecting and evaluating the chromatographic data, a computing integrator is needed to assemble the chromatographic information in a machine readable form. The chromatographic data base is assembled with dimensions of absolute retention time as a descriptor for peak identification and peak area as a descriptor for concentration. Different chromatograms are com-

pared using this data base. When there is a consistent large change in the area of a few peaks in the chromatogram of the organic volatiles obtained from a pathological sample, compared with their mean average peak area in normal samples, then visual inspection of the chromatogram usually suffices for clinical diagnostic purposes. But there are exceptions, when a more sophisticated procedure, computer-aided pattern-recognition, is required. Reliable visual comparison of large numbers of profiles, containing more than 200 peaks, obviously is not feasible. Techniques involve the use of clinically defined samples to provide training sets, which are used to establish pattern vectors characteristic of the sample type. These pattern vectors are then used to classify a further set of samples, not used in the training set, to test the efficiency of the pattern-recognition algorithm. The K-nearest-neighbor-pattern recognition procedure was used throughout the work described in this report. A full description of the programs and operations has been detailed elsewhere (32).

RESULTS

The transevaporator sampling technique was developed out of a series of experimental improvements in the micro-solvent extraction method. Removal of the extraction solvent by syringe was inconvenient and the first transevaporator was designed for this purpose. The base was the extraction tube and a narrow-bore tube extended from the glass-wool trap to just above the ether-sample interface. Helium carrier gas was used to transfer the ether layer to the glass-wool trap and then to strip away the ether solvent. At room temperature, ether extracts about 7% v/v of water, which can cause problems at the desorption stage by

blocking the precolumn. To retain the water and also to distribute the sample uniformly over a large surface area, the biological fluid was injected into a micro-column packed with Porasil E and the extraction solvent forced through the column by gas pressure. The micro-column retains most of the water and the high relative molecular mass polar organics and inorganic salts present in the biological fluids.

The transevaporator is able to provide two profiles, a modified headspace and a solvent elution profile in a sequential manner from the same sample. The modified headspace profile contains very few peaks and is of little value for diagnostic purposes compared with the solvent elution profile.

As an analytical tool, the transevaporator sampling apparatus is a semi-quantitative technique capable of providing reproducible organic volatile profiles from biological fluids. In absolute terms, the percentage recovery of test compounds depends on both the volatility and the chemical nature of the compounds being studied. For aqueous standards containing propan-1-ol, butan-1-ol, decan-2-one, heptan-2-one, and benzaldehyde prepared to cover the range 0.5-5.0 ppm, the percentage recovery was consistent for each compound throughout the concentration range and averaged 20, 40, 50, 55, and 70%, respectively, for the test compounds. The reproducibility of the solvent extraction profile obtained from 50 µl of a pooled normal serum sample is illustrated in Figure 4 for three separate replicate analyses.

The chromatograms in Figure 4 also indicate the complexity of the organic volatile fraction from serum. The intercomparison of chromatograms requires the use of pattern recognition techniques

and computer interpretation to handle the immense amount of information generated in a screening program.

To make the data compatible between chromatograms, a fixed volume of sample is extracted for each analysis and the peak areas are normalised after elimination of background peaks and of odd peaks that appear in only a few chromatograms. The success of the sampling technique as a diagnostic aid is very dependent on the reproducibility of the profile obtained. For the pooled normal serum (10 replicate analyses), the median average relative standard deviation was 18.3% and the range 6.5-51.1%. Retention times can be reproduced to better than 1% relative standard deviation in the chromatographic system. These results are typical of other biological fluids and illustrate the reproducibility that can be expected when using the transevaporator sampling technique. Another important parameter in profiling techniques is the biochemical individuality of the human population. For 15 serum samples obtained from healthy individuals, the median average relative standard deviation was 32.9% and the range 17.1-87.5% for the selected chromatographic peaks used to provide the data base for comparison with patient's serum. The data base consisted of approximately 150 detected peaks, of which about 75 were detected in all 15 serum samples and used for the calculation. The biochemical individuality is much more suppressed in serum than in urine, making the comparison of different profiles less troublesome. For diagnostic purposes, the consistent change in concentration of a peak or peaks greater than one relative standard deviation of the value obtained for the normal profiles is considered significant and used for the detection of the disease.

INVESTIGATION INTO SAMPLING METHODS

A series of methods are used by different surgeions to obtain serum samples. The possibility of contamination of the sample or loss of components from the sample due to the sampling method was statistically evaluated using three human male volunteers. The methods of obtaining the serum samples were as follows:

(a) all glass system using an aluminum needle; glass syringe, glass centrifuge tubes, glass vial (no plastic or rubber)

(b) glass vacutainer with rubber stopper, glass centrifuge tubes and glass vials

(c) as in (b) but heparin added to blood prior to centrifuge step

(d) blood drawn by a Pedi-Butterfly apparatus through a polyethylene tube to a plastic syringe, glass centrifuge tube, glass vial.

Method (a) was certainly the method of choice. The extraneous background contribution to the chromatogram was minimized and no major contaminanats were observed. Method (c) gave the worst results. The use of heparin to aid precipitation of the red blood cells can not be recommended. Several major contaminant peaks were observed. Methods (b) and (d) were intermediate between (a) and (c). Some contamination of the sample was observed compared to serum samples obtained by method (a). This background contribution was variable but in most cases statistically not unacceptable. No major contaminants were found. Methods (b) and (d) were deemed to be acceptable but not preferable. Method (a) using an all glass-aluminum system is highly recommended and

should be used whenever possible.

The loss of organic volatiles from the serum samples obtained by methods (a) to (c) was not significant. Relative concentrations of normal components (excluding contaminants) remained within the experimentally defined limits of the reproducibility of the analytical system.

SERUM FROM VIRUS INFECTED MICE

Ten serum samples were collected from mice as follows: 1-3 are normal serum samples, 4-7 are serum samples from virus infected mice, and 8-10 are serum samples from mice after a convalescent period (i.e., 17 days after virus infection).

A visual inspection was made of all peaks in the chromatograms from mice 1-10. Eighteen peaks were identified as being present in all serum samples and were absent in the blank. The eighteen peak data set was normalized and the relative standard deviation calculated for the normal mouse serum samples 1-3 (Table 1). This data was used to assign experimental and normal variations for the data set and when compared with the values in the virus infected mouse sera, only peaks different by at least one standard deviation from the normal values were considered significant. The relative standard deviation for the peaks covered the range 1-58% which is typical for the transevaporator. The mean relative standard deviation was 18% which is excellent if one considers the complexity of the sample and the trace level (ppm to ppb) levels being determined.

The eighteen peak data set was determined for the four mice (4-7) which were infected with virus. One sample was highly contaminated and was eliminated from the data set. The individual values for 4, 6, and 7 are summarized in Table 2. A comparison

of Tables 1 and 2 indicates that peaks 14 and 15 are significantly elevated above the normal value upon virus infection.

Serum samples were obtained from 8, 9, and 10, seventeen days after infection. It was assumed that the mice would have completely recovered from the infection. The individual values are summarized in Table 3. For each mouse, the normalezed peak area for peaks 14 and 15 has declined from the infected value but has not reached the averaged normal serum concentration of these peaks.

The data obtained in this study is highly predictive as the virus infected mice were easily distinguished form the normal mice samples.

SERUM FROM VIRUS INFECTED HUMANS

Ten serum samples obtained from human volunteers were chromatographed and twenty-six peaks identified as being present in all serum samples and absent in the blank. The samples available included 5 normal serum samples, 4 virus infected serum samples (6-9), and one convalescent serum sample (10). The twenty-six peak data set was normalized and the relative standard deviation calculated for the five normal serum samples (Table 4). The relative standard deviation for the selected peaks covered the range 9-65% with a mean value of 28%. The normalized peak areas for the virus infected serum samples are given in Table 5. Peaks 6, 12, 13, and 25 were found to vary significantly in concentration (the difference in peak area was greater than one standard deviation). Peaks 6, 12, and 25 were lowered in concentration while peak 13 was elevated in concentration. The normalized peak areas for the convalescent serum sample are given in Table 6. For this

sample, the serum values of peaks 6, 12, 13, and 25 have not returned to their normal values. The results obtained in this study are in keeping with our previous preliminary study which adds to the confidence of the general predictive value of this technique for assessing virus infection.

CONCLUSIONS

The transevaporator sampling apparatus developed for the isolation of the organic volatile fraction of serum samples from mice or humans prior to their separation by high-resolution gas chromatography, has a small sample requirement of 5-500 μl, and is ideally suited to profiling studies of serum for the identification of chemical markers of value as a diagnostic aid for the recognition of upper respiratory virus infection. During the project period, a total of 46 human serum and 10 mice samples were analyzed and several diagnostic peaks identified which were characteristic of the virus infected state. Using pattern recognition techniques, 87% of the samples were successfully characterized as virus infected or normal after the training algorithm had been developed.

REFERENCES

1. Garrod, A. E. "Inborn Errors of Metabolism," Henry Froude, London, 1909.
2. Pauling, L., Science, 1968, 160, 265.
3. Pauling, L. Robinson, A. B. Teranishi, R., and Cary, P., Proc. Natl. Acad. Sci., U.S.A., 1971, 68, 2374.
4. Teranishi, R., Mon, T. R., Robinson, A. B., Cary, P. and Pauling, L., Anal. Chem., 1972, 44, 18.
5. Robinson, A. B., Partridge, D., Turner, M., Teranishi, R., and Pauling, L., J. Chromatogr., 1973, 85, 19.

6. Durin, H., Robinson, A. B., and Pauling, L., Clin. Chem., 1975, 21, 1970.

7. Kaji, H., Hisamura, M., Saito, N., and Murao, M., J. Chromatogr., 1978, 145, 464.

8. Zlatkis, A., Lichtenstein, H. A., Tishbee, A., Bertsch, W., Shunbo, F., and Liebich, H. M., J. Chromatogr., 1973, 11, 299.

9. Zlatkis, A., Lichtenstein, H. A., and Tishbee, A., Chromatographia, 1973, 6, 67.

10. Zlatkis, A., Bertsch, W., Lichtenstein, H. A., Tishbee, A., Shunbo, F., Liebich, H. M., Coscia, A. M., and Fleischer, N., Anal. Chem., 1973, 45, 763.

11. Matsumoto, K. E., Partridge, D. H., Robinson, A. B., Pauling, L., Flath, R. A., Mon, T. R., and Teranishi, R., J. Chromatogr., 1973, 85, 31.

12. Novotny, M., McConnell, M. L., Lee, M. L., and Farlow, R., Clin. Chem., 1974, 20, 1105.

13. Robinson, A. B., and Pauling, L., Clin. Chem., 1974, 20, 961.

14. Novotny, M., Lee, M. L., and Bartle, K. D., Chromatographia, 1974, 7, 333.

15. Liebich, H. M., Al-Babbili, O., Zlatkis, A., and Kim, K., Clin. Chem., 1975, 21, 1294.

16. Liebich, H. M., and Al-Babbili, O., J. Chromatogr., 1975, 112, 539.

17. Liebich, H. M., J. Chromatogr., 1975, 112, 551.

18. McConnell, M. L., and Novotny, M., J. Chromatogr., 1975, 112, 559.

19. Goldberg, E. M., and Sandler, S., Chromatographia, 1975, 8, 331.

20. Liebich, H. M., J. Chromatogr. Biomed. Appl., 1978, 163, 125.

21. McConnell, M. L., Rhodes, G., Watson, U., and Novotny, M., J. Chromatogr., 1979, 162, 495.

22. Zlatkis, A., Bertsch, W. Bafus, D. A., and Liebich, H. M., J. Chromatogr., 1974, 91, 379.

23. Dowty, B., Carlisle, D., Laseter, J. L., and Storrer, J., Science, 1975, 187, 75.

24. Liebich, H. M., and Woll, J., J. Chromatogr., 1977, 142, 505.

25. Politzer, I. R., Githens, S., Dowty, B. J., and Laseter, J. L., J. Chromatogr., 1975, 13, 378.

26. Zlatkis, A., and Andrawes, F. A., J. Chromatogr., 1975, 112, 533.

27. Zlatkis, A., and Liebich, H. M., Clin. Chem., 1971, 17, 592.

28. Liebich, H. M., and Hulsgen, G., J. Chromatogr., 1976, 126, 465.

29. Stafford, M., Horning, M. G., and Zlatkis, A., J. Chromatogr., 1976, 126, 495.

30. Stoner, E., Cowrun, D., and Craig, L. C., Anal. Chem., 1975, 47, 344.

31. Dunges, W., and Kiesel, K., in Zlatkis, A., Editor, "Advances in Chromatography," Chromatography Symposium, Houston, 1979, p. 697.

32. Zlatkis, A., Lee, K. Y., Poole, C. F., and Holzer, G., J. Chromatogr. Biomed. Appl., 1979, 163, 125.

TABLE 1. 18 PEAK DATA SET FOR ORGANIC VOLATILES IN NORMAL MOUSE SERUM (MICE Nos. 1-3)

Peak Identification Number	Mean Normalized Area	Standard Deviation	Relative Standard Deviation (%)
1	3.79	0.21	5.5
2	32.57	2.36	7.3
3	5.62	0.71	12.6
4	1.93	0.46	23.9
5	4.97	0.50	10.9
6	13.16	0.19	1.4
7	3.75	0.90	24.0
8	0.98	0.38	38.9
9			
10	1.11	0.64	57.9
11	2.02	0.22	10.6
12	2.00	0.66	32.8
13	10.01	0.98	9.8
14	3.74	0.34	9.0
15	1.54	0.04	2.9
16	3.55	1.65	46.5
17	6.38	0.79	12.4
18	2.34	0.31	13.4

TABLE 2. 18 PEAK DATA SET FOR VIRUS INFECTED MICE (Nos. 4, 6, 7)

Peak Identification Number	NORMALIZED PEAK AREA		
	Mouse 4	Mouse 6	Mouse 7
1	3.88	3.44	1.58
2	37.35	29.94	27.76
3	5.47	4.24	3.49
4	1.61	2.23	1.78
5	6.37	5.42	5.74
6	14.02	13.82	13.09
7	3.02	4.29	2.99
8	0.61	1.49	1.13
9	0.70	1.24	1.12
10	0.90	1.05	1.10
11	2.05	2.53	2.76
12	2.11	2.26	2.04
13	8.55	10.50	12.21
14	4.39	4.97	5.28
15	1.98	1.92	2.76
16	3.53	4.65	5.89
17	2.41	4.67	7.26
18	1.06	1.83	2.07

TABLE 3. 18 PEAK DATA SET FOR CONVALESCENT MICE (Nos. 8, 9, 10)

Peak Identification Number	NORMALIZED PEAK AREA		
	Mouse 8	Mouse 9	Mouse 10
1	2.39	4.52	3.10
2	24.57	28.36	34.26
3	4.29	5.30	5.59
4	1.57	1.38	1.43
5	4.12	4.37	5.09
6	14.83	14.37	14.40
7	3.80	4.55	3.64
8	1.66	0.94	1.40
9	1.18	0.58	0.62
10	0.96	1.30	1.02
11	2.91	1.99	1.87
12	1.56	2.29	1.85
13	12.81	12.42	10.37
14	4.10	1.86	3.46
15	1.95	1.35	1.75
16	6.64	3.06	2.78
17	7.65	8.19	4.87
18	3.00	2.90	2.48

TABLE 4. 26 PEAK DATA SET FOR ORGANIC VOLATILES IN NORMAL HUMAN SERUM

Peak Identification Number	Retention Time Minutes	Mean Normalized Area	Standard Deviation	Relative Standard Deviation (%)
1	19.43	3.68	0.93	25.2
2	20.34	9.14	2.99	32.2
3	20.63	8.73	4.55	52.1
4	23.44	10.38	2.76	26.6
5	28.19	2.54	0.36	14.3
6	28.58	7.26	0.92	12.6
7	29.13	5.54	0.50	9.1
8	32.56	2.29	0.68	29.9
9	33.31	1.28	0.40	31.3
10	33.55	1.36	0.34	25.0
11	37.03	5.76	0.98	17-1
12	37.48	2.27	0.30	13.2
13	39.60	3.08	0.57	25.2
14	40.59	6.24	0.80	28.8
15	42.70	2.84	2.12	34.0
16	43.17	6.39	0.90	31.6
17	44.75	2.62	0.68	10.7
18	48.54	2.99	1.02	25.8
19	50.77	1.51	0.36	34.0
20	51.15	2.52	0.69	23.9
21	53.06	2.29	1.49	27.3
22	53.46	1.57	0.33	65.2
23	56.68	1.24	0.53	20.8
24	58.74	1.24	0.53	42.9
25	23.95	3.14	1.46	46.1
26	24.11	1.80	0.69	35.9

TABLE 5. 26 PEAK DATA SET FOR VIRUS INFECTED HUMAN SERUM

Peak Identification Number	VOLUNTEER NUMBER			
	6	7	8	9
	NORMALIZED AREAS			
1	3.32	3.40	3.42	3.37
2	10.38	12.76	7.95	11.03
3	13.18	14.54	11.06	11.96
4	14.20	11.75	20.16	13.45
5	2.01	2.52	2.18	2.78
6	6.03	5.29	5.30	5.82
7	4.76	5.77	4.20	5.14
8	1.10	4.06	4.87	1.94
9	1.00	1.68	1.86	2.14
10	1.27	1.89	1.98	1.91
11	6.17	4.97	4.15	3.93
12	1.61	*	1.45	1.21
13	2.90	3.54	4.75	3.46
14	1.72	1.50	2.03	2.18
15	3.97	7.06	5.08	7.12
16	3.39	2.11	1.66	1.66
17	6.49	5.62	5.28	5.40
18	1.98	1.68	1.57	2.26
19	1.84	1.27	2.01	3.78
20	1.33	*	0.85	1.06
21	1.52	4.02	1.21	1.47
22	4.28	*	0.83	3.36
23	2.10	1.69	2.65	1.84
24	0.82	*	0.83	0.90
25	1.45	1.62	1.30	1.26
26	1.14	1.33	1.37	1.28

*peak areas less than the integrator threshold value

TABLE 6. 26 PEAK DATA SET FOR A CONVALESCENT SERUM SAMPLE

Peak Identification Number	Normalized Peak Area
1	3.34
2	7.77
3	10.81
4	19.70
5	2.13
6	5.18
7	4.11
8	4.76
9	1.82
10	1.94
11	4.05
12	1.41
13	4.64
14	1.98
15	4.97
16	1.62
17	7.44
18	1.53
19	1.96
20	0.83
21	1.18
22	0.81
23	2.59
24	0.81
25	1.27
26	1.33

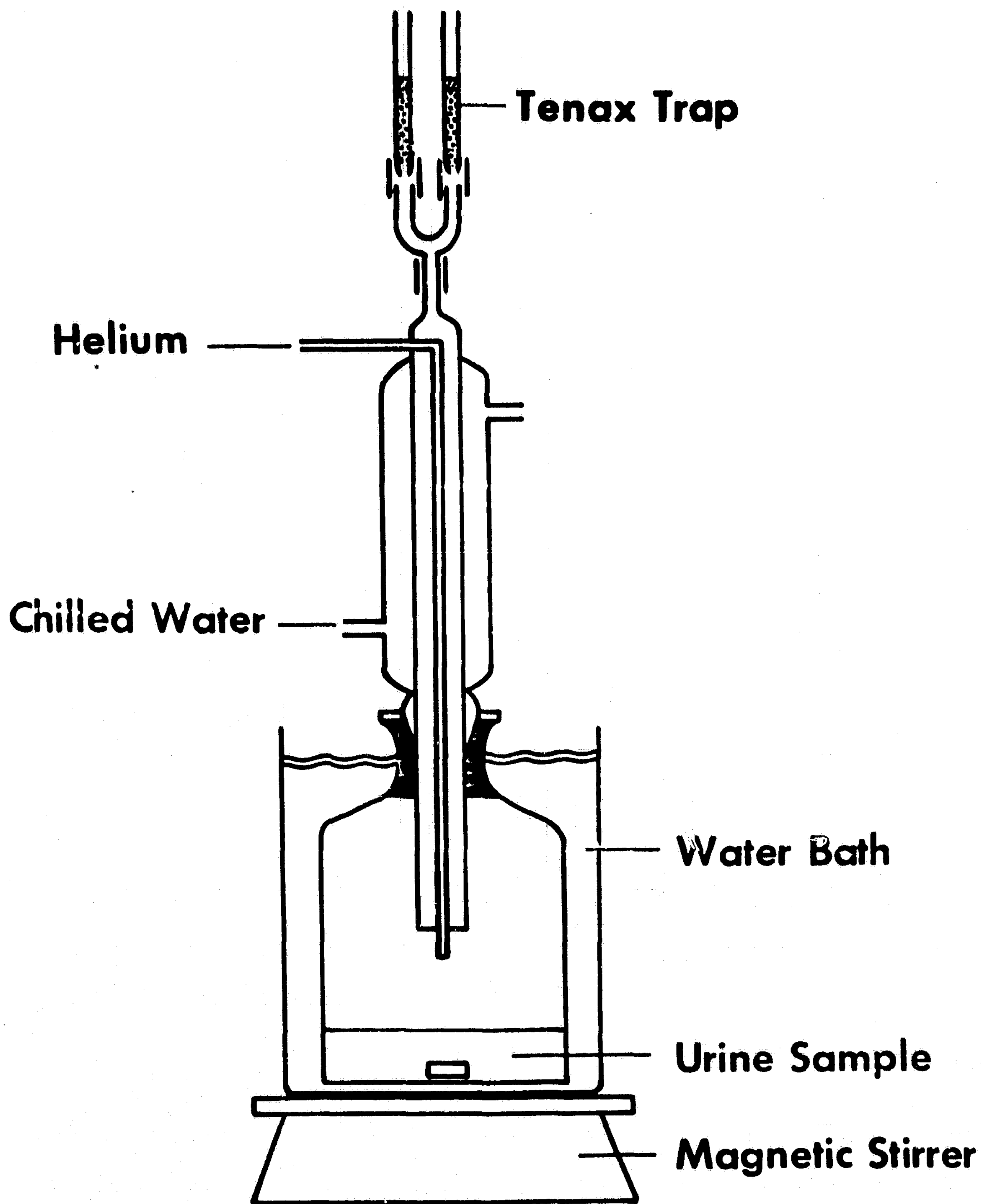

Figure 1

Headspace Sampling Apparatus

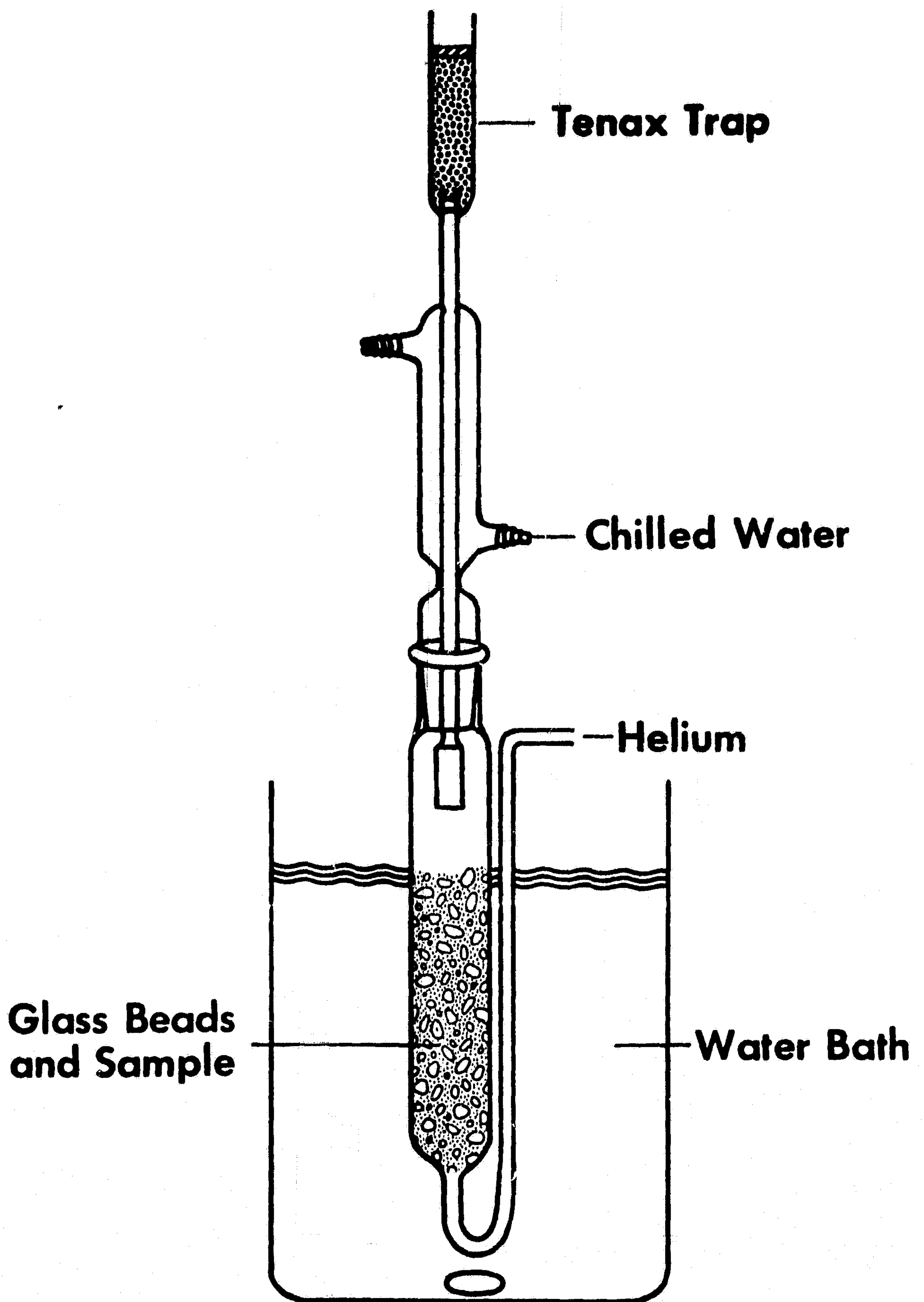

Figure 2
Gas-phase Stripping Apparatus

TRANSEVAPORATOR

MODIFIED HEADSPACE | SOLVENT EXTRACTION MODE

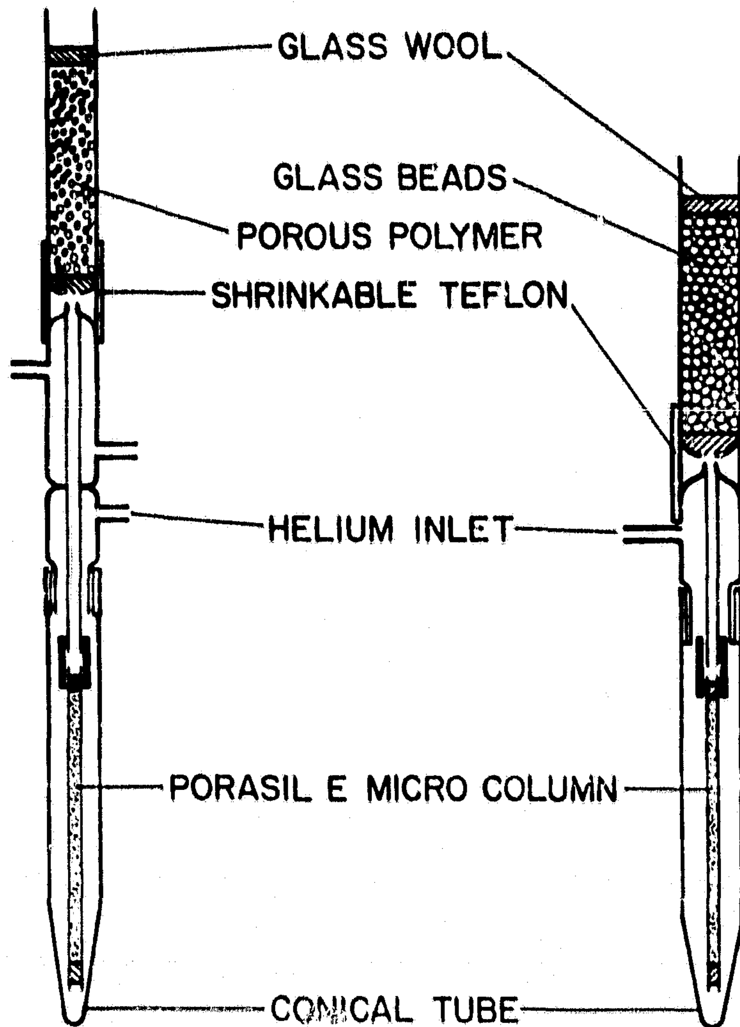

Figure 3

Transevaporator Sampling Apparatus

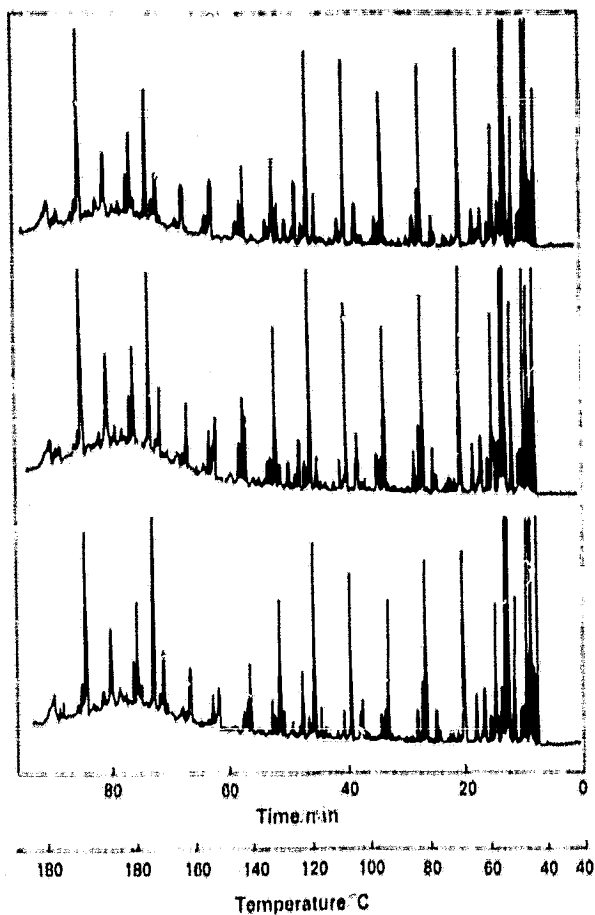

Figure 4. Repeatability of the transevaporator sampling technique in the solvent elution mode for three 50 µl samples of a pooled normal serum. The three replicate separations were performed on a 106 m x 0.25 mm i.d. glass capillary column coated with Silar 10C. The column was programmed with an initial isothermal period at 40°C for 6 min, then programmed at 2°C min^{-1} to 180°C and held at this temperature for 30 min. Helium carrier gas flow rate, 1.5 ml min^{-1}.